The contribution of morphology to our understanding of the pathogenesis of experimentally produced corneal vascularization

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In many disorders of the cornea, blood vessels invade this normally avascular tissue. Several theories have been proposed to explain the pathogenesis of corneal vascularization. Since none had received general acceptance, experimental studies of the phenomenon were begun several years ago in hamster cheek pouch chambers. These investigations which employed sequential morphologic studies during the process of corneal vascularization gave rise to a new hypothesis, namely, that corneal vascularization is usually a manifestation of the inflammatory response and mediated by leukocytes. This report briefly reviews the evolution, evidence, and current status of this theory.

Key words: corneal vascularization, leukocytes, hamster cheek pouch chamber.

Since the beginnings of the scientific investigation of disease, morphologic approaches have formed the backbone of pathology and have played a crucial role in the delineation of diseases and in our understanding of pathologic processes. However, even when numerous human lesions in different stages of evolution are studied by light as well as electron microscopy, critical questions often remain unanswered. Pure morphologic observations on entities commonly do not provide insight into the events that precede the lesions in question. One may liken the problem to the impossibility of trying to reconstruct the story of a full-length movie by examining a restricted number of frames taken toward the end of the film. In conditions where experimental models are available, this difficulty can fortunately be overcome to some extent by studying animals that have been killed at sequential time intervals prior to the onset of the entity under consideration. In this conference which centers around applications of anatomic techniques in experimental ophthalmic pathology, I will emphasize this approach in trying to elucidate the chain of events that precede the invasion of the cornea by blood vessels. In the

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present review I will concentrate on the main issues and particularly on events that antecede corneal vascularization. For the sake of brevity, details about controls, experimental procedures, and less significant observations will not be given, since they are fully discussed elsewhere.

First, I will provide some background. The avascularity of the normal cornea and its vascularization in certain pathologic states has been common knowledge for a long time. Yet it was only after the beginning of World War II that attempts were made by a few investigators to explain these related questions. In 1940 Meyer and Chaffee were impressed by the fact that the cornea and certain other avascular tissues are rich in mucopolysaccharides which were thought to possibly inhibit vascularization. Bachsich and his colleagues later expanded this view and suggested that this inhibitor of vascularization became altered in pathologic states which permitted blood vessels to invade the cornea. In 1949 two different theories emerged. Cogan proposed that the avascularity of the normal cornea resulted from a barrier which the compact lamellae of corneal collagen offer to vascular invasion and that vessels entered the cornea if the lamellae became separated by edema, which consistently accompanies corneal vascularization. Campbell and Michaelson, on the other hand, believed that corneal vascularization was dependent upon one or more diffusible factors, capable of directing capillary growth, which the normal avascular cornea lacks. Other theories have largely centered around variations of the aforementioned themes, and particularly about the nature of inhibitors and promoters of corneal vascularization.

Several years ago I became interested in the problem and decided to test the existing hypotheses experimentally. Initially I decided to implant corneas into hamster cheek pouch chambers, since this experimental technique had provided useful information about the microcirculation of neoplasms and embryonic tissues. A variety of corneal tissues were inserted into cheek pouch chambers of more than 300 hamsters. The transplants included normal corneal autografts, allografts, and xenografts; corneas injured by cautery; corneas denatured by repeated freeze-thawing, boiling, or autoclaving; as well as corneas soaked in hydrocortisone, ethanol, or sodium hydroxide. The implanted specimens were observed at regular intervals through the transparent chamber windows until the animals were killed and the tissues were examined microscopically. Surprisingly the host reaction to the transplanted tissues was independent of the nature of the explants, and so was the chain of events that followed. Polymorphonuclear leukocytes and a variable amount of granulation tissue often surrounded the explants. Concomitantly, there was commonly a slight leukocytic infiltrate in the adjacent viable or nonviable corneal tissue. The degree of cellular infiltration into the cornea varied considerably from experiment to experiment and even within the same cornea. Some corneas had a slight to moderate cellular infiltrate within a few days, whereas others were relatively acellular for almost 2 weeks.

Other significant observations were as follows. Corneas did not necessarily become vascularized when the corneal lamellae were separated in a swollen stroma (casting additional doubt on Cogan's theory of corneal vascularization). Newly formed blood vessels often grew towards and into the corneal explants (in keeping with the concept that specific substances possess the ability to stimulate vascular growth towards the site of maximum concentration). Eventually most (but not all) normal, injured, and nonviable corneal grafts became vascularized. The time at which corneal vascular invasion began after transplantation and the rate at which blood vessels invaded the cornea varied considerably. Both of these parameters appeared to be host dependent, but vascularization was suppressed in corneas that had been soaked previously in hydrocortisone or sodium hydroxide. Prior to vascular invasion, granulation tissue was evident adjacent to the
corneal explants and often beneath them. I was particularly impressed by the observation that corneal vascularization was always associated with a cellular infiltration into the corneal stroma. Moreover, when vascularization involved only part of the cornea, the blood vessels were in the site of the cellular infiltrate. If cells did not penetrate the corneal tissue under investigation, it consistently remained avascular even if in a markedly edematous state. The observation suggested that leukocytes liberated some substance, either directly or indirectly, which induced directional vascular growth into the cornea.

In view of the new concept of corneal vascularization that emerged, several additional projects were undertaken in my laboratory. In this work I joined forces with Fromer, who was then a graduate student at Duke University. At first it was thought worthwhile to compare the events that precede and accompany corneal vascularization in several apparently diverse established experimental models of corneal vascularization. These included exposure of the cornea to silver nitrate (a focal nonprogressive lesion), alkali (a focal progressive lesion), alloxan (a possible metabolic inhibitor), intracorneal antigens in sensitized and nonsensitized animals (an immunologic injury), and the maintenance of rats on a riboflavin-deficient diet (nutritional deficiency). In these models corneas were processed at variable intervals after corneal injury for light microscopic analysis of the sequence of events that preceded and accompanied corneal vascularization. In all instances leukocytes entered the corneas before capillaries.

