SEROLOGICAL STUDIES IN KERATOPLASTY*

BY

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It has been shown that human cornea carries blood-group specific antigens corresponding to those of red blood cells (Nelken, Michaelson, Nelken, and Gurevitch, 1956, 1957) and the clinical significance of these findings has been discussed by various authors (Havener, Stine, and Weiss, 1958; Mehri, Becker, and Oglesby, 1959). Sudden clouding of the graft, *maladie du greffon*, could be produced experimentally in rabbits by prior injection of incompatible red blood cells (Nelken, Nelken, Michaelson, and Gurevitch, 1961). The possible role of additional corneal antigens in the aetiology of the "disease of the graft" was described by the present authors (Nelken, Michaelson, Nelken, and Gurevitch, 1958). In a further study of the antigenic structure of the cornea, organ-specific antigens, species as well as individual specific antigens, have been found (Nelken and Nelken, 1962).

The present communication deals with these individual specific antigens in correlation with clinical findings in keratoplasty.

Materials and Methods

Preparation of corneal extracts was as follows:

Rabbit eyes were enucleated under sterile conditions immediately after death. Human eyes were enucleated as soon as possible after death, but in no case later than 12 hours after death. The cornea was removed and after rinsing in physiological saline solution it was frozen at -20°C . in sterile tubes containing 0.5 ml. of this solution. Before use the frozen cornea was cut into thin slices with the aid of small scissors and a scalpel. This material, in the saline in which the cornea was originally frozen, was homogenized in a small Pyrex glass tissue-grinder with metal knives; 4.5 ml. of saline was added during the homogenization.

In order to prevent heat production during homogenization the whole Pyrex cup was immersed in ice-water. The final white pasty homogenate consisted of particles of 2–12 μ in diameter. The corneal homogenate was frozen in 0.5 ml. quantities and kept at -20°C . until used. No homogenate was ever frozen and thawed twice. Before use the tube with the homogenate was thawed and centrifuged at $1,000 \times g$ for 10 minutes in a refrigerated centrifuge. The supernatant fluid served as antigen.

Antibodies were determined in sera of patients after keratoplasty. The passive haemagglutination test (Nelken and Nelken, 1962) was used. Blood was obtained from the patients under sterile conditions and allowed to clot for at least four hours at room temperature.

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The serum was separated from the blood, inactivated at 56°C., and stored at -20°C. in small aliquots.

Anti-human corneal serum was prepared in rabbits as previously described (Nelken and Nelken, 1962).

Experiments and Results

Individual specific antigens were examined in two ways.

First, three different rabbit anti-human corneal sera were examined against fifteen different human corneal extracts. One corneal extract served as antigen while the other corneal extracts were used as inhibiting antigens. No differences of titre could be observed when any of these antigens served either as coating antigens or as inhibiting antigens. The rabbit anti-human corneal serum by the tanned-cell agglutination and inhibition methods did not reveal any differences between the different extracts of human cornea.

Secondly, serum of post-keratoplasty patients served as antibody. Thirty-three patients were examined. Blood was taken before the operation and at 5-day intervals after the operation up to three months. The cornea of the donor's second eye was saved and kept at -20°C. In 2 cases anti-human corneal antibodies could be detected before the operation. In 15 other cases anti-corneal antibodies were found after the operation by the tanned-cell haemagglutination technique and by complement fixation.

Positive sera were examined against several corneal extracts. The sera of 14 patients had approximately the same titre when examined against different corneal extracts. Typical results of such an experiment are summarized in Table I.

