Host response to allogeneic implants in the anterior chamber of the rat eye

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The anterior chamber of the eye has long been considered an immunologically privileged site, but the nature of this privilege has been re-examined recently. In our study, we used rats with defined genetic differences as the donors and the recipients. Implants were from split-ear sections, and the host response was measured both in vivo, by implant survival time, and in vitro, by the mixed-leukocyte reaction and the ^{51}Cr-release cytotoxicity assay. Controls consisted of syngeneic implants and allogeneic orthotopic skin grafts. This study has shown that (1) host sensitization occurs approximately 7 days after implantation, (2) cytotoxic effector cells are produced by day 14, with a peak on day 21, (3) implant survival time is long in spite of the presence of competent effector cells, and (4) the survival time of implants is directly related to the degree of genetic histoincompatibility. The reasons for the long implant survival in hosts with a demonstrable immune reactivity has not been established, but the survival time appears to be related to the route of sensitization (vascular rather than lymphatic), which lends itself to the production of serum blocking factors and the procurement of supressor T cells.

Key words: anterior chamber of the eye, transplantation immunity, cellular immunity, immunologically privileged site, mixed-leukocyte reaction, chromium-release assay

The unusual, oftentimes privileged, immunologic treatment of foreign-tissue grafts within the anterior chamber of the eye is an accepted phenomenon. Although certainly sufficient literature attests to this finding, recent re-examination of this subject has been justified by the development of experimental tools that allow a closer examination of the immune mechanisms involved. Although there is disagreement among the various groups of workers in this field with regard to several aspects of the immunology of the anterior chamber of the eye, there appears to be a concensus that the hypothesis that afferent arc blockade explains the privileged nature of the anterior chamber is no longer tenable. The disagreement has centered on whether the major immune mechanism is efferent blockade alone or a combination of efferent and afferent blockade.

Because intraocular structures do not have lymphatics, the time of vascularization is of paramount importance in the assay of allograft survival. Several reports have documented that anterior-chamber implants became vascularized within a few days after implantation yet survived for long periods.[1–3] Kaplan and Stevens[4] have concluded that (1) the privileged nature of anterior-chamber implants could very well result from the presentation of the antigen to the host's immunologic system by the vascular route

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rather than the lymphatics, paralleling the classic findings of Barker and Billingham[5] on the host response to isolated (alymphatic) vascular pedicle skin grafts, and (2) that the survival times of anterior-chamber implants were influenced by several factors including implant age, donor-host genetic differences, and the type of allogeneic tissue placed within the anterior chamber. On the other hand, Franklin and Pendergast[6, 7] have interpreted their results of anterior-chamber implantation in outbred rabbits to mean that the anterior chamber is not privileged, since in their system, the implants were rejected at a similar time and in a similar histologic fashion as were control orthotopic skin grafts.

We have tried to relate the in vivo survival of anterior-chamber implants to an in vitro analysis of the immune response. The results of these comparisons indicate that the host's immunologic system actually reacts quickly to tissue allografts placed within the anterior chamber yet rejection by the host definitely is delayed. The possible mechanisms of this delay are discussed.

Materials and methods

Animals. Female rats weighing 150 to 200 gm belonging to the following inbred strains, with these specific Ag-B designations, were used: Brown Norway (BN) Ag-B^3, Lewis (Le) Ag-B^1, Fischer (Fi) Ag-B^1, ACI Ag-B^4, Buffalo (Buf) Ag-B^6, and MAXX Ag-B^3.

Anterior chamber implantation. The technique of implantation of ear-skin allografts into the anterior chamber has been described.[3] Briefly, 1 mm^2 split-ear implants were inserted into the anterior chamber through a lateral incision made directly above the limbus with a No. 11 scalpel blade. By careful manipulation and pressure on the corneal surface, it was possible to "float" the implant to the side opposite the incision, where contact at the junction of the iris and cornea ensured vascularization.

Skin grafting procedure. The donor was killed by exposure to chloroform, shaved, and rinsed with water. The skin was removed with scissors and forceps and tacked underside-up to a cutting board, where the panniculus adiposus and the panniculus carnosus of the superficial fascia were removed by rubbing the skin with a 4 by 4 in. gauze pad. The skin was kept moist at all times with sterile isotonic saline. When all extraneous tissue had been removed, full-thickness grafts 2 cm in diameter were taken from the skin with a punch and scissors.

