Local graft versus host reactions within the anterior chamber of the eye: the formation of corneal endothelial pocks

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Introduction into the rabbit's anterior chamber of lymphoid cells sensitized to the histocompatibility antigens of the recipient leads to the development of an intraocular graft versus host reaction. This takes the form of a more or less severe uveitis, and the production of small focal areas of endothelial cell destruction on the posterior surface of the cornea. In the milder responses, these present as discrete pocks on the endothelial layer, which heal rapidly. In the more severe reactions, the foci of endothelial destruction may be so numerous as to overlap, resulting in complete immunologic destruction of the entire endothelium. The dose-response relationships in this experimental system are discussed, as are its uses as a model for the study of the mechanism of endothelial damage during the course of specific corneal graft rejection.

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

It was demonstrated earlier that the insertion of allogeneic lymphoid tissue into the anterior chamber of the rabbit eye results initially in the development of a local graft versus host (GVH) reaction accompanied by severe uveitis. The tissue destruction which accompanies this immunopathologic process follows the interaction of sensitized lymphocytes with the histocompatibility antigens of the host tissue. The phenomenology and mechanisms of local GVH reactions have been most clearly described by Elkins, using a model based upon the introduction of allogeneic lymphoid cells beneath the renal capsule of the rat.

Since the most significant event in the specific rejection of corneal grafts is the lymphocyte-mediated destruction of the endothelial layer, we have modified the experimental approach to the development of GVH reactions within the anterior chamber in order to favor involvement of
the corneal endothelium, so that further information might be obtained on the mechanism of its immunologic destruction. This was accomplished by introduction into the anterior chamber of a suspension of sensitized allogeneic lymphocytes, resulting in the formation of pocks of local destruction of corneal endothelium, in addition to the uveitis earlier observed. This approach was taken by analogy with the demonstration that sensitized allogeneic lymphoid cells could effect the formation of destructive pocks on the chorio-allantoid membrane of the chick embryo. The study of GVH reactions on the corneal endothelium should also contribute to our understanding of the mechanism of this phenomenon, since only in the eye can direct observation by biomicroscopy serve as an important supplement to histopathologic studies.

Materials and methods

The general outline of the protocol employed in these experiments is presented graphically in Fig. 1. The animals employed 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 three, twenty such groups being employed in this study.

Presensitization by skin transplantation. The initial step in the experiment was the transplantation of 2 by 4 cm. full-thickness skin grafts from the ear of one rabbit (A) to the ear of a second animal (B). This procedure was performed under local lidocaine anesthesia, employing interrupted 6-0 silk suture. The skin grafts were examined daily, and standard criteria were employed to evaluate initial take and the timing of graft rejection.

Lymphocyte transfer. One day after rejection of the skin graft on the ear of cell donor rabbit B, the now palpably enlarged draining preauricular lymph node was removed by careful dissection under general anesthesia with promazine (Sparine) and pentobarbital (Nembutal). The lymph node was immersed in Hanks' balanced salt solution, and under sterile conditions was cut into small fragments and the cells teased out by gentle manipulation with a forceps against a sterile stainless-steel mesh. After removal of larger fragments by permitting them to settle for 1 hour at 4° C, the supernatant lymphocytes were washed twice in balanced salt solution and their concentration adjusted appropriately, usually to 2.5 x 10^6 cells per milliliter. Cell viability was usually better than 90 per cent, as judged by the trypan blue dye exclusion test. Under general anesthesia, 0.1 ml. of the cell suspension in a heparinized syringe was injected into the anterior chambers of the original skin donor rabbit A, the skin graft recipient and cell donor rabbit B, and an unrelated third-party rabbit C. This was accomplished by first removing about 0.1 ml. of aqueous by insertion through the limbus into the anterior chamber of a 27-gauge hypodermic needle on a tuberculin syringe and then injection of the cell suspension through the same needle. Only one eye was injected in each animal.

Clinical observations. The injected eyes were examined daily by hand light and biomicroscope to follow the development of keratic precipitates, of endothelial damage and resulting corneal involvement, and of the usual manifestations of uveitis. Following intracameral inoculation, groups of animals were killed at intervals for morphologic studies.

Assessment of endothelial damage. Eyes were 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. The stain employed in these endothelial preparations involved silver impregnation followed by hematoxylin staining.

Anterior chamber (A/C) inoculation of serum from sensitized donors. In order to control the foregoing experiments, and to confirm the cellular-immune nature of the GVH reaction, serum transfer experiments were performed. Sets of animals were studied as outlined in Fig. 1, but instead of lymph node cells, serum was obtained from...
rabbit B and 0.1 to 0.2 ml. injected into the anterior chambers of rabbits A and C, using the same experimental timing. In a few cases, mixtures of serum and lymph node cells from the sensitized donor were employed. Subsequent clinical and histologic examination followed the procedures outlined above.

