Immune factors in corneal graft rejection

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This panorama of the immune factors in corneal graft rejection covers a spectrum of mechanisms which play a role in the opacification of corneal grafts. Great emphasis has been accorded to the role of transplantation immunity. The clinical and experimental evidence implicating transplantation immunity in the rejection of corneal grafts has been critically reviewed. In addition, evidence is presented indicating that some immune mechanisms which result in inflammatory sequelae other than classical transplantation immunity can lead to rejection of corneal grafts. Evidence also suggests that certain well-described pseudoimmune or immunomimetic reactions, which are devoid of the classic criteria required for hypersensitivity reactions, contribute to rejection of corneal grafts.

Key words: corneal graft rejection, transplantation immunity, inflammation, immunomimetic, allograft, xenograft, autograft, “second-set” phenomenon, keratoplasty, chimerism, avascularity, vascularization, afferent and efferent limbs (arcs), central lymphoid tissue response, antigen-antibody reaction, Shwartzman reaction, Auer phenomenon.

The literature on immune factors in corneal graft rejection was last reviewed in 1962. The volume of subsequent investigation addressed strictly to this subject necessarily prohibits an inclusive and detailed review. This paper reviews the relationship of the immune response to corneal graft rejection with emphasis on the immunologic factors which predispose corneal transplants to rejection reactions. In sharp contrast to previous summaries, these factors are discussed within a framework which not only includes transplantation immunity per se but extends beyond it to the common mechanism involved in graft failure: inflammation—specifically those inflammatory reactions with immunologic features which may affect the viability of a graft.

Corneal graft rejection is the irreversible opacification of grafted tissue. Table I summarizes the major biologic and technical factors which cause or contribute to rejection of a graft. The common denominator of these factors is inflammation; the basic manifestation of inflammation is altered capillary permeability, which results in cell and serum exudation from the intravascular to the extravascular compartment. If the inflammatory process, regardless of its etiology, is sufficiently severe and prolonged, the grafted corneal tissue will incur irreversible damage culminating in failure of the graft (Fig. 1).
Table I. Factors implicated in penetrating keratoplasty failures

<table>
<thead>
<tr>
<th>Biologic factors</th>
<th>Technical factors</th>
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<tr>
<td><strong>Preoperative</strong></td>
<td><strong>Postoperative</strong></td>
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<tr>
<td>Selection of unfavorable candidate with local or systemic disease</td>
<td>Immunologic Transplantation immunity</td>
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<td>Use of unsuitable donor material</td>
<td>Other immune factors</td>
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<td></td>
<td>Retrocorneal membrane</td>
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<td></td>
<td>Suture toxicity</td>
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<td>Uveitis</td>
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<td>Hyphema</td>
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<td>Glaucoma</td>
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<td>Infection</td>
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<td></td>
<td>Epithelialization of anterior chamber</td>
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<td></td>
<td>Anterior synechiae</td>
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<td></td>
<td>Vitreous touch to donor endothelium</td>
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<tr>
<td></td>
<td>Delayed wound repair</td>
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<td></td>
<td>Recurrence of original disease in graft</td>
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![Diagram](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933699/)

**Fig. 1.** Schematic representation of inflammation and corneal graft failure.

Immune factors in corneal graft rejection

The classic immune factors which result in inflammatory processes deleterious to the host are the hypersensitivity reactions, subdivided for convenience into immediate and delayed (cellular-mediated) types. Table II presents the traditional, tissue-damaging reactions which exemplify immunologic hypersensitivity.

During the past decade there has been a gradual increase in awareness of the possible importance of other inflammatory pathogenetic mechanisms in graft rejection. While these mechanisms exhibit immune characteristics, they do not entirely fulfill the criteria for classical hypersensitivity reaction—they resemble, or mimic, immunologic hypersensitivity—and thus, are designated as immunomimetic reactions. The classic hypersensitivity reactions, on the one hand, are triggered or initiated by a direct combination or interaction of specific antigens and antibodies, or, by the interaction of specific antigens and immunologically activated cells. Immunomimetic reactions, on the other hand, are devoid of this specificity, (i.e., specific antigen-antibody combination) as initiators of the acute inflammatory pathway; Table III lists several major reactions which may be termed immunomimetic.

**The contemporary problem**

Despite the volume of investigation directed to corneal grafting and despite the considerable advancements in ocular surgery over the past 20 years, the incidence of graft failure remains inexplicably high in both good and poor prognostic categories. Results in the most favorable prognostic categories are satisfactory. However,
results in scarred and vascularized recipient corneas are, to be realistic, dismal. This comparison is especially sobering in light of the latest data available on success rates in modified renal allografts, for which the donor kidney is provided by a relative of the recipient (Fig. 2). The range of successful grafts in scarred, vascularized host corneas is 11 to 63 per cent after one year; in sibling-donor renal allografts, the expectation of one-year survival is approximately 90 per cent and at 2 years, 80 per cent. This achievement is not an unreasonable objective for corneal transplantation, nor is there justification for considering it unattainable within the next decade.

