The early stages (1 day to 3 weeks) in the development of laser-induced choroidal subretinal neovascularization were studied in the monkey eye. Histopathology revealed that the intense laser beam disrupted the choroid/Bruch's membrane/retinal pigment epithelium (RPE) complex and initiated a repair process. Although all lesions received the same energy density, the initial choroidal wound varied among the lesions: in some, the necrotic choroid was surrounded by hemorrhagic retinal detachment with RPE denudation; in others, the necrotic choroid was surrounded only by minimal damage to the RPE monolayer. Formation of the choroidal wound was followed by an inflammatory response. Later, newly formed choroidal tissue filled the wound and continued to proliferate towards the subretinal space. RPE cells from the edges of the wound proliferated over the newly formed subretinal tissue and closed the wound. In lesions with a large area of damaged RPE, coverage of the wound was slow; fluid accumulated in the subretinal space, and the lesions demonstrated pooling of fluorescein on angiography (leaky lesions). In lesions with minimal damage to the RPE monolayer, closure of the wound was rapid, and the proliferating choroidal tissue did not reach the subretinal space. There was no subretinal fluid accumulation and no pooling of fluorescein on angiography (nonleaky lesions). Our results indicate that both the amount of damage of the choroid/Bruch's membrane/RPE complex and the ability of RPE cells around the damaged area to proliferate and restore the continuity of the RPE layer determine the evolution of newly formed choroidal fibrovascular tissue into a subretinal membrane with or without pooling. Invest Ophthalmol Vis Sci 31:899-908, 1990

Subretinal neovascularization (SRN) is the final common pathway of several forms of macular degeneration. The natural history and pathogenesis of SRN are difficult to study in humans, but can be investigated in an animal model.

SRN can be induced in the primate eye by intense argon laser photocoagulation. The experimental neovascularization closely correlates with the development of subretinal vessels in humans after therapeutic laser photocoagulation, and also has many features in common with other diseases characterized by the disciform response—ie, it may lead to serous detachment, spontaneous hemorrhage, and cicatrization. Therefore, we used this primate model to study the early stages in the development of laser-induced SRN, in an attempt to understand the pathologic events leading to the various manifestations of the disciform response.

Materials and Methods

Choroidal SRN was induced by intense laser photocoagulation in 13 cynomolgus monkeys of both sexes, maintained and treated in accordance with the ARVO Resolution on the Use of Animals in Research. Eight high-intensity laser burns were applied at and around the macula of each monkey as previously described. The lesions were monitored 1, 3, 7, 10, 14, and 21 days after laser photocoagulation by fundus photography and fluorescein angiography. The eyes were enucleated at the above time points, opened, and fixed overnight by immersion in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M
phosphate buffer (pH 7.4). The eight laser lesions in each monkey eye were separately resected as previously described, dehydrated in graded alcohols, and embedded in glycol methacrylate (Polysciences, Warrington, PA). The lesions were serially cross-sectioned (3-μm sections), stained by periodic acid-Schiff, and viewed by a light microscope.

Serial Reconstruction

An orthogonal reconstruction of the laser lesions was prepared from camera lucida drawings of the serial cross sections; drawings were made every 50 μm. Three features of the lesion were noted on the drawings: the break in Bruch's membrane, the extravascular fluid (hemorrhage), and the changes or degeneration of the retinal pigment epithelium (RPE). On each drawing, a line was drawn parallel to Bruch's membrane; this line was thickened in a different color beneath the areas where each feature was observed. We obtained a representation of the lesion in orthogonal projection by constructing an array of the lines from all the sections, suitably spaced to represent the final magnification.

Results

The angiographic pictures and histologic findings of the laser lesions are described at various time points throughout the early stages of development of the subretinal neovascular membrane.

