The fine structural changes in the corneal endothelium during graft rejection

Hajime Inomata,* George K. Smelser, and Frank M. Polack

Fully penetrating corneal grafts were made between unrelated albino rabbits. Rejection was induced, and was regularly accompanied by invasion of the host cornea by superficial vessels. Advancing rejection was evidenced by stromal haze and thickening in the rejected portion of the graft. It was possible to study the clinically normal unrejected portion, the interface between it and the affected portion, and the obviously abnormal portion of the graft, as well as the host region and scar. The endothelium of the clear portion of the graft was found to be infiltrated by lymphocytes which penetrated between cells, but did not reach Descemet's membrane. The endothelial cell apparently closed over the lymphocytes after the invasion. The leukocytes, therefore, were in the endothelial intercellular space. In the interface between the clear and turbid portions of the grafts, fibrin present in the anterior chamber was found on the endothelial surface accompanied by mononuclear cells—mainly lymphocytes. They pushed between adjacent endothelial cells; in some instances they reached Descemet's membrane. The endothelial cells' cytoplasm was remarkably little affected in this area. Mitochondria, endoplasmic reticulum, and the terminal web appeared qualitatively normal. The cells, however, became attenuated in some portions and greatly thickened in others. Their surface adjacent to the anterior chamber was frequently covered by minute processes not found normally. In the region which appeared clinically hazy, it was obvious that Descemet's membrane was exposed to the aqueous humor in places. The membrane was otherwise covered by mononuclear leukocytes and remaining endothelial cells. Many of the latter were greatly rounded and apparently in a process of desquamation.

Key words: penetrating corneal transplants, graft rejection, skin grafts, corneal endothelium, Descemet's membrane, anterior chamber, histopathology, electron microscopy, light microscopy, rabbits.

From the Department of Ophthalmology, Columbia University, New York, N. Y., and the University of Florida, Gainesville, Fla.

This investigation was supported in part by National Institute of Health Research Grants NB 01202-12 and NB 08026-01, and Public Health Service Research Career Program Award No. 5-K6-NB-19-609-06 from the National Institute of Neurological Diseases and Stroke.


*Recipient, FIGHT FOR SIGHT Postdoctoral Research Fellowship No. F210 (G-2) of the National Council to Combat Blindness, Inc., New York, N. Y.
munologic stimulus by transplanting a piece of skin from the corneal donor. The possibility is also considered that a uveitis may play a role in the late graft opacification.2

Although the histopathology of the graft rejection has been studied,1-5 this has been limited by the resolution of the light microscope and many questions still remain unanswered, such as: (1) the actual way in which endothelial cells are damaged, (2) whether they desquamate first, or show changes on contact with leukocytes, (3) the character of the ultrastructural changes in endothelial cells and lymphocytes, and (4) the source of these lymphocytes. The present work deals with these changes as revealed by the electron microscope, and will be confined to the endothelial cells because these appear to be the first seriously affected.

Materials and methods

Fully penetrating corneal grafts, 4.5 mm. in diameter, were made between unrelated albino rabbits. Two weeks after transplantation, if the grafts were clear and no surgical complications could be detected, a skin graft was taken from the donor of the cornea and transplanted to the host. By this method an immunologically provoked graft rejection was brought about. A precise account of the surgical procedure has been previously reported.4 Rejection became clinically apparent about 2 weeks after the skin graft, and was accompanied by vascularization of the host cornea which reached near, or entered the scar surrounding the transplant. It was commonly observed that the rejecting graft first became affected on the side nearest the host vascularization, and this portion became cloudy and swollen while the other, as yet unrejected, portion remained clear and thin. The interface between the 2 areas was marked by precipitates on the endothelial surface (Fig. 1). It is this stage of active rejection which has been studied.

Rabbits were anesthetized by intravenous injection of pentobarbital (Nembutal)* and the eye bearing the graft was enucleated, immediately placed in ice-cold fixative solution, and opened at the equator. The lens and iris were removed from the anterior half of the eye. Fairly large pieces of cornea were fixed in 1 per cent osmium tetroxide buffered with 0.15M sodium cacodylate, pH 7.4, at 4° C., for one hour. They were cut into somewhat smaller pieces before fixation was completed and dehydrated rapidly through a graded series of alcohols, and embedded in Epon 812. The final blocks of tissue of 1 to 2 mm. were cut with sharp razor blades where the tissue was in 80 per cent alcohol. The following areas of host cornea and graft were obtained and placed in separate bottles: (1) clinically normal host, (2) vascularized host, (3) host-graft junction, (4) turbid graft, (5) clinically normal graft, and (6) the "interface" or transition between apparently normal and turbid grafts. Sections, 1μ thick, stained with Azure II were made of all specimens for orientation and localization of electron micrographs. Thin sections were cut by glass or diamond knives and stained with uranyl acetate and lead citrate, and examined by means of a Siemens Elmiscope I. Four rejecting and 5 good normal grafts were studied, as well as control normal corneas.