Results

Clinical response.

A/C inoculation of sensitized lymph node cells into allogeneic skin donor rabbit A. The injection of $2.5 \times 10^5$ lymph node cells into the anterior chamber of the rabbit against which the cells had been sensitized led to the development within the first 24 hours of a mild uveitis, as evidenced by a slight ciliary congestion, 1+ aqueous ray, and a mild congestion of iris vessels. There were scattered dust-like keratic precipitates (kp's) on the corneal endothelium visible by retroillumination, but the cornea was clear and of normal thickness. At 48 hours after injection, the mild initial reaction had substantially subsided, so that there was now only a ± iritis and fewer kp's than on the preceding day.

On the third day following intracameral injection of cells, a more severe inflammatory reaction was evident in the anterior segment of the eye. More and larger keratic precipitates could now be seen readily with slit lamp, even without retroillumination, while the corneal thickness was seen to be increased beyond normal limits, more evident in the lower portion of the cornea than in the upper half. There was now a 1 to 2+ iritis, with appreciable congestion of iris and ciliary vessels and a positive aqueous flare. The inflammatory reaction continued to increase, so that on the fourth day after injection the iritis was more severe and the cornea was now 1.5 to 2 times its normal thickness, with yet-larger kp's visible on the corneal endothelium. Small nodules of cells could now be seen on the anterior face of the iris as well as in the pupillary margin.

The inflammatory reaction appeared to peak at between 5 and 7 days after inoculation of sensitized lymph node cells into the anterior chamber. There was now appreciable corneal edema, often masking the intense uveitis, while the thickness of the cornea was sometimes 3 times normal values. At this time, small capillaries could be seen starting to invade the cornea from the limbus. After reaching its peak, the inflammatory reaction was visibly reduced by 8 to 10 days after inoculation, with corneal edema and uveitis subsiding. Some two weeks after inoculation, the eye was generally quiet except for a few fine kp's, mainly in the lower third of the cornea.

During the course of these experiments, a moderate degree of individual variation in intensity of response was seen among the experimental animals tested. The foregoing description represents an attempt to depict the average response, with some animals presenting milder and some more severe reactions than those indicated. Following extremely severe reactions, secondary glaucoma was sometimes seen due to posterior synechia, occasionally leading to persisting corneal edema and buphthalmus.

A/C inoculation of autologous lymph node cells (rabbit B). Just as in the allogeneic situation, the injection of $2.5 \times 10^5$ lymphoid cells into the eye of the cell donor was followed within 24 hours by the onset of a mild (1+) iritis, with a slight degree of ciliary and iris vessel congestion and 1+ ray in the anterior chamber. Again, fine dust-like keratic precipitates could be seen by retroillumination scattered on the surface of the corneal endothelium. However, in contrast with the allogeneic inoculation, the reaction to autologous cell inoculation cleared entirely within 48 hours after injection, so that no further ray or cells and no iritis could be seen at this time. Only a very few punctate keratic precipitates could be seen, all of which disappeared within 72 hours following inoculation. From this point on, the eyes were completely normal throughout the subsequent course of the experiments.

A/C inoculation of allogeneic lymph node cells into third-party rabbit C. The introduction of $2.5 \times 10^5$ cells into the eye
of a normal rabbit unrelated to either the lymphoid cell donor or to the donor of the sensitizing skin transplant resulted in an initial mild response to the trauma of injection similar to that seen in the other two groups. Starting on the third day after inoculation, uveitis and involvement of the corneal endothelium were seen to develop in most of the animals employed. The individual variation within this group was more extensive than that seen within group A. In rare instances, the anterior segment inflammation was as severe as that seen in group A animals, while in other instances the reaction was only minimal or even nonexistent. In general, the average response within this group, while qualitatively similar to that seen in group A animals, was appreciably milder and resolved more rapidly.

**A/C inoculation of serum or serum-cell mixtures.** The injection of serum from sensitized animals resulted in similar responses in all the animals tested. There was a mild, transient response to the trauma, which subsided in all instances within 24 to 48 hours. Henceforth, the eyes were completely normal, and no evidence of endothelial damage was visible clinically. When serum was mixed with lymphoid cells, both derived from skin recipient animals of group B, and inoculated into the anterior chambers, the reactions seen were substantially the same, in both duration and intensity, as those observed using cells alone.