Transplantation immunity (tissue incompatibility)

The term transplantation immunity or, more precisely, tissue incompatibility, denotes a state of recipient intolerance to living tissue transplanted from a member of the same species (allograft) or a member of a different species (xenografts). Transplantation biologists characterize transplantation immunity as an active immunologic process against histocompatibility antigens present in the donor tissue but absent in the host. The bases for this characterization are:

1. Autografts succeed in general, while allografts and xenografts uniformly fail.10, 11
2. Allo- and xenografts flourish during an initial period of grace or "latent period" (usually from 8 to 10 days for skin allografts between randomly selected rabbits).10, 11, 12
3. The "second-set" phenomenon—the accelerated and intensified rejection of a second graft from the same donor as compared to the initial graft.12, 13
4. Specificity of the graft rejection reaction—sensitization to an initial graft is specific for the tissue from the original donor; the course of a second, indifferent graft from a donor
unrelated to the first will proceed normally.10

(5) The systemic nature of transplantation immunity—the accelerated rejection of second-set, solid-tissue grafts regardless of their location.10

(6) The capacity to transfer transplantation immunity in inbred strains with sensitized lymphoid cells.14

(7) Induction of actively acquired immunologic tolerance in the host to subsequent allografts.15,16

(8) Abolition of tolerance to tissue allografts by injecting an animal with lymphoid cells from normal adult members of the same strain.17

(9) Abrogation of transplantation immunity by immunosuppressive regimens.18,19

These findings establish the primacy of immunologic mechanisms in the rejection of transplanted tissues, a primacy which is generally applicable to the broad scope of the transplantation field. In contrast, evidence implicating transplantation immunity in corneal graft rejections is considerably less tenable. While experimental data are abundant, clinical evidence supporting the role of tissue incompatibility in keratoplasty rejection is at best fragmentary and incomplete.

Unique problems inherent in corneal transplantation. Experimental investigation of immune factors in corneal graft rejection presents unique difficulties which are not encountered by the transplantation biologists. For instance, experimentalists in corneal transplantation have not yet developed an ideal animal model. Transplantation biologists can readily and reproducibly induce rejection of unmodified tissue and organ grafts. In contrast, corneal investigators must introduce undesirable variables to achieve reproducible rejection; consequently, the model does not simulate the human counterpart. The majority of experimental work on corneal transplantation immunity has been done in the following three models: (1) corneal neovascularization induced in the recipient cornea prior to corneal grafting (lamellar, interlamellar, or penetrating keratoplasty); (2) penetrating allografts followed by subcutaneous implantation of homologous skin; (3) Interlamellar or penetrating xenografts, which yield reproducible corneal graft rejection.

Although each of these models offers advantages and disadvantages in corneal transplantation, the systemic nature of transplantation immunity—the accelerated rejection of second-set, solid-tissue grafts regardless of their location—presents unique difficulties which are not encountered by the transplantation biologists. For instance, experimentalists in corneal transplantation have not yet developed an ideal animal model. Transplantation biologists can readily and reproducibly induce rejection of unmodified tissue and organ grafts. In contrast, corneal investigators must introduce undesirable variables to achieve reproducible rejection; consequently, the model does not simulate the human counterpart. The majority of experimental work on corneal transplantation immunity has been done in the following three models: (1) corneal neovascularization induced in the recipient cornea prior to corneal grafting (lamellar, interlamellar, or penetrating keratoplasty); (2) penetrating allografts followed by subcutaneous implantation of homologous skin; (3) Interlamellar or penetrating xenografts, which yield reproducible corneal graft rejection.

Table II. Classic immunologic hypersensitivity reactions resulting in tissue damage

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<tr>
<th>Immediate (antibody mediated)</th>
<th>Delayed (cellular mediated)</th>
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<tr>
<td>Antigen-antibody complexes (immune aggregate disease): arthus, serum sickness</td>
<td>Tuberculin reaction (prototype)</td>
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<tr>
<td>Cytotoxicity</td>
<td>Contact dermatitis</td>
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<tr>
<td>Anaphylaxis</td>
<td>Transplantation immunity</td>
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<td></td>
<td>Other protein antigens (Jones-Mote)</td>
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<td></td>
<td>Graft vs host reaction</td>
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<td>Autoallergic disease</td>
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Table III. Inflammatory pathogenetic mechanisms with immune features but not typical of classic hypersensitivity reactions

| Local antibody formation |
| Aggregated, denatured, or polymerized gamma globulin activation of complement pathway |
| Endotoxin as a substrate for complement |
| Schwartzman reaction |
| Structural alteration (Auer phenomenon) |
| Localization of circulating immune complexes at sites of altered capillary permeability |
transplant investigation, none is clearly superior to the other models. The investigator's selection of a model usually depends upon the particular demands or objectives of the given experiment. It should be emphasized again that none of the above models is directly analogous to corneal grafting in the clinical area; therefore, any comparisons with these models are fraught with hazards.

Another critical problem peculiar to human corneal transplantation is the marginal functional reserve present in the donor endothelium, which must survive if the graft is to succeed. The number of endothelial cells in the entire human cornea has been estimated at approximately 500,000. As a point of comparison, the average 7.5 mm. donor button contains about 195,000 endothelial cells. These cells are fragile and highly vulnerable to trauma or noxious stimuli. By contrast, the functional mass and reserve of a transplanted kidney (glomeruli and tubules) easily exceed the corneal endothelial reserve by several orders of magnitude. Thus, chronic rejection of a renal allograft may proceed for months to years before its functional reserve is compromised.

Compounding the already unique difficulties of corneal transplantation is the lack of physiologic dependence of corneal metabolism and structure upon vascularization. Consequently unlike many other transplanted tissues, rejection of a technically adequate corneal graft is heralded by loss of clarity and by opacification rather than by extrusion or sloughing. Undoubtedly there are exceptions to this general statement, depending upon the severity of morphologic changes in the recipient cornea. Alkali-burned corneas are a notoriously poor prognostic category for keratoplasty, frequently demonstrating necrosis and donor cornea slough. This complication is usually ascribed to defective recipient wound repair; in actuality, it may be caused by vascular ischemia in a severely altered host cornea. Some evidence indicates that treatment of the recipient cornea with subconjunctival heparin prior to grafting facilitates host cornea revascularization and yields greatly improved results.