Results

Light microscopy. Particular attention was paid to structural changes in endothelial cells at the interface between the clear and turbid portions of the rejecting graft. Full-thickness sections of the rejecting graft revealed that the swelling was due to edema of the anterior third of the stroma, more prominent in the turbid than in the clear portion, but seen in both (Fig. 2). The endothelial cell layer, however, showed the most remarkable changes in structure. These cells in the clear portion of the graft formed a single layer with uniform cellular thickness, as is seen in the normal cornea. On the other hand, the turbid portion showed an uneven posterior surface and cellular infiltration (Fig. 3). The interface between the 2 areas was marked by precipitates on the endothelial surface (Fig. 1). It is this stage of active rejection which has been studied.

Rabbits were anesthetized by intravenous injection of pentobarbital (Nembutal)* and the eye bearing the graft was enucleated, immediately placed in ice-cold fixative solution, and opened at the equator. The lens and iris were removed from the anterior half of the eye. Fairly large pieces of cornea were fixed in 1 per cent osmium tetroxide buffered with 0.15M sodium cacodylate, pH 7.4, at 4° C., for one hour. They were cut into somewhat smaller pieces before fixation was completed and dehydrated rapidly through a graded series of alcohols, and embedded in Epon 812. The final blocks of tissue of 1 to 2 mm. were cut with sharp razor blades where the tissue was in 80 per cent alcohol. The following areas of host cornea and graft were obtained and placed in separate bottles: (1) clinically normal host, (2) vascularized host, (3) host-graft junction, (4) turbid graft, (5) clinically normal graft, and (6) the "interface" or transition between apparently normal and turbid grafts. Sections, 1μ thick, stained with Azure II were made of all specimens for orientation and localization of electron micrographs. Thin sections were cut by glass or diamond knives and stained with uranyl acetate and lead citrate, and examined by means of a Siemens Elmiscope I. Four rejecting and 5 good normal grafts were studied, as well as control normal corneas.

Results

Light microscopy. Particular attention was paid to structural changes in endothelial cells at the interface between the clear and turbid portions of the rejecting graft. Full-thickness sections of the rejecting graft revealed that the swelling was due to edema of the anterior third of the stroma, more prominent in the turbid than in the clear portion, but seen in both (Fig. 2). The endothelial cell layer, however, showed the most remarkable changes in structure. These cells in the clear portion of the graft formed a single layer with uniform cellular thickness, as is seen in the normal cornea. On the other hand, the turbid portion showed an uneven posterior surface and cellular infiltration (Fig. 3). The interface between the 2 areas was marked by precipitates on the endothelial surface (Fig. 1). It is this stage of active rejection which has been studied.

Rabbits were anesthetized by intravenous injection of pentobarbital (Nembutal)* and the eye bearing the graft was enucleated, immediately placed in ice-cold fixative solution, and opened at the equator. The lens and iris were removed from the anterior half of the eye. Fairly large pieces of cornea were fixed in 1 per cent osmium tetroxide buffered with 0.15M sodium cacodylate,
Fig. 1. A rejecting rabbit corneal homograft. It was first affected on the side nearest host vascularization. The rejected portion is opaque, while the rest of the graft remains clear. The interface between the two areas is indicated by arrows.

Fig. 2. A light micrograph of a thick section cut through the interface (arrow) between the clear and turbid portion. It shows endothelial damage and edema of the anterior third of the stroma (×110).

Fig. 3. A higher magnification of Fig. 2 showing remarkable endothelial changes. Endothelial cells in the turbid portion become rounded or spindle-shaped and some of them appear extremely dark (clear arrow), whereas cells in the clear portion remain flat and are much thinner and uniform in thickness (×260).

Fig. 4. An electron micrograph showing a deformed endothelial cell (End) in the turbid portion. The central portion of the cell is thickened and the peripheral portion is extremely thin. The round endothelial cell contains numerous mitochondria and rough-surfaced endoplasmic reticulum. An increased number of microvilli extend toward the anterior chamber (AC). DM, Descemet's membrane (×2,500).
Peripheral portion was extremely thin. Small spaces were observed in the endothelial cell layer which appeared to be vacuoles under the light microscope, and darkly staining cells were often observed at the interface of the two areas in the rejecting graft.