The method of grafting was similar to that reported previously.[3] The recipient rat was anesthetized, and a circular graft bed approximately 2 cm in diameter was made on the upper back, exposing the panniculus carnosus. Great care was taken to prevent bleeding within the bed. The graft was fitted to the bed and secured by three "spots" of Eastman Kodak adhesive No. 910, an alkyl-2-cyanoacrylate fixative. The graft became attached within minutes, and a liberal quantity of white petroleum jelly (Moroline) was applied as a moisturizer before the area was bandaged with gauze and adhesive tape. On the morning of the sixth day the bandage was removed, with care taken not to disrupt the graft.

The graft was evaluated twice daily by inspection, an easy and suitable method for determining rejection in most cases. Only when the entire graft appeared avascular or necrotic was it scored as rejected. Sloughing of the graft was not used as a criterion for rejection because of its high degree of unreliability in this laboratory and others.[8] Biopsies of the graft were from edge to edge and full thickness. We found it inadvisable to biopsy a graft more than once, since the trauma caused by the initial biopsy made the interpretation of a second biopsy specimen difficult.

Second-set grafts were prepared in a similar manner and applied, shortly after the rejection of the primary graft, to a fresh graft bed.

Mixed leukocyte reaction (MLR). This reaction was brought about as described by Wilson and Fox[9] with minor modifications. Responder cells were harvested from the spleens of untreated, skin-grafted, or anterior-chamber–implanted Le recipients, and stimulator cells were obtained from the spleens of BN donors. The spleens were homogenized, the cells were washed, and the leukocytes were prepared by density centrifugation in Ficoll-Hypaque. Stock solutions of both responder and stimulator cells at a concentration of 2×10^6 cells/ml were prepared in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). A 0.1 ml amount of both cell populations was mixed in the wells of Linbro tissue culture Disposo-trays, six replicate wells being used for each test condition. Mitomycin C was not used in this short (48 hr) MLR assay because preliminary studies had demonstrated that sensitized cells have a significantly greater response than do nonimmune cells in mixed culture during the ini-

Table I. Survival of primary and secondary skin allografts between strains of inbred rats with diverse immunogenetic barriers

Strain combination		*Immunogenetic locus*	*Barrier strength*	*Median survival time (days)*	
				1st set ± S.D.	*2nd set ± S.D.*
BN	Le	Ag-B	Strong	8.7 ± 0.2 (73)*	6.8 ± 0.2 (51)
BN	Fi	Ag-B	Strong	10.0 ± 0.4 (28)	6.3 ± 1.1 (14)
BN	BUF	Ag-B	Strong	8.5 ± 0.5 (6)	6.0 ± 0 (7)
BUF	ACI	Ag-B	Strong	9.7 ± 0.5 (7)	7.6 ± 0.5 (8)
Lew	Fi	Non Ag-B	Intermediate	8.8 ± 0.3 (34)	6.2 ± 0.3 (11)
F/344	Le	Non Ag-B	Intermediate	8.2 ± 0.5 (8)	6.6 ± 0.5 (9)
Lew	MAXX	Ag-B	Weak	13.0 ± 0 (4)	—
BN	MAXX	Non Ag-B	Very weak	20† (5)	

*Number in parentheses represents number of animals.
†Intact and viable at time of writing.

Table II. Correlation between visual and histological examination of orthotopic skin grafts between BN donors and Le recipients

Type of graft	*Correlating viability ratio: histological/visual*			
	Viable	*±**	*Nonviable*	*Total*
Primary	14/14†	2/4	11/11	27/29
Secondary	5/5	5/5	9/9	19/19
Combined	19/19	7/9	20/20	46/48

*Borderline viability.
†14/14 means that of 14 grafts considered to be viable by visual inspection, all 14 were considered viable by histological examination.

tial 48 hr of incubation. Negative control cultures consisted of unsensitized Le cells with BN spleen cells, and positive control cultures contained lymphocytes from skin-grafted Le rats in mixed culture.

