Efferent limb protection of corneal allografts from immune rejection. **Stephen R. Waltman and Joel M. Engelstein.**

Twenty-six rabbits with clear 6.5 mm. penetrating corneal allografts had skin grafts from the same donor eight weeks later. Ten animals rejected their corneas within 16 days. Two and one-half weeks after skin grafting eight of the remaining 16 rabbits had 5.5 mm. corneal buttons excised and resutured within the first graft. These grafts were all rejected 23 to 26 days after skin grafting. Of the eight grafts which were not excised, four remained clear and four were rejected 27 to 50 days after skin grafting. Without intervening skin grafts, all 10 re-excised corneal allografts remained clear. These results indicate that when efferent immune protection is short-circuited by sensitization with skin grafts some corneal grafts are protected from the efferent immune arc by corneal anatomy. When this anatomy is interrupted, efferent protection is abrogated, resulting in an increased graft rejection rate and decreased graft survival.

In rabbits, and man, nonrejected penetrating corneal allografts (PKP's) do not sensitize the host. Therefore, a major factor protecting PKP's from immune rejection is a block in the afferent limb of the immune arc. In the present study, subsequent skin grafts from the same donor animals were used to sensitize the host and eliminate this afferent limb protection. We then studied the efferent limb of the immune arc in well-healed corneal transplants.

**Materials and methods.** Six and one-half millimeter penetrating corneal allografts were exchanged between pairs of virgin New Zealand white rabbits weighing 2.5 to 3.5 kilograms. The members of each pair were from two partially inbred strains. Continuous 8-0 silk suture was used, and topical antibiotics and dilating drops were applied daily until suture removal on Day 10.
TABLE I. Time of rejection of corneas after skin graft

<table>
<thead>
<tr>
<th>Re-excised corneas (days)</th>
<th>Non-re-excised corneas (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>26</td>
<td>50*</td>
</tr>
<tr>
<td>26</td>
<td>NR†</td>
</tr>
<tr>
<td>26</td>
<td>NR</td>
</tr>
<tr>
<td>26</td>
<td>NR</td>
</tr>
<tr>
<td>26</td>
<td>NR</td>
</tr>
</tbody>
</table>

*Clotted following trauma.
†NR = not rejected.

Twenty-six animals with clear corneal grafts had 12 mm. ear skin grafts exchanged between partners eight weeks postoperatively. Skin and corneal grafts were examined daily. Rejection was scored when the skin grafts were less than 10 per cent viable or when there was a definite ciliary flush leading to corneal graft clouting.

Two and one-half weeks after skin grafting, the 16 animals with clear corneal grafts were randomly divided into two equal groups. In the experimental group, a 5.5 mm. corneal button, inside the original graft, was excised, rotated 180 degrees, and sutured in place with a continuous 10-0 nylon suture (Fig. 1). The corneal group was not operated (Fig. 2).

Nine rabbits with clear 6.5 mm. corneal autografts had 5.5 mm. buttons excised and sutured with 10-0 nylon. Ten additional rabbits had 6.5 mm. corneal allografts without subsequent skin grafts. Eight weeks postoperatively they had a 5.5 mm. central corneal button excised and resutured with 10-0 nylon. All animals were observed daily for three months.

Results. All 26 animals rejected their skin grafts within seven to fourteen days with a mean rejection time of 9.2 days. This is comparable to skin graft survival time in rabbits with clear corneal allografts.

Ten of the animals rejected their corneal grafts during the first 16 days after skin grafting. Of the 16 remaining animals with clear grafts and noninflamed eyes, eight had corneal re-excisions (Fig. 2). All of these animals had clear grafts and quiet eyes within two days after this procedure. All went on to reject their resutured corneas between Day 23 and Day 26 following the initial skin graft (Fig. 3). The rejection began with a ciliary flush, and 24 to 48 hours later a line of keratitic precipitates could be seen advancing across the endothelium.

Of the eight control animals whose corneas were not re-excised, three rejected their grafts between Days 27 and 29, and one rejected at Day 50 (following trauma). Four animals retained clear grafts for the duration of the study. These results are shown in Table I. The survival times are significantly different for these two groups (p < 0.01). Furthermore, there are significantly more clear grafts in the control group (p < 0.025).

None of the nine re-excised autografts clouded. In the 10 animals without skin grafts, all of the ten re-excised corneal allografts remained clear for the three-month observation period (Fig. 4).

Discussion. The unique anatomy of the cornea allows corneal allografts to survive while other transplanted tissues are rejected. In previous experiments it has been shown that nonrejected corneal allografts do not lead to sensitization and second set rejection of subsequent skin grafts. Rejected corneal grafts, however, do sensitize the
Fig. 3. Rejection of re-excised 5.5 mm corneal allograft in animal sensitized by a skin graft.

host and accelerate skin graft rejection. Stark and co-workers reported that nonrejecting corneal transplants in humans did not lead to detectable lymphocytotoxic antibodies, but those patients with evidence of immune graft rejection did develop these antibodies. This indicates that unrejected corneal allografts do not sensitize the host, and a block in the afferent limb of the immune arc exists. Following skin grafting and rejection of the skin graft, however, the animal is sensitized, afferent protection is short-circuited, and the efferent limb can be studied.

In this study only 10 of 26 animals rejected their corneal allografts within 17 days when skin grafts were performed eight weeks later. This is in contrast to previous results where 75 per cent of corneas were rejected by this time using a different strain of rabbits, and is an example of strain variations. It emphasizes the difficulties in comparing experimental results between different groups of animals. When the corneal allograft was left unaltered, half of the rabbits retained clear grafts for the duration of the experiment. The other half had the onset of graft rejection delayed. In the sensitized host then, a well-healed corneal allograft is protected from immune rejection by the efferent limb of the immune arc.