One Hour and 1 Day after Laser Photocoagulation

Fluorescein angiography and histopathology of the laser lesions 1 hr postlaser were similar to those of 1 day postlaser. Therefore, these lesions will be described together. Nineteen lesions from three monkeys were studied at these time points: six lesions 1 hr postlaser and 13 lesions 1 day postlaser.

![Fig. 1. Fundus photograph of a monkey eye immediately after intense laser photocoagulation for induction of SRN. A halo of hypopigmentation (arrow) surrounds each laser spot.](Fig. 1)

![Fig. 2. Late-phase fluorescein angiogram of intense laser lesions in a monkey eye 1 day after photocoagulation. Each of the eight laser lesions demonstrates pooling of dye of similar spot size.](Fig. 2)
Fig. 3. Light micrographs of intense laser lesions in a monkey eye 1 day after photocoagulation. The center of both lesions (bent black arrows) shows total disruption of the choroid (C), Bruch’s membrane (short white arrows), and its overlying RPE. (a) Representative hemorrhagic lesion. The damage to the retina (R) in the center of the lesion extends to the internal limiting membrane (arrowhead). The center of the lesion is surrounded by hemorrhagic detachment of the retina (bent white arrows) and RPE degeneration or denudation (short black arrows). (b) Representative nonhemorrhagic lesion. In the center of the lesion the outer retina is coagulated (asterisk), with minimal damage to the inner nuclear layer (INL). The center of the lesion is surrounded by minimally damaged RPE (curved white arrows). R, retina (periodic acid-Schiff, ×160).

ious lesions revealed that in 8 of 19 (42%) lesions, the hemorrhage of the necrotic choroid extended beyond the center of the lesion, resulting in various degrees of retinal hemorrhagic detachment, and total denudation of the RPE in the detached areas (Figs. 3a, 4a). In 11 of 19 (58%) lesions, the center of the lesion was surrounded only by a relatively small area of damaged RPE (Figs. 3b and 4b).

Three Days after Laser Photocoagulation

At this time point, 12 lesions from three monkeys were investigated by both fluorescein angiography and light microscopy. On angiography, 4 of these 12 lesions (33%) demonstrated pooling of dye. Eight of the 12 lesions (66%) demonstrated staining without pooling of dye (Fig. 5). Histopathology revealed that the center of all 12 lesions (where the choroid had been previously disrupted [Fig. 3]) was at this time filled by newly formed fibrovascular tissue infiltrated by macrophages (Fig. 6a); a few PMNs also were seen. The periphery of the laser burns, however, varied among the lesions. In the lesions that demonstrated pooling of fluorescein, various degrees of hemorrhagic retinal detachment were present around the break in Bruch’s membrane (Fig. 6a). The RPE at the detached areas was denuded, and the fluid-filled spaces between the choroid and the detached retina were infiltrated with macrophages (Fig. 6b). In the lesions that were only stained on angiography, the center of the lesion (ie, the break in Bruch’s membrane) was surrounded by degenerating or proliferating RPE cells (Fig. 6c).

Seven to 10 Days after Laser Photocoagulation

At this time, 18 lesions from three monkeys were studied by both fluorescein angiography and histology. On angiography, all lesions demonstrated staining but no pooling of dye (Fig. 7). Histopathology revealed in 8 of the 18 (45%) lesions various degrees of choroidal neovascular tissue invasion into the subretinal space around the break in Bruch’s membrane (Fig. 8a). In 10 of 18 (55%) lesions, the choroidal neovascular membrane was localized around the break in Bruch’s membrane (Fig. 8b). At this time, almost no fluid was present between the newly formed subretinal membrane and its overlying retina.
Hemorrhagic detachment
1d
Fluid & RPE Degeneration
42%

