Vascular Casts of Experimental Subretinal Neovascularization in Monkeys

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The origin, course, and pattern of experimental subretinal neovascularization (SRN) in monkeys were studied with scanning electron microscopy (SEM) of Mercox preparations of the choroidal vascular bed. This technique allowed visualization of the entire circulation of the SRN lesion from both the retinal and scleral aspects. The SEM data is comparable to that of fluorescein angiography, but provides details not detectable by angiography. The afferent arteriole was traced back to the posterior ciliary artery of origin. In the early stages, the efferent vessels appeared to connect with the choriocapillaris; whereas later the efferent vessels connected directly to the choroidal venous system. The study of such plastic casts enables more accurate assessment of some aspects of the vascular architecture of the SRN frond. Invest Ophthalmol Vis Sci 24:481-490, 1983

Subretinal neovascularization (SRN) is a pathologic process common to many disciform macular diseases.1 The ultrastructure of the abnormal blood vessels found in experimental SRN has been described by us,2 although we found it difficult to evaluate the branching patterns and the relationship of the new vessels to the choroidal vessels because of their extension in three dimensions. To resolve this problem, we used a technique of plastic casting of the choroidal vasculature that permitted clear demonstration of the ocular vessels.3-7 This technique was found to be particularly helpful in the evaluation of experimental SRN.8 In the present study, the branching pattern and distribution of neovascular vessels during lesion growth and regression were studied by scanning electron microscopy (SEM) of the vascular casts.

Materials and Methods

Ten rhesus monkeys of both sexes were used in this study. The method used to produce SRN was the laser photocoagulation technique previously described.9,10 All the animals were examined periodically with fluorescein angiography, and the lesions divided into active and regressive stages on the basis of permeability to fluorescein. Animals examined at 5, 8, 9, 10, 12, and 27 weeks after photocoagulation had active leaking vessels seen on fluorescein angiography. One animal did not develop SRN, and a cast preparation was performed 11 weeks after photocoagulation. Animals with previously active SRN, which had undergone involution, were studied by cast preparation 81, 82, and 158 weeks after laser photocoagulation.

From 5 to 158 weeks after photocoagulation, the animals were killed under sodium pentobarbital (30-70 mg) anesthesia. Both common carotid arteries were cannulated with a 16-gauge plastic cannula. The jugular veins were transected and perfusion was undertaken with 1500 cc physiologic saline and sodium heparin (10iu/cc) at a constant pressure of 140 mm/Hg. After the complete replacement of blood, a mixture of 60 cc methyl-methacrylate monomer (Merox) and 1% accelerator was perfused until the resin appeared at the jugular veins. The vessels in the neck were then clamped until the resin hardened, which usually took from 10 to 20 min. The eyes were enucleated and kept intact in 15% KOH at 37-40 C for 3 weeks with daily changes of the KOH. After this time, the cast was rinsed, air dried, and the entire posterior pole isolated under a dissecting microscope. The cast was mounted onto an SEM stub and coated with 30 nm of gold-palladium. The cast was then examined with a JEOL SEM 35 scanning electron microscope at an accelerating voltage of 10 or 15 KV (inclined angle 0°, 10°, or 15°, working distance 39 mm). Following examination from the choroidal side, the cast was remounted, coated with an additional
Fig. 1. Fluorescein angiogram 1½ weeks after onset of neovascularization (Monkey M5). Left, taken during early phase of angiography, site nos. 1, 2, 3, and 4 were studied with the scanning electron microscope. Right, late venous phase; leakage of dye is evident.

25 nm of gold-palladium, and examined from the retinal side.