Persistence and rejection of donor epithelium, endothelium, and stroma. When the previous reviews were published, the ultimate fate of the transplanted corneal cellular elements was uncertain. The alternatives conjectured at the time were (1) that the donor cornea served as a scaffold or matrix (allostatic graft) for eventual replacement of host cells or (2) that donor cells survived intact during the lifetime of the graft (allovital graft). During the past decade, four independent laboratories have demonstrated the persistence of donor keratocytes and endothelial cells (true chimerism) in successful experimental penetrating corneal allografts and even in xenografts. A majority of the donor endothelial and stromal cells in the recipient cornea survived for extended periods, some up to 21 months, and possibly they could survive for the animal's lifetime, providing the graft was not rejected. Therefore, penetrating corneal allografts are allovital in nature, rather than allostatic. The significance of this finding is that it verifies the creation of a condition called chimerism, which implies a stage of continual exposure of the host immune apparatus to the foreign antigens, due to the ability of foreign cells to replicate. In contrast to a nonviable nonreplicating antigen stimulus which is unsustained, graft antigens from viable cells result in a sustained antigenic stimulus.

Recent evidence indicates that the donor epithelium in successful corneal allografts survives and retains functional integrity in an avascular recipient bed for at least six months. These experiments also showed that specific rejection of the epithelial layer could be induced by producing neovascularization of the donor tissue. The findings suggest that epithelial rejection is produced by transplantation immunity, proceeds at a variable tempo, and can be monitored by the characteristic epithelial
migration line made visible by methylene blue staining. These data have dispelled the clinical myth of nonsurvival of the donor epithelium in the recipient cornea. Although in most cases the epithelium of the donor eye is removed prior to transplantation, the adage has long prevailed that donor corneal epithelium does not survive in the recipient after corneal grafting.1

The ingeniously designed experiments of Khodadoust and Silverstein31 demonstrated the rejection pattern of the component corneal layers (epithelium, stroma, and endothelium) and the capacity of each layer to induce and succumb to allograft rejection. These experiments support Mau- menee’s1 view that lamellar grafts in a vascularized bed will both induce and succumb to transplantation immunity. They also represent the first experiments which characterize the microscopic rejection patterns of the corneal elements where the contiguous layer not under study was of recipient identity. This permitted analysis and characterization of the rejection process of each layer individually.

**Reasons for success of corneal grafts**

The classic experiments of Billingham and Boswell32 demonstrated the ability of corneal tissue to induce, as well as to succumb to reactions of transplantation immunity. Furthermore, they established the validity of the cornea as an “immunologically privileged” site for transplantation. The privilege of the cornea as a recipient site is not restricted to grafts of corneal tissue, for even skin allografts transplanted interlamellarily in the cornea survive 3 to 4 times longer than those placed orthotopically. Billingham and Boswell32 attributed this phenomenon to the avascularity of the cornea, which could account for the high degree of clinical graft success in good prognosis corneas. Other factors which have not been satisfactorily excluded as an explanation for clinical success of keratoplasty are the number and potency of the histocompatibility antigens present in the cornea as compared to other tissues and organs. Also, Billingham and Boswell admittedly did not exclude the “afferent limb” of sensitization as a factor for the “immunologic privilege” of the cornea as a recipient site. In the absence of an intact afferent lymphatic system, transplantation sensitization cannot be induced in the host.33, 34

**Table IV. Routes and forms for antigen egress from transplanted tissues and organs**

<table>
<thead>
<tr>
<th>Blood-borne pathway (venous)</th>
<th>Lymphatic pathway</th>
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<tr>
<td>Free antigen molecules</td>
<td>Free antigen molecules</td>
</tr>
<tr>
<td>Antigens bound to recipient cells</td>
<td>Antigens bound to recipient cells</td>
</tr>
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**Afferent, central, and efferent arcs of transplantation immunity.** The immune response produced by transplantation is usually visualized as being composed of afferent and efferent limbs (arcs), as well as a central portion. The afferent arc consists of that portion of the arc where antigen(s) are leaving depots in the graft until they reach the central immune centers of the host. The central portion of the arc represents the cellular proliferative activity stimulated in the lymph nodes and spleen by the histocompatibility antigens. The efferent limb is represented by the effector cells which leave the central immune centers to reach specific target tissues.

**General knowledge about transplantation immune arc.**

**Afferent arm.** Conceivably, there are two routes and two forms by which antigens or antigenic determinants reach the central immune centers of the host (Table IV). The pathway probably varies, depending on such factors as the site of the tissue or organ transplant, the vascularity of the tissue, and whether it has lymphatic drainage. Also, the pathway varies depending upon whether soluble or insoluble (sessile) antigens are involved. While no single route preferentially covers all the possible circumstances, several recent experiments...
should be cited to indicate the complexity of the analysis of the afferent limb. With regard to renal homografts, Najarian and associates\textsuperscript{35} have advanced evidence that antigenic material can be procured or detected in the recipient renal-vein plasma within hours of kidney transplantation in dogs. This is not, however, conclusive proof that the venous antigen egress pathway is either a necessary or sufficient afferent pathway for induction of transplantation immunity.