Fig. 4. Orthogonal serial reconstructions of representative intense laser lesions in a monkey eye 1 day (1d) after photocoagulation. The orthogonal projection of the various areas comprising the lesions was reconstructed from camera lucida drawings of serial cross sections of the lesions. Each line (arrowheads) of the reconstructed lesions represents one camera lucida drawing. Drawings were made every 50 μm. (a) Reconstruction of the hemorrhagic lesion shown in Figure 3a. The extravascular fluid (vertical lines) extends beyond the necrotic choroid (inner circle) around the break in Bruch’s membrane (BM) and coincides with the area of RPE degeneration (horizontal lines). Squares denote area of both extravascular fluid (causing retinal hemorrhagic detachment) and RPE degeneration. (b) Reconstruction of the lesion shown in Figure 3b. The extravascular fluid (vertical lines) is restricted to the necrotic choroid, which is outlined by the break in Bruch’s membrane (thick circle). Note that the size of the break in Bruch’s membrane is similar in both lesions (300-μm diameter), whereas the area of RPE degeneration around the break in Bruch’s membrane in the hemorrhagic lesion (squares) is larger than the area with RPE changes in the nonhemorrhagic lesion (horizontal lines).

All lesions showed proliferating RPE cells at the edges of the newly formed fibrovascular tissue (Fig. 8c). The fibrovascular tissue itself was infiltrated by macrophages.

Two to Three Weeks Following Laser Photocoagulation

At this time, 20 lesions from three monkeys were investigated both angiographically and histologically. On angiography, 9 of the 20 (45%) lesions demonstrated pooling of fluorescein, whereas 11 of the 20 (55%) demonstrated only staining of the scar (Fig. 9). Histology of the lesions that demonstrated pooling of dye revealed the presence of newly formed fibrovascular tissue in the subretinal space around the break in Bruch’s membrane. The subretinal neovascular membrane was separated from the detached overlying retina by fluid. Multilayered plaques of RPE cells were found at the edges of the neovascular membrane (Fig. 10a); a monolayer of RPE cells could be observed in some regions between the subretinal neovascular membrane and its overlying fluid. In the lesions that demonstrated only staining of the scar, the RPE barrier was almost restored, enveloping the neovascular membrane that was concentrated around the center of the lesion. In these lesions, no fluid separated the neovascular membrane from its overlying retina (Fig. 10b).

Discussion

The natural history of choroidal SRN in humans involves three stages of development: the early stage, in which the newly formed vessels are undetectable; the disciform stage, in which fluorescein angiography demonstrates pooling of dye in the subretinal space; and the reparative (involutional) stage, in which the pooling of dye diminishes gradually until only staining of the scar is observed.45
Disciform lesions containing subretinal vessels may develop in a variety of ocular diseases. The differences between the various pathologies with SRN lie in the timing of the neovascular membrane development. Whereas in some cases the whole process may take years (eg, in patients with age-related macular degeneration), in others it may take months (eg, in myopic patients). Moreover, within the same disease, there are large variations in the duration of the whole process from the predisciform stage to the cicatricial stage.

Laser-induced SRN in the monkey model also goes through three stages of development. Immediately after photocoagulation, the lesions demonstrate on angiography pooling of dye. The pooling disappears within days, and then for 2–3 weeks, fluorescein angiography demonstrates staining of the lesions without pooling of the dye. This is the first stage of the neovas-
cular membrane development. Then the lesions begin to pool dye in the subretinal space. The pooling persists for some weeks. This period is the second stage of the neovascular membrane development. At the third stage, the pooling diminishes gradually, until only staining is seen on the angiogram. As in humans, the timing of the neovascular membrane development from its induction by the laser light to its total involution varies greatly among the lesions (from 2 to 57 weeks). Furthermore, approximately 60% of the lesions contain subretinal vessels, but never pool dye in the subretinal space. In some ways, these lesions simulate occult SRN in humans.