Even in riboflavin-deficient rats, in which corneal vascularization was delayed until after the animals became severely debilitated, capillaries were identified in the superficial corneal stroma after the leukocytes. Although the various models differed in several respects, the localization, depths of stromal involvement, and direction of the vascular invasion from the limbus paralleled remarkably well the pattern of the leukocytic infiltration which these injuries induced. Focal corneal injury (e.g., silver nitrate cautery) provoked a localized leukocytic and vascular infiltration into the damaged corneal stroma from the closest portion of the limbus. Models that caused a diffuse, circumferential leukocytic infiltration into the cornea (e.g., topical alloxan administration) stimulated a vascular invasion into the periphery of the entire cornea. When leukocytes infiltrated the entire thickness of the corneal stroma (as when antigen was instilled into corneas of sensitized animals), so did the vascular ingrowth. The degree of vascularization was also directly proportional to the leukocytic infiltrate. The injection of antigen into the cornea of sensitized animals, but not nonsensitized animals, provoked a pronounced leukocytic and vascular infiltration into the cornea to a degree which surpassed that of other models.

Moreover the onset of corneal vascularization was also temporally related to the time of the initial leukocytic infiltration. For instance, injection of antigen into sensitized animals produced an early leukocytic infiltration and corneal vascularization; a longer latent period preceded both the leukocytic and vascular invasion in rats on a riboflavin-deficient diet.

In no documented experimental model that has been thoroughly studied by sequential histologic analysis to date has corneal vascularization not been preceded by a leukocytic infiltrate. Others have documented the leukocytic infiltration prior to the onset of vascular invasion in rats on a vitamin A–deficient diet. Since our original report, I have observed the same phenomenon after topical administration of colchicine to the eye and in rats maintained on a high-tyrosine diet. The only models in which corneal vascularization may possibly occur without a leukocytic infiltration are those in which neoplastic cells are instilled into the cornea. Despite the fact that adequate sequential morphologic studies have not yet been documented under these conditions, such models have little bearing on naturally occurring corneal vascularization. Neoplasms of the
cornea are extremely rare, and neoplastic cells themselves possess the capability of stimulating vascular proliferation. Other experiments showed that corneal vascularization does not follow corneal cauterization with silver nitrate in the absence of leukocytes. Weanling rats were made leukopenic by an adequate dosage of total body x irradiation. When the leukopenic effect of irradiation reached its maximum (i.e., when circulating leukocytes were virtually absent), the corneas were cauterized with silver nitrate. Under such conditions neither leukocytes nor blood vessels invaded the corneas. On the other hand, if the corneal cauterization was performed before the circulating leukocytes were totally eliminated by x irradiation, both the leukocytic and vascular invasion occurred. Important control experiments in which the corneas were cauterized after only the heads of rats received a similar dosage of x irradiation ruled out the possibility of irradiation-induced limbal vascular endothelial damage as the explanation for the vascular suppression observed by x-ray treatment.

Other rats were given subconjunctival methylprednisolone acetate immediately after, or 24 hours after, corneal injury with silver nitrate. This corticosteroid inhibited the infiltration of leukocytes and the subsequent vascular invasion of the corneal stroma, if administered immediately after cauterization. On the other hand, it did not prevent the invasion of the cornea by blood vessels if it was instilled 1 day after cauterization, at which time leukocytes had already infiltrated the cornea. However, under such circumstances the corneal leukocytic and vascular ingrowth was less severe than in the nonglucocorticoid-treated corneas.

An extension of these studies provided additional support for the contributory role of leukocytes in corneal vascularization. When polymorphonuclear leukocytes or a heat-labile fraction isolated from them was injected into corneas of rats that had had their circulating leukocytes eliminated by total body x irradiation, blood vessels invaded the corneas. Lymphocytes isolated from thymus, spleen, and lymph nodes of normal rats did not have this vascular effect.

In summary, several lines of evidence support the hypothesis that corneal vascularization is a manifestation of the inflammatory response and that leukocytes play a crucial role in stimulating vascular invasion of the cornea. Several investigators have alluded to the presence of one or more diffusible factors capable of initiating directional capillary growth which the normal avascular cornea lacks. Our studies not only support this view but in addition suggest a leukocytic origin of a chemical mediator in corneal vascularization. It remains to be determined whether the cornea is a special site with susceptibility to factors from the polymorphonuclear leukocyte that possess angiogenic activity or whether the phenomenon is more generalized.

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Corneal radiofrequency burns: effects of prostaglandins and 48/80

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Vascularization of the rat cornea was produced by means of radiofrequency burns adjacent to the limbus. Animals with and without lesions were tested for a vascular response to topical PGE, and compound 48/80, with carbon used as an indicator. There was no response to PGE, until 6 hours, at which time dense labeling of limbal vessels was observed. This response gradually decreased and by day 15, when labeling returned to control levels. No further permeability changes were seen for the duration of the experiment to day 90. Compound 48/80 gave four-plus labeling of the limbus and one-plus labeling of corneal vessels at all times during the experiment. The presence or absence of a thermal lesion did not change the degree of labeling. Histamine and bradykinin did not produce vascular labeling either with or without a corneal lesion.

**Key words:** cornea, vascularization, prostaglandins, histamine.

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