Results

Active Leaky Stage

The eyes were examined 8 weeks after laser photocoagulation (this was usually 4 to 5 weeks after the fluorescein angiogram suggested the presence of SRN). Neovascular tufts were found at the site of photocoagulation that showed leakage on the fluorescein angiogram. Site nos. 1, 2, 3, and 4 (Fig. 1) were studied. The cast of the specimen obtained from site no. 1 showed a well-developed, branching neovascular network (Fig. 2A); these new vessels originated from the choroidal vasculature and extended into the subretinal space. The afferent vessel (Fig. 2A, arrow A) was derived from the choroidal artery, which showed many imprints of endothelial nuclei and fine grooves. The efferent vessel (Fig. 2A, arrow E), collecting numerous venules, showed no obvious nuclear imprints on the surface of its replica; neither were nuclear imprints obvious at the tip of the SRN capillary, probably due to the absence of smooth muscle in the vessel walls. One plastic globule could be seen at the tip of the SRN frond (Fig. 2B, arrow), which may be due to the escape of resin.

Fluorescein angiogram of site no. 2 showed a small site of leakage at the edge of the photocoagulation site (Fig. 1). The cast specimen of this lesion (Fig. 3) demonstrated a small neovascular network; the vessels feeding and draining this network could be visualized in the area where fluorescein leaked and pooled. At the base of the neovascular network, one of the two main vessels connected to the choriocapillaris (Fig. 3, arrow E). From the scleral side, another main vessel, presumably the afferent vessel, connected to the arterial branch of the choroidal artery (Fig. 4A) and could be traced back to one of the short posterior ciliary arteries (Fig. 4B). Thus, the pattern of circulation at this site was from the posterior ciliary artery to the frond, then draining into efferent venules to the choriocapillaris.

Although no other sites showed marked leakage on fluorescein angiography, the cast preparation of site no. 3 demonstrated a tiny, delicate neovascular tuft at the edge of the lasered site (Fig. 5). These new vessels were approximately equidistant from the arteriolar and choriocapillaris components. In addition, in site no. 4 some slender sprouts from the normal choriocapillaris were seen at the edge of the burn site (Fig. 6). These sprouts are closely associated with the venous side of the circulation.

Non-leaky Regressive Stage

Eighty-two weeks after photocoagulation, SRN (which had previously leaked fluorescein profusely) now failed to leak and pool fluorescein, suggesting the lesion to be in regression (Fig. 7). The corresponding vascular cast showed a tuft with a glomerular appearance, consisting of tightly packed vessels that grew over the SRN site and connected with another SRN lesion (Fig. 8A). Although its connection to the choroidal vasculature was masked by the packed network of the capillaries in the right SRN site, the afferent (Fig. 8B, arrow A) and efferent (Fig. 8B, arrow E) vessels can be identified in the left SRN site. It became obvious from the stereo pair pictures of the left SRN site, disregarding the right SRN frond communicating channel, that the circulation of this SRN
Fig. 2. A, Cast study performed 8 weeks after laser photocoagulation (Monkey M5). Retinal view of site no. 1. The defect of methylmethacrylate cast of the choriocapillaris (c) was the laser burn site, and SRN vessels were prominent around the defect. The afferent vessel (arrow A) obviously originated from the choroidal arterial branch, which showed numerous imprints on endothelial nuclei, and the efferent vessel (arrow E), which showed no obvious surface nuclear imprints, also could be observed (X120). B, Higher magnification of the inferior SRN tips shown in Figure 2A. Note the plastic globule (arrow) at the tip of the SRN frond (X236).
Fig. 3. Retinal views of site no. 2 (Monkey M5). The afferent vessel (arrow A) terminated in the neovascular frond. The frond then drained via the efferent vessel (arrow E) as it coursed to the choriocapillaris (x180).

Fig. 4. A, Scleral view of site no. 2 (Monkey M5). The afferent vessel (arrow A) connecting the branch of the choroidal artery (arrow Art) coursed to the rim of the burn site (x146). B, Low magnification view of Figure 4A. The boxed area corresponds to site no. 2. Surrounding the optic nerve (N) note one of the short posterior ciliary arteries (SPCA), after many branches, ultimately supplies the afferent vessel (arrows) to the neovascular frond (x20).
Fig. 5. Retinal view of site no. 3 (Monkey M5). The simple SRN frond appears in the lasered region. Arrows A and E show the afferent and efferent vessels respectively (X160).

was arterial through the frond and drained into venules without going through the choriocapillaris.