The results presented in this study suggest that the first stage of the subretinal membrane development in the monkey model is an inflammatory response, which initiates a growth phase. In this phase, choroidal fibrovascular tissue proliferates, probably to replace damaged choroidal tissue. Indeed, the fundusscopic picture of the experimental lesions immediately after photocoagulation, showing a central hypopigmented area surrounded by a halo of fainter hypopigmentation, correlated on histology to a central area of necrotic choroid/Bruch's membrane/RPE complex surrounded by a ring of damaged RPE with or without hemorrhagic detachment. The pooling of fluorescein demonstrated by all lesions at this time correlated histologically to the presence of fluid in these damaged areas, probably because of choroidal vascular disruption by the laser beam. The formation of this wound and accumulation of debris were followed by an inflammatory response. Only later was newly formed fibrovascular tissue seen, first in the choroid at the center of the lesion and later in the subretinal space around the break in Bruch's membrane.

An increase in the number of inflammatory cells in the choroidal vessels, choroidal stroma, and Bruch's membrane also has been found in human eyes with degenerative changes in the choroid/Bruch's membrane/RPE complex and with SRN. It could not be determined, however, whether the infiltration of the inflammatory cells into the damaged areas only represented a response to the degenerative changes or also induced proliferation of choroidal vessels into the subretinal space. The results in the monkey model may lend some support to the previously suggested hypothesis that the accumulated debris and exudate in Bruch's membrane and subpigmented epithelial space of the human eye, probably due to aging or other damage to the RPE and the choroid, induce an inflammatory response, which in turn initiates choroidal fibrovascular proliferation into the subretinal space.

The pooling of dye during angiography of the subretinal neovascular membrane correlated in the monkey eye to the presence or absence of fluid in the subretinal space. Histopathology revealed that the fluid seen in the necrotic areas immediately after laser irradiation was absorbed within days (probably by the choroidal vasculature) and replaced by choroidal neovascular membrane. At the same time, the pooling that was seen on angiography immediately after photocoagulation disappeared. As long as no fluid was present in the subretinal space, the neovascular membrane demonstrated only staining on angiography. Only when some fluid had accumulated between the subretinal vessels and their overlying retina (as was demonstrated histologically in approximately 40% of the lesions beginning in the 3rd week post-laser) did these neovascular membranes begin to pool dye during angiography. This was the beginning of the second stage of development of the subretinal neovascular membrane, equivalent to the disciform stage in humans. In this phase, fluid that apparently leaked from the subretinal vessels accumulated in the subretinal space.

Later, in the monkey model, retinal pigment cells were shown to envelop the newly formed subretinal vessels; the subretinal fluid was gradually absorbed; and the pooling of dye during angiography of the neovascular membranes gradually disappeared. This was the third stage of the neovascular membrane development—the reparative, or involutional, stage. In humans, occult subretinal vessels also have been found to be enveloped by RPE cells, but the relationship of this process to the involution of SRN has not been determined. In the monkey model, our results indicate that the differences in duration of the involutional stage in the various laser lesions result from differences in the timing of envelopment of the subretinal vessels by RPE cells, accompanied by the absorption of fluid from the subretinal space.
Fig. 8. Light micrographs of intense laser lesions in a monkey eye 8 days after photocoagulation. (a) Representative lesion in which the newly formed fibrovascular tissue has invaded the subretinal space around the break in Bruch’s membrane (bracketed by short arrows). The center of the scar (bent arrow) is filled with fibrovascular tissue (FV) that extends into the subretinal space around the break in Bruch’s membrane and separates the retina (R) from the choroid (C). No fluid is present between the subretinal neovascular membrane and its overlying retina. Arrowheads, macrophages (X160). (b) Representative lesion in which the newly formed fibrovascular tissue (FV) is concentrated around the break in Bruch’s membrane (bracketed by short arrows). No fluid is present in the subretinal space. Multiple layers of RPE cells (long arrows) block the edges of the neovascular membrane. Arrowheads, macrophages; C, choroid; R, retina (X160). (c) Higher magnification of the edge of another lesion 8 days after photocoagulation. The edge of the fibrovascular tissue (FV) that invaded the subretinal space is blocked by multiple layers of RPE cells (long white arrow). The subretinal fibrovascular membrane (FV) is infiltrated by macrophages (arrowheads). Short black arrows, Bruch’s membrane; Ph, photoreceptor layer (X640).