The studies of Barker and Billingham,\textsuperscript{33, 34} for example, indicate that the afferent lymphatic pathway for antigen egress is imperative for development of transplant immunity. By an ingenious technique they formed a flap of skin in guinea pigs which is deprived of lymphatic drainage but retains a narrow vascular pedicle. They found that the flaps can support long-term survival of inlay skin allografts. If lymphatic connections are allowed to re-establish themselves, the foreign graft is rejected. Moreover, the hosts of long-term "intra-flap" skin allografts did not develop sensitivity as evidenced by the "first-set" type of rejection of subsequent test grafts. Cronkite and his colleagues\textsuperscript{36} confirmed the importance of afferent lymphatic channels to the sensitization process in their studies involving extracorporeal irradiation of thoracic duct lymph in cattle. Skin grafts over the posterior quarters of cattle, whose thoracic duct lymphocytes are being systematically and continuously destroyed by passage through an extracorporeal irradiation device, are preferentially protected from rejection. These findings support the prominent, if not exclusive, role of lymphatic over blood vascular channels as afferent conduits in the development of allograft sensitivity.

Strober and Gowans\textsuperscript{37} showed that blood-borne small lymphocytes can act as conveyors of antigenic "information" to the central immune centers. It is unknown exactly how this capture of information occurs. One alternative is that it occurs as the lymphocyte contacts the vascular endothelium of the graft; another is that it occurs during a period of independent migratory activity outside vessel walls among the cells of the transplant. This does not exclude the alternative that some free antigen molecules can migrate to the draining node and initiate a central proliferative immune response.

Central lymphoid tissue response. Once the antigenic stimulus has reached the appropriate lymphoid centers, a burst of cellular activity ensues, with development of large "blast" cells (hemocytoblasts or immunoblasts). Early, this reaction is primarily manifested in the draining lymph node, but subsequently spreads to involve distant lymph nodes as well as the spleen. This cellular proliferative activity has been intensively studied with tritiated thymidine labeling, as well as with electron and light microscopy.\textsuperscript{38-40} Subsequently, the immunologically activated cells, which are most likely small lymphocytes (effector or "killer" cells), circulate freely in the blood to attack their specific target tissues. The cellular proliferative activity can be detected before rejection of the graft becomes apparent, and continues long after the allograft has been destroyed.

Efferent limb of transplantation immunity. There is fairly general agreement that the immunologically activated small lymphocytes are the mediators or effectors of transplantation immunity.\textsuperscript{41-42} It is of extreme interest that the cellular milieu at the target tissue contains no greater proportion of "specifically sensitized" lymphocytes than an unrelated or indifferent skin homograft on the same animal.\textsuperscript{35} Therefore, it appears that there is no specific attraction or "homing" by sensitized lymphocytes for their specific targeted tissue and, furthermore, only a small fraction of the total cellular elements in the inflammatory infiltrate are members of the original specifically sensitized population (approximately four to ten per cent of the mononuclear cells).\textsuperscript{43, 44} Therefore, there is no specificity demonstrable for effector cells in terms of localization or attraction at the
target tissue and the bulk of the cellular infiltrate is comprised of "nonsensitized" cells.

The efferent arc of rejection is much more complex in human beings than in animals. Probably human renal allograft rejection has received the greatest attention and has been investigated more fully than other organs. This ongoing rejection of renal allografts is extremely complex. The picture in human recipients is further clouded by intensive immunsuppressive regimens. For our purposes, it will be sufficient to mention the four types of rejection classified by Porter according to their apparent time of onset.

**IMMEDIATE AND ACUTE.** This type of rejection occurs within minutes or hours after restoration of the circulation and is felt to be due to preformed circulating antibody. This deleterious antibody is present in the recipient either because of a major blood group incompatibility, multiple previous blood transfusions or pregnancies, or exposure to environmental antigens that can provoke cross-reactions with foreign histocompatibility antigens in the transplanted organ (e.g., streptococcus).

**EARLY ACUTE REJECTION WITHIN THE FIRST TWO WEEKS.** Microscopically, there is diffuse edema (interstitial) and a predominance of immunoblasts. The glomeruli and arterioles are unaffected. There are no immunoglobulins or complement components demonstrable except within the infiltrating cells.

**LATE ACUTE REJECTION OCCURRING AFTER TWO WEEKS.** Here the cellular infiltrate is less dense and composed of plasma cells and smaller lymphoid cells (immunocytes). Necrosis of the arteriolar walls with intimal thickening and disruption of the internal elastic lamina are apparent. These events follow the adherence of platelets along endothelial surfaces, a process which is known to occur in the presence of humoral antibody. Immunohistochemical techniques have demonstrated the presence of IgG, IgM, and B,C globulin subendothelial regions.

**INSIDIOUS AND LATE REJECTION OVER MONTHS AND YEARS.** The microscopic picture reveals glomerular changes entirely consistent with membranous or lobular glomerulonephritis. Glomerular capillary wall localization of complement, IgM, and IgG is common. These changes are difficult to differentiate morphologically from the various types of glomerulonephritis and may represent recurrence of the host's original kidney disease.

Thus, in human renal allograft a wide spectrum of pathogenetic circumstances are seen with changes compatible with both delayed and humoral antibody types of hypersensitivity.

**Transplantation immunity arcs as applied to corneal grafts.**

**Afferent limb.** There is almost a total absence of information concerning antigen egress from corneal allografts. However, some studies with the use of the intracorneal inoculation of soluble tracer-labeled proteins indicate that, at least for soluble antigens, one of the afferent pathways for egress of antigens from the cornea is via afferent lymphatics to the draining node in the neck. Collin, in studies designed to demonstrate the presence of lymphatics in the vascularized rat cornea, was able to show that human serum albumin was detectable in the draining lymph nodes of the neck as early as six hours after intracorneal injection. This was detectable in rats with both avascular and vascularized corneas. In rats with vascularized corneas the isotope-protein complex was detected earlier (within six minutes) and in 1,000 x greater concentration than the avascular cornea. The investigator presented a fairly convincing case from these data for the presence of lymphatics in the vascularized cornea. By contrast, other studies done by Flax (personal communication, April 26, 1969) in the avascular rabbit cornea with use of qualitative techniques (dyes and/or autoradiography) indicate that the afferent route for antigen egress from the cornea is not via a draining node but by the venous route in the aqueous
veins and perilimbal plexus. He was unable to detect any antigen whatever in the draining node after intracorneal inoculation. Certainly, this area is a fertile field of needed investigation; for the present, no absolutisms are permitted.