Electron microscopic observations. In the turbid portion of the rejecting graft, the endothelial cells were rounded or spindle-like so that their central portions were greatly thickened (Fig. 4). Sometimes leukocytes seemed to force the endothelium into rounded protuberances by inserting themselves between the endothelium and Descemet's membrane (Fig. 5). The irregularly thickened nuclei were located in the enlarged portion of the cells, whereas in the normal cornea the cells were uniformly thin and flattened, and contained relatively flat oval nuclei. The round cells in the turbid portion contained numerous mitochondria, ribosomes, and rough-surfaced endoplasmic reticulum. These cytoplasmic organelles appeared to be greater in abundance than in normal endothelium, whereas, in contrast, the Golgi complex and vesicles of various sizes seemed to be fewer. Mitochondria in the rounded cells were not swollen. The increased quantity and irregular arrangement of mitochondria and rough-surfaced endoplasmic reticulum in diseased endothelium seemed to be the result of cell shrinkage. The terminal web was seen in the cytoplasm close to the posterior surface membrane, from whence an increased number of microvilli protruded into the anterior chamber. Junctions between adjacent endothelial cells disappeared. The attenuated edges of the adjacent cells closely approximated each other in their peripheral portion (Fig. 4), but in other places, Descemet's membrane had lost its endothelial covering.

Lymphocytes entered the intercellular space between adjacent endothelial cells and reached Descemet's membrane (Fig. 5), suggesting that they had "squeezed" into this narrow cleft, perhaps forcefully, rather than having entered freely an open gap between cells. The endothelial cell vacuoles seen by light microscopy were dilatations of intercellular spaces between adjacent endothelial cells or between Descemet's membrane and detached endothelium (Figs. 5 and 6). The "interface" between the turbid and clear portions of the rejecting graft was marked by precipitates on the endothelial surface. Electron microscopy showed the precipitates to be aggregations of lymphocytes, monocytes, and fibrin. The appearance of the endothelial cells in the interface area was very variable, indicating that this area was undergoing rapid changes, and developing the most striking damage. Lymphocytes or monocytes accompanied by fibrin invaded the intercellular spaces and separated endothelial cells from each other and/or from Descemet's membrane (Fig. 6). The cytoplasmic organelles were fewer in the severely damaged endothelial cells which, however, still had a well-preserved terminal web (Fig. 7). Some endothelial cells seen in the area of interface were characterized by an over-all increase in electron density (Figs. 10 and 11). Distorted and swollen mitochondria and rough-surfaced endoplasmic reticulum were still recognizable within the dark cytoplasm. The density increased until it was difficult to recognize organelles. Cells separated from each other, and finally could be found free in the anterior chamber. When lymphocytes insinuated themselves between adjacent endothelial cells, the cell junctions disappeared (Fig. 8). Lymphocytes which had separated endothelial cells reached Descemet's membrane and seemed to spread under the endothelium; the hemidesmosomes were then absent, and Descemet's membrane became covered by two layers of cells, lymphocytes, and, covering them, endothelial cells. Fig. 9 is an electron micrograph from a clear portion of the rejecting graft, revealing lymphocytes within the endothelial cell layer which appeared to be otherwise normal under the light microscope. In this micrograph, the lympho-
Fig. 5. A lymphocyte (Lym) squeezed into the endothelial cell layer in the turbid portion. The lymphocyte partly separates the endothelial cell (End) from Descemet's membrane (DM) (x7,000).

Fig. 6. Severly damaged portion of endothelial cell layer. Lymphocyte (Lym) accompanied by fibrin (Fib) invades the spaces between endothelial cells (End) and separates them from each other and/or from Descemet's membrane (DM) (x11,000).

Fig. 7. A deformed endothelial cell (End) in the turbid portion of the cornea. This cell contains few cytoplasmic organelles; however, the terminal web (TW) is well preserved. DM, Descemet's membrane; AC, anterior chamber (x17,000).
Fig. 8. Endothelial cell layer of the turbid portion. Lymphocytes (Lym) insinuate under the endothelial cell layer, separating the endothelial cell junctions (End) and reaching to Descemet's membrane (DM). AC, Anterior chamber (×9,500).

Fig. 9. Endothelial cell layer of the clear portion of the cornea. A lymphocyte (Lym) infiltrates the endothelial cell layer, but is completely surrounded by thin cytoplasmic processes of adjacent endothelial cells (End) and does not directly touch Descemet's membrane (DM). Endothelial cells maintain tight junction (arrow) between each other in spite of the lymphocytic infiltration. AC Anterior chamber (×13,000).
Fig. 10. A dark cell in the turbid portion indicated by the clear arrow in Fig. 3. The dark cell appears to be an altered endothelial cell (End) that can be distinguished on the basis of its cytoplasmic constituents from lymphocyte (Lym) and macrophage (Mac). Fibrin deposits (Fib) are present in the anterior chamber (AC). DM, Descemet's membrane (x7,000).