The key question, therefore, is: why in some laser lesions are the subretinal vessels immediately enveloped by RPE cells, whereas in others the envelopment process is much slower?

The results presented in this study suggest that the duration of the envelopment process in the various laser lesions is determined by the initial damage caused by the laser beam to the choroid/Bruch’s membrane/RPE complex.

Even though all lesions received exactly the same intensity of laser light that caused a similar break in Bruch’s membrane (300 ± 10 μm in diameter), the amount of subretinal hemorrhage and RPE denudation around the break in Bruch’s membrane differed from lesion to lesion. These variations may result from minimal differences in the focus of the laser beam, leading to variations in the area of RPE cells that absorbed the laser light and therefore disinte-
with RPE denudation around the break in Bruch's membrane.

Figure 11 describes schematically the repair process of two representative wounds, one with minimal damage and the other with extensive damage. The various laser lesions occur between these two extremes.

When laser irradiation resulted in hemorrhagic retinal detachment with RPE denudation around the break in Bruch's membrane (Fig. 11a) (42% of the lesions), the inflammatory response that followed was extensive, as was the induced choroidal fibrovascular proliferation into the subretinal space. The restoration of the RPE barrier was slow, as a large area around the subretinal neovascular membrane had to be restored. Fluid accumulated between the newly formed subretinal vessels and their overlying membrane, allowing pooling of dye on angiography (leaky neovascular lesions).

When laser irradiation resulted in only minimal damage to the RPE around the break in Bruch's membrane, with no hemorrhagic retinal detachment (Fig. 11b) (58% of the lesions), both the inflammatory
Fig. 11. Schematic presentation of subretinal neovascular membrane formation in two representative lesions. (a) Lesion with extensive damage to the choroid/Bruch's membrane/RPE complex. One day after photocoagulation (1 d), the choroid, Bruch's membrane, RPE, and outer retina, at the center of the lesion, are disrupted (gray area). Hemorrhagic retinal detachment with RPE denudation (curved arrows) surrounds the break in Bruch's membrane (interruption of thick line). Small arrows denote RPE cells. Fourteen days after photocoagulation (14 d), the center of the lesion is filled with newly formed choroidal fibrovascular tissue (dotted area) that extends toward the subretinal space around the break in Bruch's membrane (interruption of thick line). Fluid (arrows) is detaching the subretinal neovascular membrane from its overlying retina. RPE cells (short arrows) proliferate around the subretinal neovascular membrane. (b) Lesion with minimal damage to the choroid/Bruch's membrane/RPE complex. One day after photocoagulation (1 d), the choroid, Bruch's membrane, RPE, and outer retina, at the center of the lesion, are disrupted (gray area). The break in Bruch's membrane (interruption of thick line) is surrounded only by some degenerated RPE cells (small arrows), without serous detachment of the retina. Fourteen days after photocoagulation (14 d), the RPE barrier is almost restored (short arrows) and blocks the invasion of the choroidal neovascular membrane (long arrows) into the subretinal space. No fluid is present in the subretinal space.

In summary, our results suggest that the evolution of newly formed choroidal fibrovascular tissue into a subretinal membrane, with or without pooling, depends both on the amount of damage to the choroid/Bruch's membrane/RPE complex and on the ability of RPE cells around the damaged area to proliferate over the subretinal vessels and restore the continuity of the RPE layer.

Key words: laser-induced subretinal neovascularization, monkey, inflammatory response, retinal pigment epithelium, fluorescein pooling

Acknowledgments
The authors wish to thank Prof. Alan C. Bird and Dr. Thomas E. Ogden for their critical reading of the manuscript and helpful comments, and Miss Ruth Singer for typing and editing the manuscript.

References