Central lymphoid tissue response. Polack's\textsuperscript{49, 50} has demonstrated an active cellular proliferative activity in the lymphatic tissue (central immune center) of rabbits bearing rejecting corneal xenografts. He has noted lymphoid follicles and germinal centers in the conjunctival tissues. Also, lymphoid hyperplasia was apparent in the lymph nodes of the neck and mesentery as well as spleen. Nests of lymphocytes were also present in the interlobular spaces of the liver. Rejecting penetrating corneal allografts produced notable hyperplasia of subconjunctival lymphatics (follicles of lymphocytes), but a systematic study of lymphatic tissues has not been done during the course of corneal allograft rejection.

Efferent limb. The efferent arm of corneal graft rejection has been investigated with both electron and light microscopy. Khodadoust and Silverstein\textsuperscript{50, 51} have reported on the cellular infiltrate involved in the rejection process of the individual layers of the cornea where the layers could be studied independently of the influence of adjacent tissue. In the case of pure epithelial and pure stromal rejection, a predominance of polymorphonuclear leukocytes with an admixture of lymphocytes and plasma cells was present within the advancing epithelial rejection line or the stromal rejection band, respectively. However, in the case of the rejection of the almost pure endothelial layer, the rejection line was composed almost exclusively of lymphocytes and plasma cells. Their findings for the epithelium and stroma are quite surprising in view of the fact that in most allografted tissues, a cell which morphologically resembles a lymphocyte is thought to be the "graft killer" cell and is found in abundance throughout the vicinity of rejecting solid tissue grafts.\textsuperscript{50}

This predominance of polymorphonuclear cells suggests a more prominent role for antibody-mediated hypersensitivity as opposed to a delayed hypersensitivity mechanism for the rejection of epithelial and stromal corneal allografts.

Polack's\textsuperscript{52} classic histopathologic study on the early stages of penetrating corneal allograft rejection revealed that the reaction began at the periphery of the graft after blood vessels from the host cornea reached the donor-host scar. Histologic preparations revealed that the scar tissue was first invaded by vessels and then infiltrated by lymphocytes, monocytes, and plasma cells. The vessels which reach the scar do so usually through the anterior one third of the stroma. The cellular infiltrates extended down to Descemet's membrane and the endothelium before invading the graft stroma. The cellular infiltrate next extended into the stroma and through Descemet's membrane to reach the peripheral endothelial cells of the donor causing graft edema. The endothelial cells on flat preparations were invaded predominantly by plasma cells and lymphocytes. The advancing line of cellular infiltrate appeared to destroy the endothelium as rejection progressed until finally the entire endothelial layer of the graft was completely destroyed.

Ultrastructural studies of the corneal endothelium during corneal allograft rejection have also emphasized the paramount role of the lymphocyte in the destruction and dissolution of donor endothelial cells.\textsuperscript{52} In contrast to Polack's\textsuperscript{51} finding, however, these studies showed that, although inflammatory cells were present in the stroma, none were seen to pass through intact or disrupted Descemet's membrane. They concluded that the effector lymphocytic cells gained access to the donor endothelium via the anterior chamber rather than through gaps in Descemet's membrane, as indicated by Polack's light microscopic studies.

In summary, then, there is general agreement, at least for the endothelium, that
the main vector of transplantation immunity is the lymphocyte, which is capable of causing disruption and slough of endothelial cells into the anterior chamber. It is curious that with regard to this particular corneal layer, there are many analogies to the rejection of skin allografts in terms of the vector which mediates cell death, as well as the slough of the target tissue from its moorings.

**Experimental evidence invoking transplantation immunity in corneal graft reaction.** The experimental evidence linking transplantation-related immune rejection in corneal grafts is substantial. Since the experimental work in this area is prolific and has been adequately cited in previous literature, I shall attempt to expedite discussion of these experimental findings by following the criteria established by transplantation immunologists. After noting each criterion, I shall review the pertinent work as applied to the cornea, and comment where appropriate.

(1) In general, autografts are successful, while allografts and xenografts uniformly fail. This generalization is not applicable to the cornea. As discussed under “Unique problems inherent in corneal transplantation” undesirable stimuli must be introduced in order to obtain a reproducible rejection model in which to evaluate transplantation immunity. There are numerous well-documented instances of high success rates in unmodified recipients of corneal allo- and xenografts with either lamellar, interlamellar, or penetrating techniques. However, if vascularity invades the donor graft, it will almost always fail in the early posttransplant period.

Perhaps we have been so obsessed with the study of transplantation immunity that the majority of our experimental efforts have been misdirected. Our major effort has usually been to design a model in which all variables capable of producing graft rejection are excluded except for transplantation immunity. A recent comprehensive study of the natural history of the corneal graft problem was designed not merely to study this one variable, but to analyze multiple variables. This multiple variable analysis of experimental penetrating allograft failure in the rabbit does show a significantly better success rate for control autografts than for experimental allografts.

Moore and Aronson, when comparing the “takes” of allo- and autografts found a disparity in results of 12 to 13 per cent, implicating transplantation immunity as a cause for graft failures. This demonstration, in avascular recipient corneas where all animals bearing an allograft also had a control autograft, has far-flung portents and broad implications, and offers strong evidence for invoking transplantation immunity as one of the factors in corneal graft rejection. Apparently, no one has specifically obtained comparative data on the success rate of penetrating auto- and allografts in animals with vascularized corneas, which would be valuable confirmatory evidence for the role of transplantation immunity in corneal graft rejection.