The microtest plates were incubated at 37° C in an atmosphere of 10% CO_2 for 48 hr. After 24 hr, 0.1 ml of complete medium containing 1 μCi of tritiated thymidine (40 to 50 Ci/mmol specific activity; New England Nuclear, Boston, Mass.) was added to each mixed culture. The cells were incubated, and after an additional 24 hr, they were harvested with a MASH II unit (Microbiological Associates, Inc., Bethesda, Md.). A stimulatory index (SI) was calculated by dividing the disintegrations per minute (dpm) in mixed cultures containing either implanted or skin-grafted Le responder cells by the dpm in mixed cultures containing untreated Le responder cells.

Cell-mediated cytotoxicity. Target spleen cells from BN rats were labeled according to the procedure of Biesecker[10] with minor modifications. A 1 ml amount of this cell suspension, containing 3 to 4×10^7 viable cells and 250 μCi of radioactive sodium chromate (^{51}Cr; specific activity 200 μCi/μg of chromium at 1000 μCi/ml; New England Nuclear), was incubated for 45 min at 39° C with end-over-end rotation in disposable screw-top centrifuge tubes in a humidified atmosphere of 10% CO_2 and 90% air. After the cells were washed three times, they were pooled and resuspended at a concentration of 10^5 viable cells/ml RPMI 1640 medium containing 10% FCS.

The assay used to measure cell killing was adapted from that reported earlier by Brunner et al.[11] Triplicate samples of labeled target cells (5×10^4 cells in 0.5 ml) were dispensed into 13 by 100 mm screw-top tissue-culture tubes. Effector spleen cells (5×10^6 in 0.5 ml) were then added to each tube, achieving an effector cell:target cell ratio of 100:1. The tubes were then rotated at approximately 6 rpm at 37° C for 6 hr in an atmosphere of 10% CO_2 and 90% air.

For the assay of ^{51}Cr release, 1 ml of Hanks' balanced salt solution was added to the tubes after incubation. The tubes were then centrifuged at 200 × g for 5 min. The radioactivity in 1 ml of the supernatant fluid from each tube was measured. The negative control (assay for nonspecific

Table III. Survival of skin implants in hosts of varying histocompatibility differences

Donor-recipient combination	*Immunogenetic barrier*	*Survival time* (days)*						
		7	*14*	*21*	*28*	*35*	*49*	*63*
BN → Le	Ag-B	20/20 (100)†	34/36 (94)	37/53 (69)	10/34 (29)	0/6 (0)	0/34 (0)	0/4 (0)
BN → Fi	Ag-B	40/40 (100)	38/38 (100)	—	12/41 (29)	0/8 (0)	—	—
BN → BUF	Ag-B	—	18/18 (100)	—	4/11 (36)	—	—	—
BUF → ACI	Ag-B	—	8/8 (100)	—	—	1/11 (9)		
Fi → Le	Non Ag-B	17/17 (100)	25/25 (100)	12/12 (100)	—	—	—	—
Le → Fi	Non Ag-B	—	24/25 (96)	18/20 (90)	15/16 (93)	16/16 (100)	10/10 (100)	4/6 (66)
Le → Le	None	—	—	—	—	7/7 (100)	—	6/6 (100)

*Reported as number implants viable/number implants performed.
†Numbers in parentheses represent % implant survival.

cytotoxicity) contained effector cells from untreated hosts, incubated as above with labeled target cells.

The percentage of specific ^{51}Cr release was calculated as follows:

$$\frac{\text{Isotope release in test system} - \text{isotope released in control system}}{\text{Isotope release by freeze-thaw} - \text{isotope released in control system}} \times 100$$

and reported as the mean ± standard deviation.

Results

Survival of orthotopic skin grafts. To obtain first- and second-set survival times as a baseline, skin grafts were made between various donor-recipient combinations. The results of these studies are presented in Table I. The median survival times of the various combinations ranged from 8.2 to 10 days, with most falling around 8.5 days. Grafts between animals with strong and intermediate histoincompatibilities had similar survival times. There was approximately a 2-day difference in the survival times between first- and second-set grafts in most combinations. In one instance, BN to Fi, there was a difference of almost 4 days.

To confirm the accuracy of visual inspection as a means of determining graft rejection, biopsy specimens of 48 grafts, both first- and second-set, were examined histologically by someone having no knowledge of the gross appearance of the graft (Table II). In 46 of the 48 cases, histologic examination confirmed the visual findings. In the two remaining cases, the graft was given a borderline viability classification visually and a moderately viable designation histologically. Since these results indicated that visual scoring of skin-graft rejection correlated well with histologic examination, all future grafts were scored by visual means. Rejection was never seen in autologous or syngeneic control grafts.

