No effect of concanavalin A on the corneal graft reaction

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Penetrating corneal homografts were performed in albino rabbits. The experimental group received grafts soaked in a solution of concanavalin A; the control group received grafts soaked in lactated Ringer’s solution. Animals with technically successful grafts were subsequently exposed to an additional antigenic stimulus from the corneal donor via skin grafting. This procedure produces a uniformly high rate of corneal graft rejection. The two groups were compared for the frequency and onset of the graft reaction to evaluate any effectiveness of concanavalin A in altering the graft reaction. Results demonstrated no difference between control and experimental groups. The significance of these findings is discussed.

Key words: keratoplasty, immune graft reaction, concanavalin A, antigen masking, immunosuppression

Immune graft rejection remains a significant cause of corneal transplant failure. The frequency of graft failure from immune rejection ranges from approximately 10% to 50%, varying with the degree of vascularization present. Prevention of the graft reaction has relied primarily on corticosteroids, although other immunosuppressants have been used both experimentally and clinically.

An alternative approach to prevention of graft rejection comes from the renal transplantation literature and utilizes a plant lectin, concanavalin A (Con A), as an antigen masking agent. Herein we report on experimentation with this approach to the prevention of graft rejection by utilizing Con A for in vitro pretreatment of donor corneal graft material.

Materials and methods

The experimental model used in this study has been described previously. Albino rabbits weighing 2 to 3 kg received 6 mm penetrating keratoplasties. Donor rabbits were enucleated under sedation with fentanyl-droperidol (Innovar; McNeil Laboratories, Inc., Ft. Washington, Pa.). After removal of the anterior segment from the globe, the donor button was immersed in either sterile lactated Ringer’s solution (control group) or a lactated Ringer’s solution containing 25 mg/L Con A (CalBiochem, Behring Corp., Lots 610049 and 910026). All solutions were prepared immediately prior to their use and were maintained before and during their use at 4° to 6° C. Solutions were buffered to maintain a pH range of 7.4 to 7.69. Donor material was immersed for 30 min prior to keratoplasty.

Recipient animals were premedicated with chlorpromazine (Thorazine; SmithKline Corp., Philadelphia, Pa.), 25 mg, intramuscularly 1 hr preoperatively and were anesthetized with heparinized pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.) intravenously. With a clean but not sterile technique, the 6.5 mm donor buttons were placed into 6.0 mm central recipient sites and were secured with a running suture of 8-0 black silk. All animals received atropine ointment and gentamicin ointment on the
Table I

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Clear grafts at 21 days</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Onset of rejection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(days after skin graft)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>10</td>
<td>12-19</td>
</tr>
<tr>
<td>Range</td>
<td>2-21</td>
<td>5-21</td>
</tr>
<tr>
<td>(n = 12, SD = 4.75)</td>
<td>(n = 16, SD = 5.14)</td>
<td></td>
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Operating table. Postoperatively all rabbits were treated with atropine ointment, 1% daily, until suture removal. On the tenth postoperative day sutures were removed, and on the fourteenth postoperative day the grafts were examined. Technical failures (poor wound apposition, extensive peripheral anterior synechiae, heavy vascularization, lack of clarity) were eliminated from the study. On the seventeenth day after transplant, recipients with technically clear corneal grafts received a 3 by 3 cm full-thickness skin graft from the original donor rabbit in order to stimulate immune rejection. The graft was placed in a subcutaneous pouch under the abdominal skin of the recipient.

Slit-lamp examinations of the animals were performed every other day by two observers who were unaware of the pretreatment groups to which the animals were assigned. Grafts were examined for rejection phenomena: epithelial rejection lines, subepithelial infiltrates, and endothelial rejection (Khodadoust line, anterior chamber reaction, edema). Vascularization was graded by the number of quadrants (1 to 4) of the graft in which vessels (superficial or deep) were present.

To demonstrate the presence of Con A on grafted tissues, a separate series of grafts were performed. These grafts were pretreated with fluorescein labeled Con A (fluorescein-isothiocyanate (FITC)--Con A).* In this preparation there is no free fluorescent label. Donor corneas were immersed in a 25 mg/L solution of FITC--Con A at 4° C for 30 min. Grafts were performed. The corneal grafts were then removed from the recipients at 2, 5, and 10 days. In addition, two buttons were examined immediately after the 30 min immersion in FITC--Con A. These two samples were agitated in sterile lactated Ringer's solution for 5 and 30 min, respectively, before microscopic examination. All specimens were embedded in optimal cooling temperature medium (OCT compound; Lab-Tek Products, Div. Miles Laboratories, Inc., Maperville, Ill.) and snap-frozen on a liquid CO₂ jet. Sections were examined with the Leitz Orthoplan fluorescence microscope.

**Results**

The experimental group consisted of 13 animals whose grafts had been treated with Con A prior to transplantation. Only two grafts remained clear at the end of the 3-week observation period. One of these two was an animal in which the skin graft sloughed after 1 week. The remainder of the grafts ultimately demonstrated endothelial rejection and graft opacification (Table I).