(2) Allo- and xenografts demonstrate an initial period of grace or “latent period” during which they flourish and remain clear (induction period). This is fairly well accepted even in corneas vascularized prior to experimental transplantation.

(3) The “second-set” phenomenon in which a second graft from the same donor is rejected more rapidly and violently than the initial graft. This has not been truly validated as precisely as desirable, although it has been shown in an indirect fashion by many workers. Billingham and Boswell showed a shorter survival period for second- and third-set corneal epithelial homografts. However, the corneal epithelial homografts in their experiments were transplanted heterotopically to recipient areas on the skin. The studies of Maumenee with subcutaneous implantation of skin also are not a convincing demonstration of the true meaning of the second-set phenomenon, i.e., corneal graft followed by a corneal graft. Leibowitz and Luzzio have shown that presensitization
with a soluble "tissue antigen" extracted from the cornea caused an accelerated and enhanced rejection in "a second-set" fashion to an interlamellar chick to rabbit xenograft.

A similar previous study by Robert and colleagues indicated that presensitization of the recipient with a structural glycoprotein isolated from rabbit and calf cornea produced an accelerated rejection of interlamellar corneal grafts in xenografting, but not in allografting. In each of the above two studies the accelerated "rejection" of the graft may have been initiated by serum proteins, species-specific type antigens, or "true" transplantation antigen. Therefore, a convincing demonstration of the "second-set" phenomenon in the classical sense has not been satisfactorily demonstrated.

(4) Specificity of the graft rejection reaction, i.e., sensitization to an initial graft, is specific for the tissue from the original donor. A second indifferent graft from a donor unrelated to the first will proceed at a normal tempo. A convincing demonstration of this postulate has not been done in an orthotopic circumstance with use of only corneal tissue. The primary reason for this is that undesirable manipulations or "tricks" are necessary to obtain reproducible rejection in experimental corneal allografts.

(5) The systemic nature of transplantation immunity. "Second-set" solid tissue grafts are rejected in an accelerated fashion regardless of the region of the body to which they are transplanted. This has been demonstrated by many workers, most notably byBillingham and Boswell.

(6) The capacity to transfer transplantation immunity between inbred strains by means of sensitized lymphoid cells. This has not been demonstrated as the rabbit is the standard animal used as recipient for experimental corneal transplantation. Development of highly inbred (isogeneic) rabbit strains has not proved feasible.

(7) Induction of actively acquired immunological tolerance in the host for the transplanted tissue. Aguilar produced allograft chimeras in rabbits and cats by the transplantation of skin in the early postnatal period. Six months after successful skin allografts, he performed penetrating corneal allografts, keeping the donor-recipient combinations identical to those used for skin transplantation. The penetrating corneal allografts were successful and remained transparent even though they were placed eccentrically at the limbus or neovascularization was induced. However, in this study, the investigator failed to demonstrate the specificity of the tolerant state, i.e., that a corneal allograft from an indifferent donor would be rejected by the recipient. Nevertheless, this is a fairly convincing demonstration of actively acquired tolerance as related to corneal grafting.

(8) Abolition of tolerance to tissue allografts by injection of the tolerant animals with lymphoid cells from normal adult members of the same strain. This piece of evidence has not been fulfilled for corneal graft rejection, for the same reason stated in point No. 6 above.

(9) Abrogation of transplantation immunity by immunosuppressive agents. Myriad reports have appeared demonstrating the efficacy of many suppressive agents in abrogating the corneal graft rejection reaction. This includes physical, chemotherapeutic, and biologic agents: 6-mercaptopurine (6-MP), azathioprine, corticosteroids, X-irradiation; antilymphocyte serum and antilymphocyte globulin; chloramphenicol; promethazine (Phenergan). However, this cannot be cited as strong or conclusive evidence for or against transplantation immunity as a factor in corneal graft rejection, as all these agents have nonspecific anti-inflammatory properties.

Clinical evidence implicating transplantation immunity in corneal graft rejection.

The inflammatory reaction is confined primarily to the corneal graft. This observation is often alluded to in support of transplantation immunity as a mechanism in corneal allograft rejection. It is true that under favorable circumstances, it can be
seen that the keratitic deposits accumulate primarily on the endothelial surface of the graft and not on the posterior surface of the recipient cornea. However, the inflammatory reaction associated with moderate to severe graft rejection involves the entire anterior segment and frequently the adnexa. Furthermore, in cases of anterior uveitis the same observation can be made, i.e., the keratitic deposits accumulate primarily on the central 7.5 to 8.0 mm. of the cornea, the analogous area of the majority of penetrating allografts. Therefore, this argument as such is fallacious, and cannot be used as clinical evidence either for or against invoking transplantation immunity in the rejection of corneal grafts.

In general, autografts are successful whereas allografts and xenografts uniformly fail. One of the problems in comparing clinical results of penetrating autografts to those of allografts is the failure of authors to clarify whether their reports include all cases of autografts or only successful cases. Albeit, there were a total of 36 cases, of which 32, or 89 per cent, were successful (Table V). These composite autograft results are no better than results of penetrating allografts performed in the most favorable (avascular) prognostic categories (see Table VI). The comparison of these two groups is valid for our purposes in this section, since presence or absence of recipient corneal vascularization in autograft cases is not germane, when the role of transplantation immunity in corneal graft failures is appraised. This evidence does not exclude transplantation immunity as a factor in corneal graft rejection, nor, conversely, does it lend any substantive supporting evidence.