**Histologic response.**

**Group A rabbits.** Twenty-four hours after intracameral inoculation of $2.5 \times 10^6$ lymph node cells sensitized against the skin of this animal, the flat preparation of corneal endothelium showed the normal and regular pattern of undisturbed endothelial cells. Scattered over the endothelium, sometimes lying upon and sometimes between cells, was a diffuse infiltration of single cells which looked morphologically to be lymphocytes. More of these cells were seen attached to what had been the inferior portions of the endothelium than in the superior regions. By the third day following anterior chamber inoculation, scattered colonies of lymphoid cells could be seen on the surface of the endothelium. Almost invariably, these lymphoid cell clusters overlay areas of focal endothelial destruction, in which the occasional pyknotic endothelial cell could be seen on a small field in which the normal regular endothelial cell pattern had been lost. Some of the cells in this small focus were several times larger than normal, and had irregular margins suggesting the onset of a reparative process. This belief was strengthened by the occasional finding of mitotic figures and of multinucleated endothelial cells, not often seen in the normal rabbit.

By four to six days after cell inoculation into the anterior chamber, a similar but increasingly more severe pattern of endothelial destruction could be seen. Across the entire face of the corneal endothelium, scattered pocks of endothelial destruction associated with local inflammatory cell infiltrates could be seen (Fig. 2). The numbers and severity of the foci of destruction invariably were found to increase as the preparations were scanned in what had been in vivo the direction of the inferior portion of the eye. Fig. 3 illustrates the
appearance at higher magnification of a mildly destructive lesion from the superior portion of a cornea, showing a scattering of inflammatory cells over a small area in which some endothelial destruction had already taken place, as evidenced by the disarray of the endothelial cell pattern. In Fig. 4, taken from a more inferior portion of the endothelium, greater numbers of single lymphocytes can be seen on the normal endothelial surface, while a large mass of inflammatory cells, presumably responsible for the clinically evident KP's described above, almost completely masks a focus of endothelial cell destruction.

By two weeks after intraocular inoculation, there were still a few scattered lymphoid cells on the surface of the corneal endothelium, but by now most of the inflammatory cells had disappeared, leaving only focal areas of disorganized and irregular endothelial cells with many multinucleated endothelial giant cells indicative of the healing process. At three weeks, few if any inflammatory cells could be seen, and the reparative process was essentially complete, leaving in its wake areas of marked irregularity of the normal endothelial cell pattern and many multinucleated giant cells as evidence of the earlier destructive process.

**Autologous rabbit B.** The inoculation of \(2.5 \times 10^5\) autologous lymphoid cells into the animal's own anterior chamber led at 24 hours to a picture similar to that seen in group A animals. There was a mild diffuse scattering of single lymphoid cells over the back of the corneal endothelium, on a field of regularly arranged intact endothelial cells. By the second day, the numbers of lymphoid cells scattered on the endothelium had diminished appreciably, with a few found on the third day and no lymphoid cells at all present on the endothelial surface six days after intracameral inoculation. At no time in this group of animals was there any evidence of aggregation of lymphoid cells into foci as had been seen in group A animals, nor was there any evidence of endothelial cell destruction, so that the normal well-ordered arrangement of endothelium was preserved throughout the experiment in this group of animals.

**Allogeneic third-party rabbit C.** The histopathologic observations in this group of rabbits resembled qualitatively the changes seen in group A animals. Thus, the initial 48 hours after intracameral inoculation of lymphoid cells saw a similar diffuse scattering of single cells on the corneal endothelium, followed on the third and subse-
quent days by the aggregation of these cells into clusters and the development of pocks of endothelial destruction in many of the animals. These responses were consistently less severe than were those seen in group A animals, and again the focal inflammatory infiltrates appeared to resolve more rapidly.

**Dose-response relationships.** We have already mentioned above that within any single animal reacting positively to anterior chamber injection of lymphoid cells, a crude dose-response effect could be seen within the injected eye itself, due to the settling of the lymphoid cells toward the inferior segment of the anterior chamber. Thus, the upper portion of the endothelial surface was invariably less severely involved than were the lower portions.

Variation of the numbers of sensitized lymphoid cells introduced into the anterior chamber of animals against whose skin the cell donors had been sensitized (group A) also showed a dosage effect. It was usually impossible to observe any significant clinical response to the introduction of fewer than 100,000 lymphoid cells, an observation which was confirmed histologically by the finding that such eyes had few if any detectable pocks of endothelial destruction. As the number of cells introduced intracamerally was increased, the severity of both uveitis and endothelial destruction and resultant corneal edema and thickening were found to increase in step. Thus, use of $5 \times 10^5$ lymphoid cells produced an almost invariable severe reaction, in which the inferior portion of the endothelium was often completely destroyed (Fig. 5). The injection of $2 \times 10^6$ sensitized lymphoid cells usually resulted in complete destruction of the entire corneal endothelial layer.

**Discussion**

In the immunologic destruction of allogeneic grafts of cornea, it is the endothelium of the donor that is most sensitive to attack by sensitized host lymphoid cells. Of the different cellular constituents of the donor cornea, it is also the destruction of corneal endothelium which carries with it the most serious consequences for the survival of the grafted tissue. It is thus extremely important for a full understanding of immunologic failure in clinical and experimental keratoplasty that the mechanism(s) by which corneal endothelium is destroyed be well understood.