TABLE I
TITRATION OF A POST-KERATOPLASTY PATIENT'S SERUM AGAINST TANNIC-ACID
TREATED ERYTHROCYTES COATED WITH DIFFERENT CORNEAL ANTIGENS

Serum of Patient (Days after Operation)	Antigen	Titre of Antibodies
10	Donor's cornea	0
	Random cornea I	0
	Random cornea II	0
15	Donor's cornea	0
	Random cornea I	0
	Random cornea II	0
20	Donor's cornea	1 : 16
	Random cornea I	1 : 16
	Random cornea II	1 : 16
40	Donor's cornea	1 : 8
	Random cornea I	1 : 8
	Random cornea II	1 : 8
50	Donor's cornea	1 : 4
	Random cornea I	1 : 4
	Random cornea II	1 : 4

In one patient a markedly higher titre appeared against the cornea of his donor than against any other unrelated corneae. This patient also suffered from a typical *maladie du greffon* at the same time. In the others there was no late clouding of the graft. The patient was a boy of 11 years who underwent a keratoplasty operation because of a severe lime-burn of his left eye. There was severe symblepharon and a total leucoma; pre-operative vision was limited to hand movements. The patient received a deep lamellar graft of 7 mm. diameter which remained clear for eight weeks.

The post-operative vision was 5/9. Suddenly, on the 57th post-operative day the graft became oedematous. The vision fell to counting fingers before the eye. The patient was taken to hospital at once and treated by cortisone generally and locally, receiving Meticorten 20 mg. a day and Metymid drops 3 times a day. The graft became vascularized and permanently opaque within two weeks.

The patient's blood group was B, Rh₁/Rh₂/Hr-. The blood group of the donor was B, Rh₁/Hr-.

Table II shows the appearance of anti-corneal antibodies against four random corneae and the sudden rise of the titre against the cornea of his donor on the 40th post-operative day.

TABLE II

TANNED CELL HAEMAGGLUTINATION TEST PERFORMED WITH PATIENT'S SERA

Serum of Patient	Antigen	Titre of Antibodies
Serum before operation	Donor's cornea	0
	Random cornea I	0
	Random cornea II	0
	Random cornea III	0
	Random cornea IV	0
25 days after operation	Donor's cornea	1 : 4
	Random cornea I	1 : 4
	Random cornea II	1 : 2
	Random cornea III	1 : 4
	Random cornea IV	1 : 4
40 days after operation	Donor's cornea	1 : 128
	Random cornea I	1 : 16
	Random cornea II	1 : 16
	Random cornea III	1 : 16
	Random cornea IV	1 : 16
50 days after operation	Donor's cornea	1 : 16
	Random cornea I	1 : 2
	Random cornea II	1 : 2
	Random cornea III	1 : 2
	Random cornea IV	1 : 2
60 days after operation	Donor's cornea	1 : 2
	Random cornea I	0
	Random cornea II	0
	Random cornea III	0
	Random cornea IV	0

Discussion

The difference between the fate of corneal homotransplants and other tissue homografts is usually explained in two ways—avascularity of the recipient bed and weak antigenicity of the small corneal transplant.

According to the above findings there is an additional factor which may account for the relative success of corneal homografts—the absence of individual specific antigens in the cornea. Circulating anti-corneal antibodies appeared in about 50 per cent. of our cases. These antibodies, however, reacted with every random cornea. This is in contrast to the serological findings after skin transplantation. The circulating antibodies found after skin transplantation show a very marked individual specificity—they react exclusively with the leucocytes of the donor (Friedman, Retan, Marshall, Henry, and Merrill, 1961; Walford, Carter, and Anderson, 1962).

In the case of *maladie du greffon* the circulating anti-corneal antibodies showed a certain degree of individual specificity; they reacted with a higher titre when titrated against the donor's cornea than against random corneae.

The relative lack of individual specificity of the human cornea may be another factor contributing to the success of corneal grafts.

Summary

Anti-corneal antibodies were studied in 33 keratoplasty patients. Anti-corneal antibodies were found in 2 cases before the operation. In 15 out of 33 patients anti-corneal antibodies were found after the operation. The antibodies reacted with the donor cornea as well as with any random cornea.

Individual specificity was found in one patient who suffered from typical *maladie du greffon* ("disease of the graft"). A correlation between the time of the appearance of the antibodies and the opacity in the graft was observed.

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