Fig. 11. Dark endothelial cells (End) indicate an increase in electron density. This density increases until the cytoplasmic organelles are almost obscured. Cells finally separate from Descemet's membrane (DM) and are found free in the anterior chamber (AC) (x10,000).
cyte was completely surrounded by thin cytoplasmic processes of adjacent endothelial cells. It did not directly touch Descemet's membrane, which was covered by thin cytoplasmic processes of adjacent endothelial cells. These cells maintained tight junctions between each other in spite of the lymphocytic infiltration, which may explain the less pronounced stromal edema overlying it.

Discussion

Uncomplicated clear corneal transplants retain their individuality and preserve their own cellular elements, while in other tissue transplants, such as skin, host cells penetrate the grafts and replace or destroy the donor elements.

The survival of the corneal graft has been demonstrated with the aid of such techniques as the incorporation of radioisotopic labels into the different corneal components, and the identification of transplanted cells by sex chromatin. According to the studies using these techniques, endothelial cells of clear homografts persist for a long period of time and grafts which develop transient or permanent opacity show almost complete replacement of donor endothelial cells by host cells. Khodadoust and Silverstein developed a technique for the transplantation and rejection of the individual corneal elements separately. In the rejection when posterior stroma and endothelium alone were grafted, the destruction of endothelial cells resulted in the immediate clouding of the superjacent stroma. The condition of the endothelium of the cornea is, in large part, responsible for the success or failure of penetrating keratoplasties. Viable and normally functioning donor endothelial cells must be present to give optimum results when transplanted.

Particular attention was paid to structural changes in the endothelium of rejecting grafts. Electron microscopy revealed that lymphocytes infiltrated the endothelium of the graft even in the portion which appeared clear as seen with a slit lamp. The endothelial cells of this area looked normal under the light microscope. However, lymphocytes in this area did not reach Descemet's membrane, but were separated from it by attenuated processes of adjacent endothelial cells which maintained their tight junctions with each other. In contrast to the clear portion, endothelial cells in the turbid part of the graft became shrunken or severely damaged by the infiltrating leukocytes, and detached from Descemet's membrane into the anterior chamber. In some places, Descemet's membrane was, therefore, exposed to the anterior chamber or covered only by lymphocytes instead of endothelium. Edema of the stroma was prominent in the turbid portion, where the endothelium was severely damaged, and was minimal or absent in the clear portion. This supports the concept that maintenance of transparency of the corneal graft can be attributed to the viability or function of the endothelium.

Leukocytes damaged or destroyed endothelial cells and infiltrated the subendothelial area, detaching the cells from Descemet's membrane. In some regions the damaged endothelium showed marked changes in the cytoplasmic constituents, such as the disappearance of organelles and an over-all increase in electron density. Although the mechanism of separation of tight junctions is unknown, it is reasonable to attribute this cellular damage directly to the immunologically active leukocytes, or their effect on the composition of the fluid immediately surrounding them. It has been shown that the introduction of leukocytic exudates produces a dramatic change in the characteristics of cultured endothelium. In addition, recently it has been reported that the endothelial cells of rabbit cornea, in vitro, lose their tight junctions when the calcium concentration of the perfusion medium is low.

Polack and associates studied the effect of experimental uveitis induced by the injection of bovine serum albumin into the vitreous humor on otherwise normal cor-
Changes in corneal endothelium

Changes in corneal endothelium caused by uveitis, homografts, and autografts.

Uveitis caused round cell infiltration of the endothelium and stroma, with edema and neovascularization of the graft. Similar changes occur also in the intact cornea in severe uveitis, and electron microscopic studies of these eyes show that plasma cells and lymphocytes from the anterior chamber infiltrate the endothelial cell layer. Inflammatory cells were also observed in the stroma, but these were never seen passing through Descemet's membrane. It was concluded that inflammatory cells from the anterior chamber caused the endothelial damage in the immune uveitis, suggesting that leukocytes in rejecting grafts may have the same origin. Plasma cells frequently seen in uveitis were absent in this type of graft rejection, and densification of endothelial cytoplasm and detachment from Descemet's membrane were prominent, whereas this was unusual in experimental uveitis. It is possible that the severity of endothelial cell disease is caused not only by mechanical disruption and cytotoxic effects but also, if not mostly, by specifically sensitized host lymphocytes.

REFERENCES