Ultramicroscopic changes in the corneal graft stroma during early rejection

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Early rejection of corneal stroma of penetrating homografts demonstrates various degrees of keratocyte degeneration. The altered cells are almost always in contact with small round or oval-shaped lymphocytes. Stromal changes are more pronounced in areas adjacent to epithelial or endothelial rejection. Lymphocytes, plasma cells, macrophages, and immunoblasts are present in large numbers at the host stroma or around limbal vessels where they seem to accumulate and replicate.

Key words: penetrating corneal transplants, graft rejection, skin grafts, corneal stroma, lymphocytes, immunoblasts, plasma cell, keratocytes histopathology, electron microscopy, light microscopy, rabbits.

Previous studies have shown the sequence of events during the rejection of experimental penetrating corneal homografts.1,2 The rejection of isolated corneal layers has been shown by Khodadoust and Silverstein3 in light microscopy and in electron microscopic studies by Inomata and associates4,5 and Kanai and Polack5 who demonstrated, respectively, the alterations present in the endothelium, graft scar, and epithelium. Rejection of corneal stroma may occur simultaneously with the rejection of endothelium or epithelium, or after these layers are in advanced rejection. The early rejection of stroma and endothelium can often be reversed by steroid therapy in the experimental animal, and the same has been true in many clinical cases. As most of the histopathologic material obtained from failed human keratoplasties are in advanced stages of rejection or scarring, it seemed pertinent to study the ultramicroscopic alterations in the rejecting stroma of experimental penetrating homokeratoplasties.

Materials and methods

Adult albino rabbits were used for these experiments. Six millimeter penetrating keratoplasties were made between pairs of rabbits, and two weeks later skin grafts were interchanged between these corneal “donor-recipient” pairs if their transplants were well healed and clear. Graft rejection developed 12 to 18 days later.1 Eyes for histologic study were selected at very early stages of rejection, i.e., vascular congestion of host limbal area, vessels to scar area of the graft, and turbidity of the transplant not involving more than one third of its diameter (or one half when the turbidity or haze was...
Figs. 1 and 2. (Fig. 1) Light microscope photograph of epithelium and stroma in area of active rejection. (An arrow shows direction of rejection.) Leukocytes and altered keratocytes are present in the subepithelial layer. (Toluidine blue, original magnification x250.) (Fig. 2) Electron micrograph showing altered keratocytes and lymphocytes in a rejection area. Lymphocytes (Ly) are in contact with keratocytes (K) or with other lymphocytes. Stromal lamellae are irregular (St). Epithelium (Ep); polymorphonuclear leukocyte (Ly). (Original magnification x5,000.)
very slight). Four eyes were studied. In two of them, epithelial rejection was present; in the other two, stromal and endothelial rejection had developed (the turbidity of the graft was greater).

The rabbits were killed with intravenous sodium pentobarbital, and the eyes were enucleated rapidly. The corneas were fixed in four per cent glutaraldehyde with phosphate buffer for 30 minutes. They were cut into smaller pieces and fixed again in one per cent osmium tetroxide with the same buffer for 90 minutes. After fixation they were dehydrated immediately through a series of graded alcohols and embedded in Epon. Thick sections, (1 μ) were stained with toluidine blue for light microscopy. Electronmicrographs were taken with an Hitachi 11-C and a Zeiss 9-52 microscope.

Results

Rejection of stromal cells was observed beneath areas of epithelial cell rejection (Fig. 1) where disruption of collagen fibers due to edema was evident. The epithelial basement membrane showed areas of fragmentation through which phagocytes seemed to emigrate from rejecting stroma to rejecting basal epithelial cells or vice-versa. The area of stromal rejection showed a conglomeration of lymphocytes, polymorphonuclear leukocytes, and keratocytes with varied degrees of morphologic alterations.

Lymphocytes showed cytoplasmic processes and were in intimate contact with keratocytes or with other lymphocytes (Fig. 2). They were round or oval shaped, with a round or oval nucleus, condensed chromatin, several mitochondria, poorly developed Golgi complex, and scattered ribosomes. Severe cellular alterations were present in keratocytes contacted by lymphocytes: they showed distended endoplasmic reticulum, vacuoles, phagocytized material, and high-density crystalline intracytoplasmic material (Figs. 3 and 4). Typical plasma cells were not found in rejection areas; however, there were leukocytes which seemed to be intermediate forms between lymphocytes and plasma cells (Fig. 4).

They had a dense nuclear chromatin, a large number of slightly distended endoplasmic reticulum, and few mitochondria. A discrete number of immunoblast-like cells were found around the rejection areas. They contained polyribosomes grouped in small clusters, scanty endoplasmic reticulum, and few mitochondria (Fig. 5).

The deeper stroma showed a moderate number of lymphocytes in contact with keratocytes or other lymphocytes. Disruption of the normal lamellar structure of the graft stroma was evident in areas of leukocytic infiltration even though edema was not evident. Here again, lymphocytes were in contact with keratocytes which showed multiple processes with rounding of their cytoplasm, vacuolation, and phagocytized material. In specimens without endothelial rejection, the deeper stroma showed a few lymphocytes and no stromal edema. On the other hand, in specimens with endothelial rejection, a moderate number of lymphocytes were in contact with Descemet's membrane (Fig. 6).