Table IV. Suitability of sensitized spleen and thymus cells from skin-grafted Le rats ($Ag\text{-}B^1$) to serve as responder cells in MLC with donor-specific (MAXX) allogeneic ($Ag\text{-}B^3$) cells

*Responder population**	*Control†*	*SI ± S.E.*
Spleen	Spleen	1.5 ± 0.1 (<0.01)‡
Thymus	Thymus	1.0 ± 0.02 (NS)

*Cells from day 7 skin-grafted animals in mixed culture (n = 3 per group).
†Nonimmune cells as the responder population in mixed culture for establishment of baseline levels (n = 3 per group).
‡p value. (NS) = Not significant.

Histological evaluation of skin implants within the anterior chamber of the eye. The viability of the implant was estimated from the appearance of the ectodermal elements within the graft, namely, the squamous epithelium lining the skin surface and hair follicles and the persistence of the sebaceous

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Table V. Need for an Ag-B immunogenetic barrier for stimulation in the MLC

Le responder cells	*Donor + stimulator spleen cells*		*SI**	*p value*
Control	BN	(9)†	1.0 ± 0.03	
	Fi	(9)	1.0 ± 0.03	
Day 7 after	BN	(3)	1.3 ± 0.05	<0.01
skin graft	Fi	(3)	0.95 ± 0.04	NS‡
Day 7 after	BN	(3)	1.4 ± 0.05	<0.01
a.c. implant	Fi	(3)	0.85 ± 0.03	<0.01
Day 14 after	BN	(3)	1.5 ± 0.08	<0.01
a.c. implant	Fi	(3)	0.9 ± 0.07	NS

Demonstration of a proliferative response in the mixed lymphocyte reaction only when the Ag-B immunogenetic barrier is crossed between responder and stimulator cell populations. Responder cells represent spleen cells from rats sensitized by either an orthotopic skin graft or an anterior chamber (a.c.) implant. Donor-recipient combinations represent BN to Le (Ag-B immunogenetic barrier) and Fischer to Le (non Ag-B immunogenetic barrier). Culture conditions: 4×10^5 total cells per 0.2 ml of medium in 1:1 responder-to-stimulatory-cell ratio, 48 hr incubation at 37° C, 1 μCi of ^{3}H-TdR added 24 hr before harvesting.

*Mean ± S.E.

†Number in parenthesis is the number of animals.

‡Not significant, $p > 0.05$.

glands. Upon implantation, the graft curled up, forming an "external" cyst lined by the surface squamous epithelium.[12]

The blood vessels supplying the implant arose from the iris or, in some cases, from the ciliary body, but not from the cornea. This was probably the result of the care taken during implantation to lodge the graft in the periphery of the anterior chamber. The graft appeared to become fixed to this area by fibrous tissue proliferation. Vascularity was apparent in viable implants, along with an infiltrate of mononuclear cells. During the rejection process, the vascularity diminished and disappeared, accompanied by a slow but progressive necrosis and absorption of the ectodermal element, leaving a formless mass of relatively anucleate collagenous tissue in the rejected graft. At this stage, epithelial elements were unrecognizable, the cyst space collapsed, and the cellular infiltrate disappeared. The mononuclear infiltrate, thought to be part of the rejection process, was evident in each specimen, but the degree of infiltration was variable, and no direct correlation could be made between this reaction and the stage of the rejection process. However, the appearance of phagocytic histiocytes (frequently multinucleated) around the epithelium, keratin debris, and hair shafts during the rejection process was found to coincide with this host response. Areas of inflammation and involvement of polymorphonuclear leukocytes apparently did not influence the implant.

Survival of implants. The first study was performed to determine the survival time of skin implants in the anterior chamber of the eyes of several rat strains (Table III). A clear distinction in the survival of the implants between Ag-B-incompatible and Ag-B-compatible donor-recipient combinations is evident. In the former group, 95% to 100% of these implants survived 14 days. The survival decreased to approximately 30% by day 28, with none surviving as long as 35 days. Implants in the latter donor-recipient combinations survived for much longer periods, but ultimately rejection occurred after approximately 3 to 4 months. That the implants did not die from nonspecific causes such as ischemia or a lack of nourishment was shown by the fact that syngeneic implants continued to survive after all the alloimplants were rejected.

