Induction of corneal graft rejection
by passive cell transfer

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An experimental model is presented demonstrating that penetrating corneal grafts in the rabbit may be rejected by passive transfer into the anterior chamber of specifically sensitized lymphoid cells. Destruction of histo-incompatible corneal endothelium is always marked by the formation of focal pock-like areas of damage in this system, rather than by the typical moving line of rejecting endothelium usually seen in spontaneous graft rejection. Where the transferred lymphoid cells are compatible with the tissues of the graft recipient, the picture is one of a severely affected graft on a field of uninvolved recipient corneal endothelium. Where the lymphoid cells are compatible with the graft and not with the tissues of the recipient, one sees a clear corneal graft surviving on a field of endothelial destruction on the recipient bed. The specificity of these reactions is illustrated in terms of the histocompatibility relationships between corneal donor, graft recipient, and the donor of the sensitized lymphoid cells.

Key words: graft-versus-host reaction, corneal endothelium, immunopathology, corneal graft rejection.

The passive transfer of histo-incompatible lymphoid cells into a recipient animal leads to a graft-versus-host (GVH) reaction with extensive immunopathologic destruction of surrounding target cells. The implantation of pieces of allogeneic lymphoid tissue onto the anterior face of the iris was shown by Prendergast and Franklin to result in a local GVH-induced uveitis. More recently, we have shown that passive transfer of a suspension of sensitized lymphoid cells into the aqueous induces in the recipient eye the formation of pock-like areas of damage to the corneal endothelium, representing the development of focal GVH reactions in the host. In both of these experimental models, the mode of destruction is similar to that described in detail by Elkins in his review of the phenomenology and mechanisms of the GVH reaction. We now describe an extension of our earlier passive cell transfer studies, to illustrate some aspects of the specificity and mechanism of

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SKIN GRAFT  CELL TRANSFER  CORNEAL GRAFT EXCHANGE

Fig. 1. Experimental protocol for the destruction of corneal grafts by passive transfer of sensitized lymphoid cells (see text for further explanation). In rabbit A, the transferred cells (3a) are autologous with the graft and sensitized against the recipient. In rabbit B, the transferred cells are sensitized against graft tissue and autologous with the recipient.

the lymphocyte-mediated destruction of corneal grafts.

Materials and methods

The animals employed for these studies were 5- to 7-pound adult albino rabbits of the New Zealand Giant strain, carefully screened to include only those animals with clinically normal eyes. Rabbits were always worked upon in groups of two or three, 10 to 12 such groups being employed for each of the experiments described below.

Corneal transplantation. The first step in each experiment was the exchange of 5 mm. penetrating grafts within the appropriate experimental groups as described in the protocols presented in Figs. 1 and 2. The keratoplasty procedure employed was that described in our earlier studies.4 The 10-0 running Ethylon sutures were removed as early as day 7 to prevent vascularization and consequent sensitization of the host. All animals were followed clinically for several weeks to be sure that the grafts remained clear and uncompromised by any signs of a rejection process.

The second step of each experiment was the transfer of a skin graft between appropriate animals in each group (see Figs. 1 and 2) in order to effect sensitization of the recipient by the histocompatibility antigens of the skin donor. One day after clinical rejection of the skin graft (9 to 10 days after transplantation), the draining preauricular node was removed, a single cell suspension prepared from this lymph node, and the cells passively transferred into the anterior chambers of the appropriate animals for each experiment, as illustrated by step 3 in Figs. 1 and 2. The techniques for skin transplantation and lymphocyte preparation and transfer were described in detail in our previous report.5

Clinical and histologic observations. The injected eyes were examined daily by hand light and biomicroscope following intracameral inoculation of lymphoid cells. Groups of animals were killed at intervals for morphologic studies, the appropriate eyes enucleated, and the corneas excised for the preparation of flat mounts of corneal endothelium according to the technique originally described by Smolin and employed in our earlier studies.2 These flat endothelial preparations were stained by silver impregnation, followed by hematoxylin.

Results

Corneal graft-vs.-host and host-vs.-graft reactions. In the first experiment (see Fig. 1), penetrating corneal grafts were exchanged between pairs of rabbits, A and B. After the grafts had healed into place, a skin graft from the ear of rabbit A to that of rabbit B led to sensitization of the draining preauricular lymph node of rabbit B. A single cell suspension of that lymph node was then prepared, and $2.5 \times 10^6$ lymph node cells were injected into the anterior chambers of the grafted eyes.

