The effect of intracorneal cartilage implants on the survival of corneal xenografts

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In an attempt to prevent neovascularization and prolong survival of corneal xenografts, various implants were inserted into the host cornea at the time of transplantation. Neonatal or adult rabbit cartilage segments or silicon bands were placed around lamellar guinea pig corneal xenografts in rabbits. The inserts delayed advancement of limbal blood vessels toward the xenografts, but neovascularization could not be prevented. Maximal delay of neovascularization was obtained when the xenograft was surrounded by neonatal cartilage at the same intracorneal level, without gaps between adjacent cartilage segments. Under these circumstances, the xenografts survived for an average of 28.7 ± 2.0 days as compared to less than 12 days without cartilage. Adult cartilage and silicon bands were much less effective in preventing blood vessel advancement towards the xenografts. Histopathological studies showed that the xenografts remained free of cellular and vascular infiltration until blood vessels had crossed the cartilage and reached the xenograft borders. The development of postoperative systemic host sensitization against the xenogenic tissue was assessed by serial leukocyte-migration inhibition testing. Hosts were found sensitized during the second and third postoperative weeks. Sensitization was detected before opacification when the xenograft was completely surrounded by neonatal cartilage but only after opacification when the xenograft was not protected in this manner. The present experiments confirm the close linkage of vascularization to graft opacification and suggest that antiangiogenic factors can delay but not prevent graft rejection.

Key words: corneal xenograft, neovascularization, neonatal cartilage, antiangiogenic factor, host sensitization, leukocyte-migration inhibition (LMI)
Corneal xenograft survival, cartilage implants

Fig. 1. Surgical techniques. a, Corneal xenograft and cartilage placed at the same intracorneal level. b, Superficially placed corneal xenograft overriding intralamellar cartilage. c, Continuous ring of cartilage (segments without gaps). d, Open ring of cartilage (gaps between segments).

neal xenografts. Vascularization of the cornea and the cartilage implants around the graft and opacification of the graft were monitored. In some rabbits, the immune response to guinea pig cornea was assessed at intervals with the leukocyte-migration inhibition (LMI) assay.

Material and methods

Animals. The donor animals were Hartley guinea pigs, weighing 200 to 300 gm, of either sex, which were sacrificed by cervical dislocation prior to removal of their corneas. From each donor animal, four full-thickness corneal discs 5 mm in diameter were dissected and placed into sterile Petri dishes. The recipients were albino rabbits of either sex weighing 2 to 3 kg. Neonatal cartilage was obtained from the distal tips of scapulae of newborn albino rabbits less than 2 weeks of age. Control, adult rabbit cartilage was obtained from the knee joints of animals more than 3 months old. The cartilage was cut into segments 2 to 5 mm long and 0.5 mm wide with sterile surgical blades, placed into sterile plastic dishes containing normal saline, and kept at room temperature for less than 2 hr until transplantation. Sterile silicon (“40 band” strip used for retinal detachment surgery; Medical Instrument Research Association, Boston, Mass.) was used as another control and cut into segments of similar dimensions and placed into sterile Petri dishes.

Surgery. Transplants were performed in 118 rabbits; 28 control animals received no implants (group 1, Table I). The xenograft was placed either intralamellarily (Fig. 1, a) or superficially (Fig. 1, b) in the host rabbit cornea, and the cartilage segments were inserted intracorneally either in a continuous fashion (Fig. 1, c) or with 0.1 to 0.3 mm gaps between adjacent segments (Fig. 1, d). These procedures were performed as follows.

After the rabbit had been anesthetized by intravenous injection of 50 to 70 mg of phenobarbital, a retrobulbar injection of 2% xylocaine was given. Surgery was performed with an ophthalmic surgical microscope and was clean, but not sterile. The animal's palpebral fissure was kept open with a speculum. An intralamellar corneal graft (Fig. 1, a) was performed with an initial incision 3 mm long, 3 to 4 mm from the limbus into half the corneal depth. The lips of the wound were undermined by scissors, creating an eccentric intracorneal pocket, 6 to 7 mm in diameter around the wound. Five or six cartilage segments were placed inside the pocket, creating either a continuous ring or a ring with gaps (open ring) (Fig. 1, c and d). The donor cornea was then placed within the center of the ring, and the wound was closed with one or two 10-0 nylon sutures (Ethicon, Inc., Somerville, N. J.).

A superficial lamellar corneal xenograft (Fig. 1, b) was made by cutting a superficial lamellar disc 5 mm in diameter and 0.4 mm in depth, with a corneal trephine, midway between the limbus and the center. The wound lips were undermined with corneal scissors to create an intracorneal pocket of about 2 mm in width around the wound. The cartilage segments were placed into the pocket to form either a closed ring or an open ring (Fig. 1, c and d). The donor cornea was placed on the wound and
secured with four interrupted 10-0 nylon sutures (Ethicon).

