Modification of the ocular immune response

I. Use of antilymphocytic serum to prevent immune rejection of penetrating corneal homografts

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Homograft penetrating corneal transplants were performed in albino rabbits. The animals were subsequently exposed to an additional antigenic stimulus from the donor animal to achieve a uniformly high graft rejection rate. The use of systemic antilymphocyte serum completely inhibited the rejection phenomenon.

Key words: penetrating corneal transplants, graft rejection suppression, skin grafts, antigenic stimulus, corneal edema, corneal vascularization, corneal opacity.

The recent medical literature is replete with reports of the use of antilymphocytic serum (ALS) as an immunosuppressive agent in the prevention of homograft reactions.1, 2 This agent has been successfully used in preventing corneal homograft and xenograft reactions with intralamellarly placed donor tissue.3, 4 The following study was instituted to test the effectiveness of ALS in preventing immune rejection of penetrating corneal transplants. This model was chosen because it parallels the usual clinical situation.

Penetrating corneal transplants were performed in pairs of albino rabbits. This was followed by an implantation of skin between the member pairs in order to induce a high rejection rate. Horse antirabbit lymphocyte serum (HARLS) was used to modify the homograft reaction.

Materials and methods

Preparation of antiserum. Albino and pigmented rabbits weighing 1.0 to 1.5 kilograms were killed by decapitation. The thymuses and mesenteric lymph nodes were removed and placed in ice-cold phosphate-buffered Ringer's solution. The tissues were cut into small fragments, ground in an all glass tissue homogenizer, and passed through a 60 mesh stainless steel filter. The cells were counted and viability determined by Trypan blue dye exclusion. In all cases more than 90 per cent of the cells were viable.

Forty billion cells in a mixture of saline and Freund's complete adjuvant (Difco Laboratories) was injected subcutaneously into a horse weighing approximately 500 kilograms. A series of booster injections of 40 to 80 billion cells in saline was given subcutaneously every 10 to 14 days for a total of four injections. Two weeks after the last injection, the horse was bled into bottles containing sodium ethylenediaminetetra-acetate (EDTA). The cells were separated from the plasma which was recalcified and allowed to clot overnight. The serum was then heated to 56° C. for 30 minutes.
and then absorbed overnight at 4° C. with 10 per cent packed washed rabbit red cells. Dilute merthiolate (1:10,000) was added to the HARLS and the serum was stored at -20° C.

Booster injections of rabbit tissue were given periodically and the horse was bled 10 days after the booster injections.

The lymphagglutinating titer of the HARLS was determined with the method of Jeejeebhoy. The serum was serially diluted in buffered saline. An equal volume of a lymphocyte suspension containing 1.1 x 10^4 cells per cubic millimeter was added to each tube of serum. The tubes were incubated at 37° C. for one hour and examined microscopically. The last tube in which there was definite agglutination was taken as the end point. The hemagglutinating titer was determined using 0.5 per cent red cell suspension in place of the lymphocytes.

The HARLS used in this series had a lymphagglutinating titer of 1:8,000 before and after absorption with packed rabbit red blood cells. The hemagglutinating titer before absorption was 1:256 and decreased to 1:16 after absorption.

Six millimeter penetrating corneal grafts were exchanged between pairs of albino rabbits weighing 3.0 to 4.5 kilograms. The grafting was performed under thiopental anesthesia. Continuous 7-0 or 8-0 silk sutures were used to secure the grafts in place. Clean, but not sterile, technique was used. The animals received daily topical atropine ointment until the sutures were removed on the tenth postoperative day. On the fourteenth postoperative day the grafts were examined and the technical failures were eliminated from the series. Ten to 20 per cent of the animals were eliminated. Skin grafts 2 by 3 cm. were then exchanged between the member pairs of rabbits to achieve a high rate of corneal graft rejection. The skin was placed subcutaneously in the ventral body wall. The rabbits were inspected every other day to be sure that the skin grafts remained in place. At the end of the third week after corneal grafting, the corneal grafts were re-evaluated and one animal was rejected from the series because its graft was not technically satisfactory. The animals were examined with a slit lamp daily, by an examiner who was unaware of the treatment schedule and the results were recorded. After a definite graft reaction had occurred, the animals were examined twice a week.

The animals received 1 ml. of HARLS intramuscularly each day for 7 to 8 days starting on the day of skin grafting. They then received ½ ml. intramuscularly every third day. Control animals received the same quantity of either normal horse serum or normal saline on the same schedule. All injections were discontinued 2 days after the animals had a definite full-blown graft reaction or on the twenty-second or twenty-third day after skin grafting if no reaction was noted by the observer.

The rabbits were weighed on the day of skin grafting and at the end of the third week after skin grafting. They were observed for any signs of toxicity such as diarrhea, wasting, failure to eat, or any evidence of bacterial infections.

The animals were divided into three groups according to the therapeutic regimen employed. Group one received intramuscularly normal saline, group two received intramuscularly normal horse serum, and group three received intramuscularly HARLS. All animals received injections according to the schedule outlined. The only animals included in the final statistics were those animals considered to have had a technically successful graft. A technical success was defined as a clear graft at Day 21 after corneal grafting. The technical failures were usually due to inadequate pupillary dilatation with anterior synechiae formation and secondary glaucoma. A few of the grafts became infected.

Results

The animals were observed daily for evidence of a homograft reaction. The onset of the rejection process was heralded by the presence of a ciliary flush and engorgement of iris vessels. This was followed by neovascularization of the host cornea from the limbus with subsequent invasion of the donor button. In the next 24 to 48 hours a line of fine keratic precipitates could be seen on the donor endothelium. The stroma then rapidly became cloudy. Over the next week the acute reaction subsided, leaving a dense opaque donor button with a few vessels present.