Because a high percentage of the BN to Le (Ag-B incompatible) implants survived 21 to 28 days, this donor-recipient combination was employed in all the other studies to detect, by in vitro techniques, the recipient's immune response to donor tissues placed in the anterior chamber of the eye.

MLR. Numerous studies of mixed leukocyte cultures (MLC's) have shown that there is an immunologically specific proliferative reaction, in which a large proportion of immunologically competent, predominately thymus-derived, circulating lymphocytes from both immunized and nonimmunized donors recognize and respond to the presence of major histocompatibility isoantigens. Immune cells in culture respond more quickly to stimulation than do nonimmune cells, and this fact was used to detect sensitization in both skin-grafted and implant-bearing hosts. The first consideration was the choice of cell type to be used for culture.

Table VI. MLR in Anterior-chamber Implant–bearing and orthotopic-skin-grafted Le rats

Type of graft	*SI at various days after grafting**						
	5	*7*	*9*	*14*	*21*	*28*	*48*
Orthotopic BN skin graft n = 9	1.58 ± 0.13† (<0.025)‡	1.30 ± 0.07 (<0.01)	1.16 ± 0.03 (<0.01)	—	—	—	—
BN anterior-chamber implant n = 13	—	1.37 ± 0.05 (<0.01)	—	1.51 ± 0.09 (<0.01)	1.27 ± 0.05 (<0.01)	1.33 ± 0.06 (<0.01)	1.37 ± 0.07 (<0.01)

*Mean baseline value of nonimmune controls in MLC 1.0 ± 0.02, n = 18.
†Mean ± S.E. of 3 animals, each tested in six replicate wells.
‡p value using Student's t test.

Both spleen and thymus cells were tested as responder cells after 48 hr in mixed culture with allogeneic spleen cells as the stimulator population (Table IV). The results indicated that immune thymus cells were unresponsive to allogeneic stimulation whereas sensitized spleen cells showed a significant response in comparison to control nonimmune cells. On the basis of these data, in all the other mixed leukocyte studies, spleen cells were used as the responder-cell population.

The next study was performed with the use of spleen cells from Le recipients that had been sensitized either by skin grafts or implants in the anterior chamber of the eye from either BN or Fi donors. The immune spleen cells exhibited a proliferative response only when there was a major Ag-B mismatch between the responder and the stimulator cell populations (Table V). This was true regardless of the manner in which the hosts were sensitized, whether by skin grafting or implantation. Therefore only those donor-recipient combinations that differed at the Ag-B locus were employed for further studies using the MLR.

The main goal of the mixed leukocyte studies was to determine when sensitization could be detected in implanted recipients. For controls, responder cells from recipients immunized by an orthotopic skin graft were assayed on days 5, 6, 7, and 10 after grafting. Table VI shows that (1) sensitization was detected by day 5, with a proliferative response in excess of 1.5; (2) the peak response occurred prior to graft rejection; and (3) the SI declined during the latter part of the rejection period. On the basis of these results, a similar study was undertaken in implant-bearing rats. Le responder cells were tested on days 7, 14, 21, 28, and 48 after implantation of BN skin into the anterior eye chamber. These data indicated that an accelerated proliferative response of spleen cells from animals receiving implants was detectable by day 7 and peaked at day 14, with an SI of 1.5. Subsequent determinations revealed a slightly reduced SI throughout the 48-day period of study.

Table VII. Determination of the optimal effector and target cell combinations in the cell-mediated cytotoxicity assay

Effector cell (Le)	*Immune status of the host*	*Target cell (BN)*	*Percent specific release*
Spleen	Normal	Spleen*	0
	Skin graft (day 7)	Spleen	15 ± 1.5†
Thymus	Normal	Spleen	0
	Skin graft (day 7)	Spleen	0
Spleen	Normal	Thymus	0
	Skin graft (day 7)	Thymus	8 ± 1.0
Thymus	Normal	Thymus	0
	Skin graft (day 7)	Thymus	0

*n = 3 for each combination.
†Mean ± S.D. of 3 assays, each in triplicate.