In some animals the protection was complete, in others only partial. This is in agreement with previous investigations.

When the efferent protection is eliminated by re-excising and restitching the corneal grafts, the rejection rate increases to 100 per cent in the sensitized host. All the re-excised grafts were clear and the eyes quiet three days postoperatively.

They then went on to show typical signs of corneal rejection with endothelial precipitates. Since all the re-excised autografts and the allografts in nonsensitized hosts remained clear it is unlikely that the trauma of re-excision, per se, was responsible for the initiation of rejection. Induced uveitis can also lead to corneal graft clodung, but autografts are affected as often as homografts, and most grafts survive. Furthermore, there are often clinical differences between pure homograft rejection and uveitis-induced corneal clodung.

Hence, the interruption in corneal anatomy resulting from a penetrating incision abrogates the protection from the efferent limb of the immune system allowing a sensitized host to reject a previously well-healed corneal graft.

These results are consistent with light and electron microscopic studies of graft rejection which indicate that the earliest morphologic changes in the rejecting grafts occur in areas of leukocytic infiltration at the corneal scar.

From the Department of Ophthalmology, Washington University School of Medicine, St. Louis, Mo., and the University of Florida College of Medicine, Gainesville, Fla. Supported in part by Grant EY-00004 from the National Eye Institute, Bethesda, Md. Submitted for publication Oct. 29, 1973. Reprint requests: Dr. Stephen Waltman, Department of Ophthalmology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, Mo. 63110.

Key words: keratoplasty, corneal transplant, immune rejection, efferent limb, graft rejection.

REFERENCES


by corneal transplantation, Invest. Ophthal-
4. Engelstein, J. M., Herberman, R. B., Tissot,
R. G., et al.: Prolonged survival of a rabbit
skin allograft following corneal transplantation
from the same donor, Ann. Ophthalmol. 5:
5. Polack, R. M.: The effect of ocular inflam-
thickness during homograft rejection and
uveitis in rabbits, Am. J. Ophthalmol. 72: 383,
1972.
7. Polack, F. M.: Histopathological and histo-
chemical alterations in the early stages of
homograft rejection, J. Exp. Med. 116: 709,
1962.
8. Polack, F. M.: Scanning electron microscopy
of the host-graft endothelial junction in corneal
graft rejection, Am. J. Ophthalmol. 73: 704,
1972.
scopic studies of graft endothelium in corneal
graft rejection, Am. J. Ophthalmol. 73: 711,
1972.

A viewer for correlation of fluorescein and
color fundus photographs. Robert W.
FLOWER AND ARNALL PATZ.*

A simple viewer for superposition of a fluores-
cein angiogram on a color fundus photograph
without compromising the most desirable charac-
teristics of either is described. The usefulness of
this device is described as an aid in correlating an
angiogram with the patient's retina, particularly in
conjunction with a slit lamp photocoagulator.

The introduction of improved resolution photog-
raphy for performing fluorescein angiography has
permitted more precise studies of the retinal
microcirculation. Also, new argon laser and Xenon
arc slit lamp photocoagulation delivery systems
now commercially available permit the photoco-
agulation of areas as small as 50 microns in diam-
eter. These two innovations have introduced new
concepts in the management of patients with
macular disorders and with intravenous neovascu-
larization emanating from the optic disc.

When photocoagulating lesions near the fovea,
precise localization is required and even for less
vital areas of the retina, precise localization in
relationship to visible retinal landmarks seen
through the fundus contact lens with a slit lamp
photocoagulator is desirable. When studying the
patient through a slit lamp photocoagulator, the
fundus details are visible with approximately the
same contrast and similar highlights as are seen
in standard color fundus photography. However,
much higher resolution of fine microvascular ab-
normalities and also more precise localization of
leakage sites is possible with fluorescein angi-
ographs. Therefore, when treating a patient with
a slit lamp photocoagulator it is usually necessary
to extrapolate from the much higher contrast
fluorescein angiographic landmarks to the fundus
view seen through the slit lamp. This extrapolation
is frequently difficult, especially where details of
the smaller branch vessels are concerned.

The difficulty of this extrapolation has been
lessened in some instances by use of color fluores-
cein photography as suggested by Allen and
Frazier1 and more recently advocated by Schatz
and co-workers2 at Hopkins. Color fluorescein
studies permit a greater degree of orientation with
respect to retinal landmarks as seen by standard
ophthalmoscopy. However, the resolution of the
fluorescein fluorescence is definitely diminished
by exchanging color film for the black-and-white
film ordinarily used in fluorescein angiography.

This report describes a fairly simple viewer
which permits superposition of a fluorescein angio-
gram on a color fundus photograph without com-
promising the most desirable characteristics of
either. The only requirement for producing the
two fundus photographs is that they both be made
using identical fundus camera optics. This assures
that the two images2 are of identical magnifi-
cation.

The viewer consists of two slide holders oriented
at right angles to each other with an independently
variable light source behind each (Fig. 1, D and
E). A 50 per cent beam splitter is located in such
a way that it makes a 45° angle with each of the
two slide holders (see Fig. 1). The reflecting
surface of the beam splitter (Fig. 1, A) is cen-
tered at the intersection of two imaginary lines

Fig. 1. Viewer with top removed. A, 50 per cent
beam splitter; B, rotatable slide holder; C, hori-
izontally and vertically movable slide holder; D
and E, independently variable light source; F,
and mounting bracket.