In another example, fluorescein angiography showed that the SRN had undergone regression (Fig. 9) 156 weeks after photocoagulation. Although these vessels were not apparent on the fluorescein angiogram, the vascular cast exhibited a persistent, although delicate, vascular loop. In the upper site, the afferent vessel (Fig. 10A, and arrow A) can be identified, but the efferent vessel (Fig. 10A, arrow E) cannot be traced to its base. It may have broken during the cast processing or not injected well with the resin, therefore being invisible. In the bottom site, the afferent (Fig. 10B, arrow A) and efferent (Fig. 10B, arrow E) vessels can be observed and traced at the base of the vascular frond. It is probable that the small luminal size resulted from decreased blood flow through the regressed vessels.

Discussion

The subretinal neovascularization and the determination of the disciform response has been the subject of some clinical pathological correlation as noted by Gass, Sarks, Small, Green, Alpar, and Drewry, and Green and Key. The material available for study is usually a result of a postmortem examination. It is recognized that subretinal neovascularization and the disciform process may run its course over many years as in senile macular degeneration with repeated exudation and serous detachment, hemorrhage, and cicatrization. Thus, subretinal neovascularization is a dynamic process with evolution and change of appearance of the subretinal vessels. The initial leaking phase and the regressed or nonleaking phase may be the result of maturation or wound healing response or may be related to the retinal compartment and extracellular control of tight vessel junctions. This is in contrast to the marked porosity of the choroidal compartment from which these vessels are initially derived. The current experimental study was designed in part to study the evolution of this process over time and to determine the three-dimensional array and appearance of subretinal neovascularization.
Our morphologic studies have included ultrastructure and horseradish peroxidase tracer analysis. One of the three-dimensional aspects of the extent and nature of the subretinal neovascularization. The pathogenesis of this problem is the ignorance of the three-dimensional aspects of the extent and nature of the subretinal neovascularization. The te-

Fig. 6. Retinal view of site no. 4 (Monkey M5). Small arrows indicate slender sprouts from the choriocapillaris. Note the close association to the venule (large arrow) (×180).

Fig. 7. Left, fluorescein angiogram 19 weeks after onset of neovascularization. Right, same fundus 66 weeks later shows that this no longer has active leakage and pooling of fluorescein.
Fig. 8. A. Cast study performed 82 weeks after laser photocoagulation (Monkey M467). Cast preparation of SRN demonstrated in Figure 7 (arrows). Now, 82 weeks after photocoagulation. Two glomerular configurations of new vessels overlying the choriocapillaris plane (c) (×45). B. Higher magnification of the boxed area in Figure 8A. Note the afferent (arrow A) vessels supplying the frond and the efferent (arrow E) vessels draining the frond and bypassing the choriocapillaris (×160).
dious techniques of serial reconstruction with ultra-
structural correlation prevented a thorough study of
the distribution and characteristics of these vessels
over time. The application of the perfusion cast tech-
nique allows an excellent understanding of the char-
acteristics and connections of these blood vessels.

As previously described, during the early stage of
this study, we faced the problem of defective casting.
However, we found that resin injections without per-
fusion of a fixative provided an improved filling of
the SRN vessels. After that, resin injection was per-
fomed on deeply anesthetized animals that had been
well perfused with saline.