We have presented here an experimental model for the study of the immunologic destruction of corneal endothelium, based upon the establishment of a local GVH reaction in the anterior chamber of the rabbit. The intracamerally introduced of a single-cell suspension of lymph node cells sensitized to histocompatibility antigens of the recipient results in the adherence of some of these cells to the endothelial surface, their aggregation, and the subsequent induction of a pock representing focal destruction of host corneal endothelium. In the course of mild reactions of this type, endothelial destruction appears to be self-limited, and rapid healing of the local injury may cause the entire response to pass unnoticed clinically. In more severe reactions, sufficient foci of endothelial destruction may result in transient corneal edema and thickening prior to resolution of the process. In the most severe reaction, the
focal reactions are so numerous as to coalesce and result in complete destruction of the endothelial layer with, of course, more long-standing effects upon the overlying cornea. Where a focal pock of endothelial destruction has occurred, it invariably leaves a long-lasting imprint upon the endothelial surface in the form of an alteration in the normally very regular arrangement of endothelial cells and the presence of many multinucleated endothelial giant cells typical of at least the rabbit’s mode of repair of defects in its corneal endothelium.\textsuperscript{11, 12}

This GVH model of the specific rejection of corneal endothelium has at least one clinically important implication. We have reported elsewhere in experimental studies of the mechanism of corneal graft rejection that an almost pathognomonic sign of specific rejection of the endothelium was the presence of a line of endothelial destruction which starts at the margin of the graft and slowly works its way across the donor endothelial surface.\textsuperscript{6, 7} This “endothelial destruction line” has since been observed frequently in clinical keratoplasty,\textsuperscript{13} and currently represents the only dependable sign by which specific immunologic destruction of a corneal graft may be diagnosed. We have suggested that the cells responsible for this endothelial destruction line arise from vessels within the cornea itself,\textsuperscript{8} although other investigators suggest that these sensitized effector lymphocytes must arise from the anterior uveal tract and find their way across the aqueous to the target endothelium.\textsuperscript{10} In the present study, we have introduced sensitized lymphocytes directly into the anterior chamber, and find nothing analogous to a linear and progressive destruction of endothelium. Rather, the lymphoid cells appear to establish themselves at random upon the endothelial surface and to effect only focal destruction of the target endothelium. Only when extremely large numbers of sensitized cells are introduced into the anterior chamber does one see the almost hyperacute and massive destruction of corneal endothelium such as is occasionally observed in experimental animals and in clinical situations. We may remark in passing that our observation that “immune” serum is unable to duplicate the destructive effect of sensitized lymphoid cells is in keeping with the large body of evidence that GVH reactions (and HVG responses as well) depend upon mechanisms of cellular immunity.

Several interesting points emerge from a consideration of the dose-response relationships in this experimental system. The first involves the dosage effect seen within the eye itself into which sensitized allogeneic lymphoid cells have been introduced. Even with a complete mixing of the cells suspension inoculum with the aqueous fluid, there follows a slow settling of the cells toward the inferior portion of the anterior chamber. There is thus a concentration gradient within the anterior chamber which is reflected both clinically and morphologically by an increasing degree of endothelial destruction in the inferior segment of the cornea as compared with that above. Of course, introduction of a sufficient number of sensitized lymphoid cells into the anterior chamber will mask this effect, and mediate destruction of the entire endothelial layer.

A second dosage effect is seen in the difference in response between the animal against whose histocompatibility antigens the lymphoid cells are sensitized, and a normal third-party recipient of the intracameral cell inoculation. Here, the “dosage” is controlled by the number of histocompatibility antigens against which the cells in the inoculum are sensitized (see Reference 2), so that it is natural that the intensity of the reaction in the third-party recipient be not only milder but more variable, depending upon its genetic relationship to both the animal employed to sensitize the lymphocytes and the lymphocyte donor itself. Finally, the results described herein do not permit us to draw any precise conclusions about the absolute number of lymphoid cells required to in-
duce the formation of a destructive pock in the recipient corneal endothelium. We know neither the proportion of lymphocytes in the inoculum which are specific for the histocompatibility antigens of the recipient animal (undoubtedly only a small fraction), nor the proportion of the cells introduced which have an opportunity to interact with the target endothelial cells. A significant proportion of these cells probably never see the endothelial surface; they either engage themselves elsewhere, as in the induction of the GVH uveitis component of the response, or settle out of the aqueous and leave the eyes to suffer their fate elsewhere. It is, however, of considerable interest that a single lymphocyte is believed able to induce the formation of a GVH pock.

REFERENCES