This paper reports the results achieved during the 30 days following skin transplantation, or a period of 6 weeks after corneal grafting. The rabbits treated with intramuscular saline had a rejection rate of 100 per cent. The graft rejection occurred between the seventh and eleventh day after skin grafting with an average of 9.8 days. Of the 10 rabbits treated with normal horse serum, 9 demonstrated homograft rejection. Eight of the rabbits rejected between the eighth and eleventh day and one on the sixteenth day with an average of 9.9 days. With the exception of the one serum-treated animal that did not reject its graft, the two control series...
Table I. Rejection rates of HARLS-treated and control animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. rabbits</th>
<th>No. rejecting</th>
<th>Average time of rejection in days (with range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>6</td>
<td>9.8 (7-11)</td>
</tr>
<tr>
<td>Normal serum</td>
<td>10</td>
<td>9</td>
<td>9.9 (8-16)*</td>
</tr>
<tr>
<td>HARLS</td>
<td>11</td>
<td>1</td>
<td>25.0</td>
</tr>
</tbody>
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*Eight animals rejected between 8 and 11 days with one at Day 16.

were essentially the same. They were similar to a group of 16 grafted animals treated with daily intraperitoneal saline injections for 3 weeks where a 100 per cent rejection rate was found. All of the animals in the control series that rejected their grafts had a full-blown violent graft reaction where the active process evolved over a period of 2 to 4 days.

There were 11 rabbits in the group treated with HARLS, only one of which demonstrated a homograft reaction. This one reaction was mild, it took more than a week to completely evolve, and the degree of final opacification was less than that seen in the control series. The lone rejection started on the twenty-fifth day which is much later than any animal in the control series. The results are summarized in Table I.

The difference in rejection rate between the treated and the control animals is highly statistically significant (p < 0.001 Chi square = 16). Fig. 1 shows the graft of a control animal and Fig. 2 an ALS-treated animal 3 weeks after skin grafting.

One animal treated with ALS and one treated with normal serum were killed on the twenty-fourth day for pathological studies. The ALS-treated animal had a graft with normal endothelium and epithelium and a stroma of normal thickness. There was minimal lymphocyte infiltration of the well-healed wound. In contrast, the graft in the horse serum-treated-control animal was markedly abnormal. The stroma was edematous and thickened, and a marked lymphocytic and plasma cell infiltrate was present at the graft margin. Several vessels were present in the graft. The endothelium was absent and was replaced by a fibrovascular retrocorneal membrane.

During the 30 day observation period, 16 to 20 per cent of each group of animals died for unknown reasons. There was no difference in mortality rate between the treated and control animals. No other animal died in the next 30 days. All the other animals were well and free of diarrhea or wasting. The group treated with ALS lost an average of 8 per cent of their body weight while the control animals lost 1 to 2 per cent of their body weight during the 3 week treatment period. A few of the animals were weighed 2 weeks after stopping the ALS, and there had been no further weight loss.

Discussion

A functioning donor endothelium is necessary for the maintenance of clarity of penetrating corneal homografts. Decompensation of the donor endothelium due to immune rejection leads to corneal edema, vascularization, and opacification. Rejection of the stromal component results in similar graft failures. The previous experiments on the use of ALS to prevent immune rejection of corneal grafts have all used intralamellarly placed donor tissue and have proved that rejection of the stromal components can be prevented with ALS. The model chosen for this report more closely parallels the clinical situation of penetrating corneal transplants and takes into account the additional problems seen with penetrating as opposed to lamellar transplants.

It is often difficult to differentiate graft failures due to technical factors from those due to immune rejection. Because a highly inbred group of rabbits was used, the antigenic differences between member pairs were often small, and almost all of the early graft failures could be ascribed to inadequate technique and eliminated from the series. With the use of an additional antigenic stimulus in the form...
of a skin graft, it was possible to obtain a uniformly high rejection rate. All the graft failures in this group could be ascribed to an immune rejection, and the effect of ALS as an immunosuppressive agent can be assessed. The HARLS proved highly effective in preventing immune rejection of penetrating corneal homografts. In the one rabbit that had a homograft reaction while being treated, the reaction was mild and prolonged compared with the reactions in the control groups.

Antilymphocytic serum raised against human cells has been used in the sup-
pression of homograft reactions in kidney transplants. Prolonged therapy is necessary and may be associated with disturbing side effects such as serum sickness and anaphylactic reactions.\footnote{Kashiwagi, N., Brantigan, C. O., Brettschneider, L., Groth, C. G., and Starzl, T. E.: Clinical reactions and serologic changes after the administration of heterologous antilymphocytic globulin to human recipients of renal homografts, Ann. Int. Med. 68: 275, 1968.} No such side effects were noted in our series and may be due to the small doses of ALS that were used or the short time periods (3 weeks) over which it was administered. Observation of these animals over a prolonged period should indicate whether the immune response has been suppressed or prevented, and whether prolonged treatment with the ALS is indicated.

The use of antihuman lymphocytic serum for routine corneal transplantation does not appear justified as yet in view of the possible systemic toxicity of the available preparations. However, in those cases of severely vascularized corneas in which the failure rate is uniformly high, its use may be justified. A series is currently underway to evaluate the efficacy of this agent in penetrating grafts in vascularized, scarred corneas of rabbits.

The authors wish to express their appreciation to I. Frank Rodman, D.V.M., for his help in procuring the antisera.

REFERENCES