**Table V. Composite results of penetrating corneal autografts**

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Success (%)</th>
<th>Failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penetrating autografts:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 contralaterals; 8 ipsilateral</td>
<td>36</td>
<td>32</td>
</tr>
</tbody>
</table>

**Table VI. Results of penetrating keratoplasty**

<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of cases</th>
<th>Success (% clear)</th>
<th>Follow-up (mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In avascular recipients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hughes, W. F.*</td>
<td>43</td>
<td>81</td>
<td>21 (mean)</td>
</tr>
<tr>
<td>Buxton, J. N., et al.*</td>
<td>36</td>
<td>94.5</td>
<td>22.7 (mean)</td>
</tr>
<tr>
<td>Moore, T. E., Jr., and Aronson, S. B.*</td>
<td>25</td>
<td>96</td>
<td>&gt;12</td>
</tr>
<tr>
<td>In scarred, vascularized recipients</td>
<td>93</td>
<td>62</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Moore, T. E., Jr., and Aronson, S. B.*</td>
<td>93</td>
<td>62</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Owens, W. C., et al.*†</td>
<td>52</td>
<td>50</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Slight vascularization</td>
<td>71</td>
<td>25.4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Moderate vascularization</td>
<td>64</td>
<td>10.8</td>
<td>&gt;4</td>
</tr>
</tbody>
</table>

*These patients had superficial and deep stromal vascularization with or without signs of anterior segment inflammation (active or inactive), Grade 3 or 4 according to authors' morphologic severity grading.
†Not clearly stated whether these are all penetrating keratoplasties.
Elliott Investigative Ophthalmology
March 1971

Pia is groundless. Therefore, the efficacy of this drug is keratoplasty cannot be used as a supportive argument for the role of transplantation immunity in the rejection of corneal grafts.

Likewise, early clinical trials have shown striking success in many hopeless keratoplasty candidates with the use of adjunctive systemic azathioprine therapy.\textsuperscript{96-98} However, these preliminary results which look promising for azathioprine should be regarded with cautious optimism. Furthermore, just as in the case of corticosteroids, as well as many other reasons,\textsuperscript{99} these examples of penetrating keratoplasty success in patients with a poor prognosis do not support nor exclude transplantation immunity as a vector in opacification of corneal grafts.

Detection of circulating antibodies in the keratoplasty recipient. Numerous studies in animals have shown that recipients of corneal allografts and xenografts develop anticorneal antibodies subsequent to grafting.\textsuperscript{100-102} The same findings have been found in humans after penetrating keratoplasty. Nelken and Nelken\textsuperscript{103} found anticorneal antibodies by use of the sensitive passive tanned cell hemagglutination technique. Fifteen of 33 cases without detectable corneal antibodies prior to surgery developed them during the postoperative course. In general, these antibodies were devoid of individual specificity for they reacted in equal titers with antigens derived from the donor cornea, as well as random human corneas. However, one case which developed "graft sickness" on the 57th day showed some degree of individual specificity of his antibodies. They reacted more strongly with antigens from the donor cornea than random corneas by four tube dilutions. They suggest that lack of individual specific antigens in the human cornea may be another factor contributing to the success of corneal grafts. Another interpretation for the lack of corneal antibodies with individual specificity may be due to its absorption and binding to the graft! Tsutsui and Watanabe\textsuperscript{104} also demonstrated the capacity of humans to form anticorneal antibodies after keratoplasty. They found anticorneal antibodies in eight of 14 cases tested which became positive five to 20 days after surgery and remained positive for one month. These authors tested the antibody only against the donor corneal antigens so that determination of individual specificity was not possible. While these serologic findings are of interest, and they may well reflect the presence and intensity of sensitization of the recipient to some donor tissue antigen, they cannot be used as support for invoking transplantation immunity in corneal graft rejection. These antibodies may develop as a response to an antigen other than a transplantation antigen present in the cornea; and even if they were directed against a transplantation antigen, there would be no authentic proof for pathogenetically implicating them in the rejection of the graft. Furthermore, there was no correlation in either study of presence of anticorneal antibody with graft failure or intensity of rejection. However, the value of these antibodies may be to provide an index or parameter to gauge adequacy of immunosuppressive therapy (e.g., corticosteroids) if a correlation could be shown between antibody titer and intensity of the corneal graft rejection reaction.

Exclusion of all other obvious known causes of corneal graft rejection. As repeatedly emphasized by Maumenee and others,\textsuperscript{5, 105} clinical recognition of a graft rejection reaction due to allograft immunity does not present a typical or distinct picture from many other causes of graft rejection reactions. Moreover, at present, there are no available laboratory parameters which can be used as predictors or indicators of a tissue incompatibility reaction occurring in graft. Thus, clinical diagnosis of an allotransplantation reaction is an empirical process of exclusion whereby the diagnosis is considered if the following criteria are met: (1) The graft must be initially transparent and apparently successful for the first two to three post-
operative weeks, and (2) there are no other obvious attributable causes for failure.

While empiricism of this type must of necessity pervade the clinical area, it should never be transposed or accepted as dogma in our scientific pursuits.

The other criteria used earlier in this review for invoking transplantation immunity in rejection of tissues and organs cannot be applied at the clinical level to the process of corneal graft rejection for obvious reasons.

**Summary of the role of transplantation immunity in corneal graft rejection.** When viewed in the total scope and compass of the field of transplantation biology, the evidence for tissue incompatibility as a dominant cause for the rejection of tissues and organs has certitude and substance based on solid data. The experimental evidence for this type of immunity as a factor in corneal graft rejection has not been validated as well as for other tissues or organs; nevertheless, the evidence is solid. At the clinical level, there is a total absence of evidence that transplantation immunity per se causes the rejection of a penetrating keratoplasty in a human. This should not imply or be misinterpreted to mean that transplantation immunity should be discarded as a clinical cause of graft rejection or that transplantation immunity is not capable of causing rejection of grafts in humans.