Cell-mediated cytotoxicity. The detection of cell-mediated immunity depends on several factors, e.g., type and conditions of the assay and the choice of effector and target cells. We had determined (unpublished observations) that the ^{51}Cr-release assay was re-

Table VIII. Cell-mediated cytotoxicity assay of spleen cells from Le rats bearing either anterior-chamber implants of BN skin or BN orthotopic skin grafts

	Percent chromium released by cytotoxic spleen cells on various days after grafting				
Type of graft	*7*	*10*	*14*	*21*	*28*
Skin graft n = 15	12 ± 2.0*	47 ± 6.0	4 ± 2.0	—	—
AC implant n = 15	—	—	17 ± 2.0	30 ± 3.0	7 ± 1.0

*Percent specific cytotoxicity ± S.D., compensating for nonspecific spontaneous release (range, 15% to 20%) as described in Materials and methods.

producible, with useful differences between the amounts of nonspecific spontaneous release and the values obtained in cytotoxic systems, under the following conditions: pH of 7.1, use of 10% heat-inactivated FCS, end-over-end rotation at 6 rpm in a 37° C incubator with a 10% CO_2 atmosphere, and an effector cell:target cell ratio of 100:1. Samples were routinely assayed in triplicate.

The extent of cell-mediated cytotoxicity also depends on the choice of effector cells and target cells. Spleen and thymus cells were tested as both effector and target cells at a ratio of 100 effector cells to one target cell. Effector cells were obtained from either implanted or skin-grafted hosts. As shown in Table VII, no killing was detected when thymocytes were employed as effector cells, but spleen cells were highly cytotoxic to splenic target cells and weakly cytotoxic to thymic target cells. The time of appearance of cytotoxic effector cells in Le rats bearing BN skin allografts was compared with the time of appearance of effector cells in Le rats bearing BN skin implants in the anterior chamber of the eye (Fig. 1 and Table VIII). Cytotoxic spleen cells, as determined by this assay system, were present in the animals bearing anterior-chamber implants by day 14, peaking at day 21 but rapidly declining to a low level by day 28. In contrast, animals with orthotopic skin grafts yielded a cytotoxic cell population considerably earlier. Cytotoxic cells were strikingly evident by day 7, peaked by day 10, and disappeared by day 14. Nonimmune cells from control animals were not cytotoxic, and they were used routinely in the control assay as an indicator of nonspecific, "spontaneous" release, which usually averaged 15% to 20%.

Discussion

Even though interest in the anterior chamber of the eye as a privileged site for foreign grafts is at least a century old, numerous early studies suffered from the lack of genetically controlled model systems. It was Medawar[12] who undertook a study of the anterior chamber of the eye under controlled immunologic conditions. Subsequently, Woodruff[13] noted that anterior-chamber implants, although they survived for a long time in normal hosts, were rapidly destroyed in presensitized hosts. From these findings, it was inferred than an afferent arc blockade of the immune response may be present, possibly because of the absence of blood vessels in these implants. In earlier work,[1, 14] we showed that anterior-chamber implants always became vascularized as early as 4 days after implantation but nevertheless survived a long time. We also showed that animals bearing anterior-chamber implants rejected orthotopic skin grafts in second-set fashion, indicating that there was no afferent arc blockade in these animals as had been assumed. Histologic examination of the implants revealed an intense infiltration by small round cells despite the continued survival of the implant, suggesting that the infiltrating cells were unable to bring about implant rejections. These findings have now been confirmed by other laboratories.[4, 6]

Franklin and Pendergast,[6] working with outbred rabbits, have argued that anterior-chamber implants undergo a rejection pro-

cess similar to that of an orthotopic skin graft in both time and histology, indicating that the anterior chamber is not a privileged site at all. However, the implant size (50 mm^2) could very easily account for these findings, for there is known to be a direct relationship between graft size and survival time.[15] With these facts, it is reasonable to assume that had Franklin and Pendergast used smaller implants (1 mm^2), as we did, survival for as long as 28 days could have occurred. Although Franklin and Pendergast gave no data on the skin-graft survival in their outbred rabbits, this generally has been 6 to 11 days in our system. Thus it would seem that even though anterior-chamber implants are ultimately rejected, their survival time is at least several times longer than that of an orthotopic skin graft. Whether one calls the anterior chamber a privileged site because there is a prolongation of implant survival there or designates it an "unprivileged" site because implants ultimately undergo immunologic rejection would thus seem to be a semantic distinction. The important point is that the mechanism is immunologic, as shown by the fact that the survival time of anterior-chamber implants varies according to the degree of genetic disparity of the host-recipient combination. Thus, in the Ag-B-compatible donor-recipient combinations, anterior-chamber implants survived for several months, but with implants between Ag-B–incompatible animals, the mean survival time was only 21 to 28 days.