The plastic cast replicas help to bridge the gap be-
tween histologic and fluorescein angiographic obser-
vations of SRN. The results of these various types of
studies are basically similar and show that the newly
formed vessels course from the lasered areas in the
form of a neovascular frond. These plastic casts
demonstrably demonstrate the afferent and efferent vessels.
A number of possibilities of the origin of SRN can
be considered. In the earliest stage, a choriocapillaris-
to-choriocapillaris shunt, an arteriole-to-choriocap-
illaris shunt, or a choriocapillaris-to-venule shunt
could exist. A fourth possibility is that there could be
SRN vessels directly linking an arteriole and venule
without involvement of the choriocapillaris. Some
early specimens demonstrated slender blind-ended
vessels at the edge of the lesion that originated from
the choriocapillaris. Clark and Clark found neo-
vascularization to occur at the capillary level, while
Michaelson, Wise, and Ashton observed neo-
vascularization to occur closer to the venule side. We
observed the slender vessels from the venous side of
the choriocapillaris more frequently than from the arterial side.

We can speculate that the slender vessels represent
the onset of neovascularization. We also suggest that
the initiating event in the development of SRN may
be the occurrence of capillary buds around the lasered
lesion on the venular side of the choriocapillaris; sub-
sequently, arteriolar connections may form. As a
matter of course, this process consists of reversal of
flow through the parent choriocapillaris via the new
arterial connection. Once the arteriole-choriocapil-
laris-venule unit is established, the SRN frond may
gradually increase in size and the arteriole-capillary-
venule may gain access to the subretinal space as the
SRN progresses to bypass the choriocapillaris system.

It is noteworthy that although few vessels are ap-
parent by fluorescein angiography, cast preparations
better demonstrate such vessels. In fact in the late
stages (Fig. 7, 8), extensive vessels, even in a glo-
merulus formation, may be apparent on cast prepa-

Fig. 9. Left, fluorescein angiogram taken 2 weeks after the onset of neovascularization. Right, 94 weeks later of the same fundus with
no leakage. Arrows indicate the sites observed under scanning electron microscope (see Figs. 10A, B).
Fig. 10. A, Cast study performed 158 weeks after laser photocoagulation (Monkey M248A). Retinal view of the upper site. The caliber of SRN vessels is less than that of the surrounding choriocapillaris. The afferent vessel (arrow A) arises from the choroidal artery. However, the efferent vessel (arrow B) cannot be traced to its base (×100). B, In the bottom site, the afferent vessel (arrow A) can be traced to the choroidal arterial branch (arrow Art). The efferent vessel (arrow E) can also be traced to the choroidal vein (×100).

roration despite the absence of their appearance on fluorescein angiography. Even in the more late stage, the vessels also exist as thin, delicate, presumably atrophic loops (Figs. 10A,B). As expected, the plastic cast technique is limited in that the injection pressure is not consistent throughout the smaller vessels. It is possible that some distension of the vessels may lead to opening of endothelial junctions or breakdown of endothelial walls as seen in Figure 2B. In spite of good perfusion of the resin and thicker coating of gold-palladium, we often encountered a problem of charging during scanning electron microscopy. It is probably due to the inherent poor connection of SRN frond to choroidal circulation. Moreover, some vessels, particularly closed-end vascular sprouts, are not consistently demonstrated by the injection of resin. Also, solid cords of SRN vessels and morphologic relationships of SRN vessels to surrounding tissues cannot be examined. Accordingly, more data is needed to explain the mechanism of the neovascu-
larization. As already demonstrated by cast preparation, the developing SRN presents a characteristic pattern. The pattern of the early immature capillary network differs from that at a later stage; clearly the growing vessels undergo gross modification during maturation. The possible role of extracellular control on the characteristics such as permeability of these vessels derived from the choroidal circulation must remain a subject for further study. We can speculate that there may be a difference between the retinal and choroidal compartments in terms of cellular or extracellular control. We note also that absence of fluorescein leakage in the regressive lesions results from actual decrease of leakage rather than from complete disappearance of the abnormal vessels. These results may help to explain some aspects of the dynamic process of subretinal neovascularization.

Key words: experimental subretinal neovascularization, rhesus monkey, methyl-methacrylate, vascular cast, scanning electron microscopy

References