Fig. 2. Experimental protocol for the destruction of corneal grafts by passive transfer of sensitized lymphoid cells (see text for further explanation). In rabbit C, one eye contains a graft autologous with the transferred cells, while the other eye contains a graft against whose antigens the lymphoid cells have been sensitized.
Thus, in rabbit A the graft and lymph node cells were histocompatible, while the tissues of the recipient bed provided the histo-incompatible target. Within 24 hours after cell transfer to rabbit A, a mild uveitis developed, with a mild congestion of iris and ciliary vessels and a 1+ aqueous ray. There were scattered dust-like keratic precipitates (kp's) on the corneal endothelium of both recipient and graft, but the cornea was clear. Within 48 hours after injection, this mild reaction had fairly well subsided and now showed fewer kp's and only ± iritis.

On the third day following intracameral injection of cells, the anterior segment inflammation was appreciably more severe. There was a 1 to 2+ iritis with a distinct aqueous flare and congestion of iris and ciliary vessels. Keratic precipitates could now be seen readily with the slit lamp even without retro-illumination, but they were almost entirely confined to the recipient corneal bed, as was an increase in the thickness of the recipient cornea. The corneal transplant itself seemed to be substantially spared from this local reaction. The inflammatory reaction continued to increase and reached a peak at between 5 and 7 days after transfer of the lymph node cells. The recipient cornea was now appreciably thickened, and had attached to it quite large keratic precipitates. The inflammatory reaction was markedly reduced by 8 to 10 days after inoculation, and continued to subside over the ensuing few days, with corneal edema and uveitis gradually disappearing. Two weeks after inoculation, the recipient endothelium and cornea were generally quiet except for a few fine keratic precipitates. Throughout the course of this reaction, however, the corneal graft remained substantially free of involvement.

Inoculation of sensitized lymphoid cells into the grafted eye of rabbit B in Fig. 1 produced quite a different picture. Here, the grafted corneal button was histoincompatible with the inoculated cells, whereas the recipient eye of rabbit B was histocompatible. In this case, following an early mild response to the trauma of injection, the inflammatory reaction commencing on the third day after intracameral inoculation of cells was confined almost entirely to the donor corneal button. Only on the endothelium of the grafted cornea, and not on that of the recipient bed, was seen the development of larger keratic precipitates and corneal clouding and thickening. This reaction increased in intensity over the next 4 to 5 days, during which vascularization of the graft developed, followed by the usual pattern of complete corneal graft rejection. The recipient cornea remained quite clear throughout the course of the local reaction involving the grafted corneal button, and the degree of uveitis accompanying this reaction was far less than had been seen in the eye of rabbit A.

Among the replicate groups of animals examined in this experiment, there was a moderate degree of individual variation in the intensity of the inflammatory response seen. We have attempted to describe above the average response, although some of the animals examined showed milder reactions and some appreciably more severe reactions than those reported. In most instances, the involvement of the corneal endothelium, either that of the donor button in rabbit B or of the recipient corneal bed in rabbit A, was not irreversible and the corneas usually returned to normal thickness and transparency.

The contrast in the reactions shown in the grafted eyes of rabbits A and B is most strikingly illustrated by the histologic picture shown in Fig. 3. These represent flat preparations of endothelium across the graft-host margin, taken on the fifth day after intracameral inoculation of sensitized lymphoid cells. Fig. 3, A is derived from rabbit A, in which the graft (on the right) was histocompatible with the donor lymphoid cells, whereas the host endothelium was incompatible. It is evident here that the graft endothelium is essentially spared,
Fig. 3. Flat preparations of endothelium six days after intracameral inoculation of $2.5 \times 10^5$ sensitized lymphoid cells. A, the cornea from rabbit $A$ in Fig. 1, in which the endothelium of the corneal graft is spared while that of the host exhibits marked destruction in focal pock-like areas (arrows). B, the cornea from rabbit $B$ in Fig. 1. Here the host endothelium is spared, whereas the graft endothelium shows pock-like areas of destruction (arrows). Silver-hematoxylin stain, $\times 50$. (Reprinted with permission from Maumenee.12)
with only the occasional lymphoid cell seen on its surface, and no significant destruction evident. In contrast, the endothelium of the recipient in Fig. 3, A is extensively damaged, with focal accumulations of lymphoid cells (arrows) forming destructive pocks on the endothelium and extensive disorganization of the normal endothelial architecture providing a witness to the destructive process.

Fig. 3, B shows the converse situation, taken from rabbit B of experiment 1, in which now the host endothelium is autologous and thus histocompatible with the transferred lymphoid cells, and the graft is now histo-incompatible. We see here that the normal endothelial architecture of the host bed is not involved in the destructive process, whereas focal areas (arrows) of lymphoid cell-induced destruction and disorganization of the normal endothelial pattern can be seen on the grafted tissue. The lymphoid nature of the inflammatory cells responsible for these focal endothelial lesions can be seen from the higher magnification of a typical area of destruction (Fig. 4).