Our in vitro investigations were perfomed to obtain a better understanding of the immune status of the host that bears allogeneic skin implants within the anterior chamber of the eye. The MLR was employed to study the afferent arc of the immune system. It has been shown that the MLR detects sensitization early in the host's immune response. Sensitivity was detected in the implant-bearing host as early as 7 days after implantation, even though the peak sensitivity occurred at 14 days after implantation.

The ability of sensitized lymphocytes to kill allogeneic target cells in vitro is an indication of the efferent immune potential of these

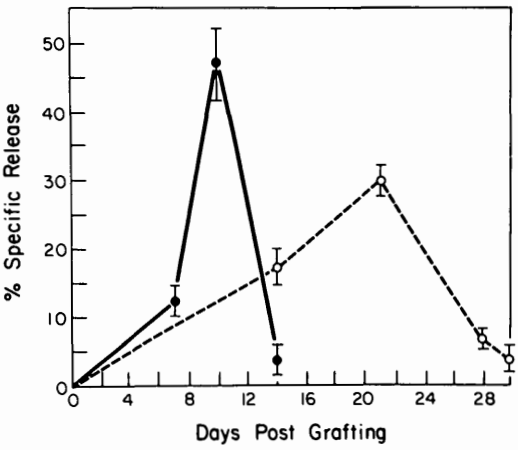

Fig. 1. Demonstration of cell-mediated cytotoxicity with the ^{51}Cr release assay with splenic effector cells from Le recipients that had received a BN orthotopic skin graft or anterior chamber skin implant. BN spleen cells were radiolabeled, and the degree of cell lysis is calculated to represent percent specific cytotoxicity, reported as mean ± standard deviation. Assay conditions are described in the text. ●——●, Effector cells from skin-grafted Le recipients, n = 15 total, 5 animals per assay point; o- - -o, effector cells from Le recipients of anterior chamber implants, n = 20 total, 5 animal per assay point.

cells. Cytotoxic lymphocytes were detected in implant-bearing rats by day 14, but the peak lytic ability appeared on day 21. After this peak, there was a rapid decline in the detectable cytotoxic cells. This coincided with the period when implant rejection was becoming evident. Similar results were observed in rats with orthotopic skin grafts except that the cytotoxic activity occurred earlier and declined rapidly when rejection was completed. Biesecker[10] reported a similar pattern in rats that had received renal allografts.

There are several interesting facets of the cell-mediated immune response of rats bearing anterior-chamber implants as compared with the response of rats bearing orthotopic skin grafts. Both of the in vitro assays employed to measure the cell-mediated immune response showed that a response was detectable earlier in the recipients of orthotopic skin grafts than in the recipients of anterior-chamber implants. This can probably be ex-

plained on the basis of the difference in the route of antigenic stimulation. The skin graft, being in contact with the lymphatics of the recipient, stimulates the immune response within a few days.[5] In contrast, it requires several days for the implant to become vascularized, and since no lymphatics are present, the route of sensitization is through the vascular system, resulting in a delay in sensitization.

The most interesting observation was that the implant remained viable for several weeks after the host developed a detectable immune response to the tissue. This was previously shown histologically, since mononuclear cell infiltrates were present within 14 days of implantation,[1-3] and was documented in this study by both the MLR and the cell-mediated cytotoxicity reaction. In contrast, orthotopic skin grafts were rejected rapidly after the cell-mediated immune response was detected.

The reason that rats bearing tissue implants in the anterior chamber of the eye are unable to complete the rejection process is not clear, although it appears to be related to the route of sensitization and the subsequent delay in producing a population of effector cells in vivo that can complete the rejection process. Perhaps the key is that the host is exposed to low-dose antigen sensitization through the spleen. Two very interesting host responses could therefore occur that would explain the delay in rejection: (1) the development of serum blocking factors and (2) the procurement of suppressor T cells. Either of these events would be consistent with both our in vivo and in vitro observations, and studies are currently underway to investigate these intriguing possibilities.

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