All the animals were treated once at the end of surgery with Neosporin ophthalmic ointment. They were examined daily with flashlight and magnifying glasses and occasionally with a slit lamp. The following stages of lamellar xenograft rejection were measured (in days): (1) the "latent" interval between the time of surgery and the first invasion of host cornea by limbal blood vessels, (2) the "neovascularization" phase, extending from the end of the latent phase until the time the xenograft was contacted by the invading blood vessels, and (3) the "opacification" phase from the time of blood vessel contact with the xenograft until the first detection of xenograft opacification (Fig. 2).

When cartilage was placed into the intracorneal pocket, the neovascularization phase was further subdivided into three distinct intervals: the time taken for the blood vessels to advance from the limbus to the cartilage, and the time taken for
them to cross the cartilage, and the time required for them to advance from cartilage to xenograft (Fig. 3).

Animals which developed postoperative infection, rupture of sutures, wound dehiscence, or other technical complications were excluded from the study. The rabbits were all sacrificed within a month after transplantation. Their eyes were enucleated and kept in formalin for histopathological studies.

The development of host sensitization after transplantation was assessed twice weekly by the LMI technique, which was adapted for use in rabbits by Basu and Carre. The antigens comprised soluble guinea pig corneal proteins, 0.2 mg/ml concentration, prepared as previously described for human corneal antigens.

Results

The rabbits are grouped according to the arrangement of xenograft and corneal implants in Table I. The table also shows the time course of neovascularization and xenograft opacification.

Blood vessels generally invaded the host cornea from the limbus at the same intracorneal level as the site of the corneal xenograft. Thus a superficial graft attracted superficial vessels, and a deep graft attracted deep vessels. Cartilage without xenograft did not cause neovascularization (not shown). As shown in Table I, the length of the latent phase was 5 to 7 days in all rabbits transplanted with guinea pig cornea. Similarly, the time taken for the blood vessels to advance from the limbus to the cartilage (groups 2, 3, and 4) or silicon (group 5) was equal in all groups and not longer than 2 days. In contrast, the time required for the blood vessels to cross the intracorneal cartilage implants varied with the type of cartilage, the arrangement of its segments, and the spacial relationship between the cartilage and the xenograft. When both neonatal cartilage and xenograft were placed at the same intracorneal level and the cartilage ring was closed (i.e., without gaps between adjacent segments), the crossing time was 16.7 ± 2.0 (mean ± S.D.) days (group 2a). Comparison with groups 4 and 5 by the t test yields p < 0.001. Adult cartilage and silicon bands similarly arranged were less effective in blocking blood vessel advancement, and the crossing time was only 3 to 6 days (groups 4 and 5). With an open ring, the blood vessels advanced through the gaps between adjacent segments without delay (group 3a). When the corneal xenograft was superficial, the superficial blood vessel advancement towards the xenograft was not impeded by the intracorneal cartilage (groups 2b and 3b). In all groups, the blood vessels resumed their rapid progress towards the xenograft once they had crossed the intracorneal barrier; they reached the xenograft within 1 to 2 days. Xenograft opacification was seen 2 to 4 days following the contact of blood vessels with the graft borders. In no case was opacification seen prior to the crossing of the cartilage by blood vessels.

Histopathological sections, stained with hematoxylin and eosin, were taken at different time intervals after transplantation. In animals transplanted with xenograft without cartilage or silicon, intensive cellular and vascular infiltration of both host and donor corneas was seen during the second postoperative week. Microscopically, the infiltration of cells and blood vessels in xenografts surrounded by a continuous ring of neonatal cartilage remained confined to the host cornea in the region between cartilage and limbus until blood vessel crossing of the cartilage. Once the blood vessels had crossed the cartilage, lymphocytes and polymorphonuclear leukocytes and subsequently blood vessels were seen to invade the xenograft. The cartilage, whether adult or neonatal, remained free of cellular or vascular infiltration throughout the experiment.

LMI testing was performed twice weekly on 10 rabbits transplanted with xenografts without cartilage and in 10 rabbits whose xenografts were surrounded by a continuous ring of neonatal cartilage. Postoperative systemic sensitization to the guinea pig corneal proteins developed at the same time in the two groups (Figs. 4 and 5); host leukocytes demonstrated sensitivity during the second, and more often the third, postoperative week. LMI was greater than 25%, which is
Table I. Positioning of xenografts and intracorneal implants with time course of resulting neovascularization and graft opacification

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Xenograft site*</th>
<th>Implant</th>
<th>Latent phase (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Intralamellar (12)</td>
<td>None</td>
<td>5-7</td>
</tr>
<tr>
<td>1b</td>
<td>Superficial (16)</td>
<td></td>
<td>5-7</td>
</tr>
<tr>
<td>2a</td>
<td>Intralamellar (23)</td>
<td>Continuous ring of neonatal cartilage</td>
<td>5-7</td>
</tr>
<tr>
<td>2b</td>
<td>Superficial (12)</td>
<td></td>
<td>5-7</td>
</tr>
<tr>
<td>3a</td>
<td>Intralamellar (7)</td>
<td>Open ring of neonatal cartilage</td>
<td>5-7</td>
</tr>
<tr>
<td>3b</td>
<td>Superficial (8)</td>
<td></td>
<td>5-7</td>
</tr>
<tr>
<td>4</td>
<td>Intralamellar (30)</td>
<td>Continuous ring of adult cartilage</td>
<td>5-7</td>
</tr>
<tr>
<td>5</td>
<td>Intralamellar (10)</td>
<td>Continuous ring of silicon band</td>
<td>5-7</td>
</tr>
</tbody>
</table>

* Number in parentheses refers to the number of hosts.