New capillary vessels were found in the anterior and middle layers of host stroma and at the limbal area (Fig. 7) where lymphocytes and plasma cells were seen predominantly outside the endothelial capillary wall. Some lymphocytes were present between the endothelial and the periendothelial vascular sheath and were surrounded by basement membrane (Fig. 8). Typical plasma cells, some in mitotic stage, were seen around capillary walls or host stroma (Fig. 9). Occasionally, a variety of cell types, i.e., lymphotic cells, plasma cells, isolated endothelial cells, macrophages, and immunoblast-like cells, were seen forming clumps in some areas of the host stroma (Figs. 10-11). Macrophages with a Golgi complex consisting of clusters of vesicles and lamellae were also seen (Fig. 12). These cells contained inclusion bodies larger than mitochondria with two limiting membranes, occasionally forming myelin-like figures with a high-density granular material in their matrix. Specimens without endothelial rejection showed no alterations in the deep stroma, whereas lymphocytic infiltration and
stromal cell destruction was evident in corneas with endothelial rejection.

Discussion

These studies demonstrate that rejection of the corneal graft does not always come all at once to its three cellular layers but may occur in different layers at varied times. In grafts with superficial stromal rejection associated with epithelial rejection, we found an increased number of lymphocytes, polymorphonuclear leukocytes, phagocytized keratocytes, and disorganized collagen lamellae. The epithelial basement membrane was fragmented with phagocytic cells invading the basal epithelial cell layer.
Figs 5 and 6. (Fig. 5) Immunoblast-like cell (Im-Ly) showing polyribosomes, scanty endoplasmic reticulum, and few mitochondria. Keratocyte (K). (Original magnification ×10,000.) (Fig. 6) Corneal graft specimen with endothelial rejection showing a lymphocyte (Ly) near or in contact with Descemet's membrane (DM) and enveloping a keratocyte (K). Collagen fibrils (cf). (Original magnification ×12,000.)

in some areas. The alteration of keratoctyes in contact with lymphocytes was similar to that observed previously in rejecting corneal epithelium. We found various degrees of ultramicroscopic cell damage or advanced cell destruction in rejection areas invaded by lymphocytes; however, we found no morphologic evidence of cell membrane damage at sites of contact between lymphocytes and keratocytes which could explain cell death. A cell membrane alteration between macrophages and lymphocytic cells in antibody synthesis has been described by Schoenberg and co-workers.

Lymphocytes and plasma cells have been
Figs. 7-9. For legend see opposite page.
accepted as the effector cells in graft rejection. The present study confirms the observation that plasma cells do not usually appear in early graft rejection. However, a number of leukocytes around the rejection area appear to be intermediate forms between lymphocytes and plasma cells, a transformation process which has been observed already in tissue culture by Weiss. These lymphocytes possess polyribosomes, mitochondria, and rough endoplasmic reticulum.

The type of lymphocyte engaged in graft rejection is the so-called small lymphocyte. It is round or oval in shape but it becomes flat with many projections developing from the cytoplasm when in contact with other cells. Immunoblast-like cells are also seen in rejection areas; they show large oval nuclei with prominent nucleoli, clusters of ribosomes, and intermediate cells, probably in the transitional stage between the immunoblast and the small lymphocyte. Typical immunoblasts contain a greater number of ribosomal clusters and are often seen in the host cornea or at the limbus.

The origin of the immunologically active or competent lymphocyte is most likely the limbal area10-13 or the regional lymph node,13 though more distant lymph nodes can become sensitized.14 Howes's studies in ferritin-sensitized rabbits challenged by intracorneal injections of protein have shown characteristic immunocompetent lymphocytes or immunoblasts present at the corneal limbus. In previous light microscopic studies, lymphocytes have been seen leaving these channels, apparently conglomerating and replicating outside limbal vessels as shown in Fig. 8. In these studies, we have found clumps of such cells at the limbus and around capillaries as well as suggestive figures of lymphocyte transformation into plasma cells at the limbal stroma and near the graft. It is interesting to note that an increased number of plasma cells have been described in the lymph node of animals with a "second-set" skin graft rejection14 when compared to a first-set rejection. In our studies, however, plasma cells have not been very prominent at the corneal limbus or in the rejecting graft.

Most profound stromal graft alterations developed when the endothelium had started to reject or had been rejected first, perhaps because in these cases graft edema is more pronounced. An increased number of lymphocytes were seen near, or...
Figs. 11 and 12. (Fig. 11) Electron micrograph of an area similar to that of previous picture demonstrating lymphocytes (Ly), plasma cells (PC), isolated endothelial cells (En), and a macrophage (Mac). (Original magnification x6,300). (Fig. 12) Macrophage containing inclusion bodies (Ib) with double limiting membrane and a myelin-like figure. Golgi-complex (G); nucleus (N). (Original magnification x16,000.)
in contact with, Descemet's membrane; however, there was no evidence of breaks such as that found in the epithelial basement membrane.5

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REFERENCES