**Corneal graft rejection by passive transfer of cells in the indifferent third-party rabbit.** In order to assure ourselves of the specificity of the rejection process and of the lack of participation of the host in the response to passively transferred lymphoid cells, an experiment was set up whose scheme is depicted in Fig. 2. A rabbit (C) was first grafted with 5 mm. penetrating corneal buttons, the donor to one eye being rabbit A and to the other eye being rabbit B. Some weeks later, after the grafts had healed in well and clearly, skin was transplanted from rabbit A to the ear of rabbit B, and after rejection the enlarged and sensitized pre-auricular lymph node draining the skin-graft site was ex-
cised and furnished the cell suspension for passive transfer into the anterior chamber of both eyes of rabbit C.

The results of this experiment were quite convincing. In the eye containing the graft from rabbit B (histocompatible with the transferred lymphoid cells), the graft was uninvolved in any specific rejection process and remained clear throughout the experiment. In contrast, the graft in the other eye, derived from rabbit A (to whose histocompatibility antigens the transferred lymphoid cells had specifically been sensitized), was engaged in a violent rejection reaction starting some three days after cell transfer to the anterior chamber. The course of this reaction was similar to that described for the analogous experiment one above, so that the graft suffered fairly extensive focal endothelial destruction in the form of the pocks previously described, and the superjacent cornea became quite cloudy and edematous.

The one difference in this experiment was seen in examination of the recipient corneal beds of both grafted eyes in rabbit C. Starting on the third day after inoculation of lymphoid cells into the anterior chamber, a mild uveitis was almost invariably seen, accompanied by a mild-to-moderate focal destruction of the host endothelium accompanied by corneal edema and thickening. The degree of involvement of the endothelium of the recipient bed was more variable but usually more mild than that seen in the first experiment, where the transferred lymphoid cells had been specifically sensitized to the histocompatibility antigens of the recipient. Thus, these rabbits C generally showed one eye containing an uninvolved graft on the mildly involved recipient bed, while the other eye showed a very highly inflamed graft on an only moderately inflamed recipient bed.

Discussion

It was shown earlier that the passive transfer of sensitized lymph node cells into the anterior chamber of a rabbit against whose histocompatibility antigens the cells were directed resulted in the formation of a local GVH reaction on the corneal endothelium. This took the form of focal pocks of endothelial cell destruction mediated by lymphocytes which had settled onto the endothelial surface. In this paper, we have taken advantage of this model of passive transfer of sensitized lymphoid cells to test their ability to effect the destruction of penetrating corneal grafts, and to study the specificity of this immunologic process. The introduction, into the anterior chamber of a grafted eye, of $2.5 \times 10^8$ lymphoid cells derived from a lymph node sensitized by a skin graft leads to an immunopathologic process which depends entirely upon the histocompatibility relationships between the cells of the graft and of the recipient corneal bed on the one hand, and the skin and lymph node donors on the other. When the corneal graft is derived from the same animal that provided the skin transplant, and the lymphoid cells are derived from the corneal graft recipient itself (rabbit B in Fig. 1), then the normal picture is seen of an immunologically rejecting graft surrounded by a clear recipient cornea with undisturbed endothelium. However, when lymphoid cells are passively transferred into the anterior chamber of a rabbit against whose histocompatibility antigens they are sensitized, in an eye bearing a penetrating corneal graft from the lymph node donor, the picture is completely different. In this instance, the donor corneal button remains uninvolved in any rejection process and crystal-clear, while all around it the recipient corneal bed is being attacked by a local GVH reaction mediated by the transferred lymphoid cells. These results illustrate quite clearly the specificity of the immunologic rejection process, and the fact that this reaction is mediated by the lymphoid cells contained in the passive transfer inoculum. This point is brought out even more clearly.
in the experiment described in Fig. 2 above, where passively transferred sensitized lymphoid cells can either spare a graft or effect its destruction in an unrelated third-party bearer of the corneal grafts, depending upon the histocompatibility relationship between graft, skin donor, and lymphoid cell donor.

It is worth additional emphasis that any destruction of corneal endothelium caused by the introduction of sensitized lymphoid cells into the aqueous presents as the random occurrence of pocks of focal endothelial destruction scattered over the face of the target cell monolayer. This is a different picture than is seen either experimentally or clinically in spontaneous corneal graft rejection, where the process almost invariably begins at the edge of the graft in closest approximation to the nearest blood vessels, and presents as a line of endothelial destruction which slowly works its way across the donor endothelial surface. This "endothelial destruction line" is almost a pathognomonic sign of specific rejection, and currently represents the only dependable hallmark by which specific immunologic destruction of a corneal graft may be diagnosed. These observations suggest that the sensitized lymphocytes responsible for graft destruction must arise in most instances from vessels within the cornea itself. Otherwise, effector lymphocytes arising from vessels in the uveal tract and finding their way across the anterior chamber would cause random focal destruction of target endothelium as described in this paper, rather than the discrete rejection line usually seen.

REFERENCES