**Other plausible immune mechanisms as a basis for corneal graft rejection other than transplantation immunity (adventitious antigen-antibody reaction)**

As alluded to earlier, any inflammatory process occurring in the anterior segment of the eye, if of sufficient duration and severity, will result in graft edema, irreversible damage, and eventual graft opacification.

Polack³ has been able to produce graft clouding and edema in both penetrating allo- and autografts by induction of a sterile inflammatory reaction. After determination that animals had achieved “takes” of their corneal grafts, a primary immunogenic uveitis¹⁰⁶,¹⁰⁷ was produced which caused graft edema for periods of two to four weeks. The clouding of the graft was equally severe in both the auto- and allografts, thus excluding tissue incompatibility as a contributing factor. Because of the self-limited nature of the immunologic stimulus, the grafts were not irreversibly damaged.

Moore and Aronson⁷ have demonstrated a similar phenomenon recently. Animals with clear penetrating allo- and autografts were systemically immunized with BSA. Subsequently, both eyes were challenged by topical application of the antigen to the cul-de-sac. Fifty-seven per cent of the allografts and 67 per cent of the autografts with structural changes in their corneas as a result of previous inflammation developed complete donor opacity on exogenous BSA challenge. This evidence further substantiates the fact that fortuitous antigen-antibody reactions occurring by random chance in an altered host cornea can lead to delayed graft failure. In this circumstance, as in Polack’s, transplantation immunity can be excluded as a contributing factor as both auto- and allografts were equally affected.

**Immunomimetic reactions as plausible mechanisms in corneal graft rejection**

Denature gamma globulin with activation of the complement pathway. In a quantitative comparison of silk and nylon sutures, Aronson and co-workers¹⁰⁸ have shown the capacity of silk suture to preferentially bind substantial quantities of human gamma globulin by the isotopic labeling experiments. Also, they have demonstrated the presence of B,C (representative of complement) on the silk suture by qualitative fluorescent antibody technique. While some binding affinity was also demonstrated for monofilament nylon, it was minimal. This demonstration explains the clinical observation of greater host reactivity to silk suture than to monofilament nylon. The pathogenetic pathway for su-
silk suture + gamma globulin \[\rightarrow\] gamma complex

gamma complex + complement \[\rightarrow\] polymorphonuclear chemotaxis and degranulation

polymorphonuclear degranulation \[\rightarrow\] hydrolytic enzyme release \[\rightarrow\] tissue necrosis

Fig. 3. Pathway of silk suture toxicity. (From Aronson, S. B., McMaster, P. R. B., Moore, T. E., Jr., and Coon, M. A.: The pathogenesis of suture toxicity, Arch. Ophthal. 84: 641, 1970. Reprinted with permission of the authors.)

Suture toxicity with resulting inflammation is undoubtedly a strong factor in corneal graft rejection and is schematically presented in Fig. 2. Experimental data in animals and humans support a strong role for this immunomimetic mechanism in the rejection of corneal grafts.7

Shwartzman reaction. The classical Shwartzman reaction may or may not be the equivalent of the immunomimetic reactions mediated by endotoxin. Recent evidence has incriminated endotoxin as a substrate for the complement pathway.109 Thus, the complement system under certain conditions is activated by endotoxin. This, in turn, leads to release of chemotactic factors which attract polymorphonuclear leukocytes (PMNs). The rupture of PMNs and release of hydrolytic enzymes result in parenchymal damage of the involved tissue. This endotoxin pathway must surely contribute to corneal graft inflammatory reactions in both the early and late postoperative period. The microbial environment (pathogenic and indigenous) is one of the sources for endotoxin release in the external eye. If this endotoxin has access to the corneal stroma through an epithelial defect at the site of a suture tract, it will initiate the inflammatory reaction described above. It has been shown recently that indigenous and pathogenic bacteria do play a role in the rejection of corneal grafts in both human beings and experimental animals.7

Structural alteration syndromes (Auer phenomenon110). Once there has been structural tissue alteration as a consequence of previous inflammatory episodes, there exists a state of permanently altered vascular permeability. This prolonged altered vascular permeability has been well validated in the case of experimental uveal inflammation by Gamble and associates.111, 112 These authors’ data suggest that recurrent immunogenic uveitis is mediated by a selective deposition of preformed circulating antigen-antibody complexes at tissue sites of altered capillary permeability. The presence of these extravascular immune complexes attracts numerous PMNs initiating the acute inflammatory pathway.113 A similar mechanism may be operative in early and delayed graft rejection. For instance, it has been shown in experimental corneal grafts that altered vascular permeability exists in the host and donor cornea in the early postoperative period.114 If the early postoperative inflammation is not suppressed and neovascularization of cornea supervenes (altered host cornea), a state of prolonged increased altered capillary permeability ensues. In such a case, preformed circulating antigen-antibody complexes will localize selectively in the parenchyma with resultant exacerbation of stromal inflammation. If this cycle were of sufficient duration, it could contribute to irreversible clouding of corneal grafts.

REFERENCES


66. Leibowitz, H. M., and Elliott, J. H.: Chemotherapeutic immunosuppression of the corneal graft reaction. II. Combined


Erratum

In the December, 1970, issue of the Journal, in the article by Raymond P. LeBlanc, R. H. Stewart, and Bernard Becker entitled "Corticosteroid provocative testing," line 11 in the right-hand column of page 946 should read: "dexamethasone, 0.1 per cent four times a day to."