Fig. 4. Development of host systemic sensitization after guinea pig corneal transplantation in rabbit eyes. The xenografts are opacified in 12 days (groups 1a and 1b). Sensitization is detectable in the second and third postoperative weeks.

considered significant, after graft rejection in the control animals. In the experimental animals LMI became significant at the same time, whereas the xenografts remained optically clear.

Discussion

The present experiment shows that under certain conditions, homologous neonatal cartilage inserted intracorneally in rabbit eyes can prolong lamellar corneal xenograft survival. Homologous cartilage itself does not induce vascularization, and it delays host blood vessel contact with xenograft. Without neonatal cartilage, the xenografts opacify within 12 days after transplantation, whereas with a continuous ring of neonatal cartilage, the xenografts remain optically clear for an average of 28.7 ± 2.0 days after surgery.

Prolongation of xenograft survival is seen only when both cartilage and xenograft are placed intracorneally at the same level. Intralamellar neonatal cartilage did not inhibit superficial blood vessel advancement towards a superficial xenograft. Moreover, the advancement of blood vessels was not impeded when gaps were left between adjacent segments of cartilage. Hence neonatal cartilage appears to have no effect on blood vessel advancement beyond the site of the cartilage itself. This is confirmed by its failure to change the length of the latent interval (blood vessel formation at the limbus and vessel advancement from limbus to cartilage) or the time needed for vessels to advance from cartilage to the xenograft. The delay appears to be partly mechanical, since it was also noted with adult cartilage and silicon.

On the other hand, the finding that neonatal cartilage causes a significantly longer
Corneal xenograft survival, cartilage implants

Neovascularization phase (days)

<table>
<thead>
<tr>
<th>From limbus to cartilage</th>
<th>Cartilage crossing</th>
<th>From cartilage to xenograft</th>
<th>Opacification phase (days)</th>
<th>Survival time, mean ± S.D. (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>10.7 ± 0.3</td>
</tr>
<tr>
<td>1-2</td>
<td>1-2</td>
<td>1-2</td>
<td>2-4</td>
<td>10.5 ± 0.2</td>
</tr>
<tr>
<td>1</td>
<td>1-2</td>
<td>1-2</td>
<td>2-4</td>
<td>28.7 ± 2.0f</td>
</tr>
<tr>
<td>1</td>
<td>1-2</td>
<td>1-2</td>
<td>2-4</td>
<td>12.5 ± 1.5</td>
</tr>
<tr>
<td>1</td>
<td>1-2</td>
<td>1-2</td>
<td>2-4</td>
<td>10.1 ± 0.5</td>
</tr>
<tr>
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<td>1-2</td>
<td>1-2</td>
<td>2-4</td>
<td>9.8 ± 1.0</td>
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<tr>
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<td>3.8 ± 0.3</td>
<td>1</td>
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<tr>
<td>1</td>
<td>3.0 ± 0.2</td>
<td>1</td>
<td>2-4</td>
<td>13.9 ± 1.0</td>
</tr>
</tbody>
</table>

Fig. 5. Development of host systemic sensitization in rabbits with guinea pig corneal xenografts surrounded by a continuous ring of intracorneal neonatal cartilage (group 2a). The xenografts remain clear for more than 3 postoperative weeks. The hosts are found sensitized during the second and third postoperative weeks.

delay in crossing time than adult cartilage or silicon bands implies the presence of biological factors which are antiangiogenic. The absence of activity of these putative factors beyond the cartilage site suggests a lack of diffusion or diminution of potency due to dilution. Graft vascularization is needed for the expression of the destructive forces of the immune response in the case of lamellar corneal xenografts and allografts. However vascularization may not be as important in penetrating corneal grafts, where rejection may be attributed to sensitized cells reaching the graft via the anterior chamber.

The present experiments confirm the close linkage of vascularization to graft opacification and suggest that antiangiogenic factors can delay but not prevent graft rejection. The histopathological sections show that when neonatal cartilage inhibits blood vessel advancement towards the xenograft, the latter also remains free of cellular infiltration. The xenograft is invaded by leukocytes only after the blood vessels have crossed the neonatal cartilage.

The time course of postoperative development of host sensitization as assessed by the LMI technique is the same whether the xenograft becomes opacified or remains optically clear. A similar time course was noted with corneal allografts in man. It appears that the LMI test cannot detect sensitization until a large number of systemic lymphocytes have become sensitized. The xenograft's antigens seem capable of stimulating the systemic immune system without overt vascularization, possibly by diffusing via the anterior chamber or peripheral lymphatics.
appears that the LMI test reflects the afferent arm more accurately than the efferent arm of the immune response. It may depend on the number of sensitized lymphocytes or on specific cells in a subpopulation of lymphocytes.13

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REFERENCES