The control group consisted of 20 animals whose grafts were treated with lactated Ringer's only, prior to surgery. At 21 days there were no clear grafts. The groups were unequal due to eliminations for technical failure or death.

Experimental and control groups were compared for (1) the frequency of rejection and (2) the onset of rejection (defined as the presence of an epithelial line). The designation of the epithelial line as the signal event in the rejection process was decided on because it was followed by endothelial rejection in all but one case. In three animals endothelial rejection occurred without a preceding epithelial line. These animals were deleted from the comparison of onset. This explains the smaller number used for this comparison (see Table I). By the binomial distribution test, the rejection rates of the two groups were not significantly different (at a 5% level).

With the two-sample t test there was no
significant difference between the time of onset of rejection in the two groups (at a 5% level). See Table 1.

The specific manifestations of rejection did not significantly differ between the two groups. Recognizable subepithelial infiltrates were not seen.

Specimens treated with FITC-Con A demonstrated intense staining of endothelial and epithelial cells at 5 and 10 min after the 30 min immersion period. Comparably intense staining of endothelial and epithelial cell layers was also evident at 2 days after transplantation. At 5 days after transplantation only endothelial fluorescence was demonstrable. No labeling was demonstrable at 10 days. Stromal cells did not label.

Discussion

This study deals with an alternative approach to the prevention of the graft reaction and was suggested by experiments in renal transplantation. Recent work demonstrates that one of the purified plant lectins, Con A (the phytohemagglutinin of the jack bean), is effective in prolonging the survival of canine renal allografts in minimally immunosuppressed hosts. In those experiments the donor kidneys were perfused in vitro with Con A under specific temperature and pH conditions prior to grafting. This in vitro perfusion, in combination with minimal host immunosuppression, was associated with significant delay in the onset of the graft reaction.

Con A is a complex protein of the lectin category, best known for their agglutinating and mitogenic properties. Characteristic of the lectins is their tendency to bind firmly to carbohydrate moieties on cell surface membranes. This latter property—the tendency to bind to cell surfaces—that suggested the use of Con A as an antigen-masking agent. Simmons et al. theorized that in the kidney model Con A may mask the immunogenicity of graft antigens by binding to the cell surfaces and interfering with the perception of antigens on the graft by immune lymphocytes or by antibodies. This would, of course, interrupt the immune arc. The precise nature of the cell membrane alterations, be they conformational changes in the HL-A antigens themselves or steric interference with receptor sites, is not known.

In addition to the foregoing explanation for the effectiveness of Con A as an immunosuppressant, this lectin has been shown to suppress the development of cytotoxic leukocytes in cell cultures and may be immunosuppressive in vivo by functionally altering T cell membranes.

This experiment attempted to test the possibility of delaying or eliminating the allograft reaction by in vitro pretreatment of the corneal donor button with Con A. Treatment of corneal donor tissues in vitro has been attempted previously with recipient serum and antilymphocyte serum. Phytohemagglutinins have not been used in the same way.

The failure to delay the graft reaction in the present experiment has several possible explanations. Although fluorescein labeling demonstrates that Con A is present in the epithelium and endothelium, its presence is demonstrable only transiently. There is no evidence that Con A permeates the corneal stroma. The continued presence of Con A may be necessary for blockage of the immune reaction. Toledo-Pereyra and Simmons suggest that the immunosuppressive capabilities of Con A are dependent on specific circumstances including pH, temperature, the solvent used, the quality of the Con A used, and the presence of mild host immunosuppression. Any one of these variables may have affected our results. The most likely of these is the absence of host immunosuppression in the present protocol. Canine renal allografts treated with Con A do not demonstrate prolonged survival without some degree of systemic immunosuppression. This suggests that modified immunogenicity of the cell requires a modified level of host responsiveness to effectively alter the graft reaction. This may hold true for the cornea as well.

Another possible explanation is that the experimental model used here (employing a "second-set" phenomenon) may overwhelm the immunosuppressive properties of Con A by the delivery of a huge dose of "unmasked"
antigen in the form of the skin graft. A more sensitive assay may be necessary to detect prolongation of graft survival.

The majority of attempts at prevention of immune graft rejection have involved local or systemic host immunosuppression. A number of systemic immunosuppressants, including antimetabolites (azathioprine, 6-mercaptopurine), 6, 10-21 antilymphocyte serum, 22-25 and estrogen, 26 have been used both experimentally and clinically. The most obvious drawback to the use of such agents in a nonlife-threatening disease is their potentially serious side effects (systemic immunosuppression, bone marrow depression, serum sickness, and possibly malignancy). Accordingly, corticosteroids have been the mainstay of rejection prevention, largely due to their multifaceted antiphlogistic effects as well as their effectiveness as topical agents. 27, 28 Nonetheless, systemic and topical corticosteroids have well-documented toxic side effects.

If donor cell membranes could be appropriately altered prior to keratoplasty by nonspecific agents designed to decrease immunogenicity, the need for long-term, seriously toxic immunosuppressants might be avoided. Although one would not expect permanent alteration of graft immunogenicity, delaying the recognition of graft alloantigens may permit events leading to graft adaptation and reduction of prolonged local or systemic therapy.

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REFERENCES


