suggest at least two lines of inquiry worthy of experimental investigation in this model. It is noteworthy that eyes developing rubeosis iridis also manifest an infiltration of lymphocytes, plasma cells, and macrophages. Since neovascularization is an established consequence of inflammation in several settings, including corneal neovascularization and granulation tissue, similar mechanisms may be involved in rubeosis iridis in this model.

Hypoxia has been incriminated in the pathogenesis of neovascularization in many normal and pathologic states, including neovascularization of the iris. As shown previously, the oxygen tension in the anterior chamber in cat eyes after vitrectomy and lensectomy is significantly lower than normal, and this may contribute to the development of iris neovascularization. In theory, retinal detachment should make the eye even more hypoxic.

This model should allow an investigation into the roles of inflammation, hypoxia, and other conditions in the pathogenesis of rubeosis iridis, as well as evaluation of therapy of iris neovascularization.

Key words: rubeosis iridis, neovascularization, vitrectomy, lensectomy, retinal detachment, neovascular glaucoma, hypoxia, diabetic eye disease

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References


Blood Vascular Abnormalities in the Degenerative Mouse Retina (C57BL/6J-rd le)

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The authors have used a light microscopic horseradish peroxidase technique to demonstrate the arborization of blood vessels in mice homozygous for retinal degeneration and their normal heterozygous littermates. The results indicate a paucity of blood vessels in the homozygous animals as early as 14 days of postnatal age. The blood vessel deficiency at this early time coincides with degeneration of the photoreceptor cells and occurs at the approximate age when blood vessels in the normal mouse retina have reached maturity. After photoreceptor degeneration is complete, total blood vessel length per unit area continues to decrease from about one half of normal at the earlier ages to less than one third the amount at 1 yr and after. Invest Ophthalmol Vis Sci 25:364–369, 1984

We have examined the retinal vascular arborization in the C57BL/6J-rd le mouse with retinal degeneration. Using the light microscopic horseradish peroxidase (LM–HRP) method of Raviola and Freddo for whole mount visualization of the retina's blood vessels, we have shown a difference in the length of patent blood vessels in the mouse homozygous for retinal degen-
eration (rd le/rd le) when compared with its normal littermate control (rd le/+ +). In a very recent abstract, Blanks and co-workers, using lectin probes specific for the retinal vasculature in the mouse,\textsuperscript{2} found a similar blood vessel paucity in the rd mouse. They observed a 40–60% difference in blood vessel length at 1 month. In this study we have found blood vessel loss and/or lack of development to occur as early as 14 days, a time that coincides with extensive photoreceptor degeneration\textsuperscript{3} and at the approximate time that the normal mouse retinal vasculature reaches maturity.\textsuperscript{4,5} We also found a progressive reduction of mean blood vessel length in the homozygote over a period greater than 1 yr after photoreceptor degeneration.

Materials and Methods. Breeding pairs of C57BL/6J-rd le mice that have an autosomal recessive gene for retinal degeneration (rd) that is linked to a light ear marker (le) originally were obtained from the laboratory of Drs. MM La Vail and RL Sidman.\textsuperscript{3} Littermate pairs of homozygous light eared (rd le/rd le) and normal dark eared (rd le/+ +) animals were set up for breeding on a 12L-12D lighting cycle. Experiments were performed on littermate pairs with ages from 4 days to 28 postnatal months. The light microscopic horseradish peroxidase (LM-HRP) technique of Ravola and Freddo\textsuperscript{1} was used on these pairs to visualize and compare the vascular arborization of the retinas with the following modifications. Horseradish peroxidase (0.5 mg/g body weight in phosphate-buffered saline, pH 7.2) was injected into the left external jugular vein. After fixation in 2% paraformaldehyde–2% glutaraldehyde and reaction with diaminobenzidine, the retinas were washed and immersed in 100% glycerol solution for 2 hrs. Photography was performed the same day and the slides stored at 4°C until used for data collection on a Zeiss MOP-3 system and a Microplan II (Laboratory Computer Systems, Inc.). Both systems are digital planimeters that consist of digitizing tablets with built-in microprocessors that calculate length and areas from two-dimensional images. One eye from each animal was divided into four to eight segments and the total blood vessel length measured in an area of approximately 0.1 mm\textsuperscript{2} using a camera lucida tube to project the image on the tablets. The group means then were compared by two two-sample t tests, with and without the assumption of the equality of variances. Differences observed between homozygous and heterozygous animals were significant to at least P < 0.0001 in age groups of 14 days and older.

Results. Figure 1A shows a whole mount retinal preparation from the right eye of a 4.2-month-old normal heterozygous mouse (rd le/+) that had a mean total blood vessel length of 103.7 mm/mm\textsuperscript{2} ± 10.2 SD, while its homozygous counterpart (Fig. 2A) had a mean length of 33.0 mm/mm\textsuperscript{2} ± 6.5 SD when compared with that in Figure 2C, its normal littermate control (104.2 mm/mm\textsuperscript{2} ± 9.25 SD).

Figure 2 shows two additional time points at which the blood vessel scarcity is evident. Figures 2A and 2B were randomly selected quadrants from littermate pairs killed at 1 month of age. The normal heterozygote (Fig. 2A) had a mean blood vessel length of 103.7 mm/mm\textsuperscript{2} ± 10.2 SD, while its homozygous counterpart (Fig. 2B) had a mean length of 36.4 mm/mm\textsuperscript{2} ± 3.74 SD. The later amounted to approximately one-half the total blood vessel length/unit area seen in the normal mouse at this time. The second set of illustrations in Figure 2 is from littersmates killed at 12 months of age. The light-eared homozygote (Fig. 2D) had one-third the amount of blood vessels (33.0 mm/mm\textsuperscript{2} ± 6.5 SD) when compared with that in Figure 2C, its normal littermate control (104.2 mm/mm\textsuperscript{2} ± 9.25 SD).

Figure 3 summarizes the mean blood vessel lengths at ages ranging from 4 days to 28 months of postnatal development. The overall mean of the normal heterozygous (rd le/+) animal greater than or equal to 14 days was 96.81 mm/mm\textsuperscript{2} ± 12.63 SD and that of the homozygous (rd le/rd le) animal was 37.79 mm/mm\textsuperscript{2} ± 16.58 SD. Thus, the total blood vessel length/unit area was 61% less in the diseased homozygote when these means were compared in the normal and diseased retina. When the overall mean of the normal heterozygous retina was compared with that of homozygous animals at individual ages, there was a decrease in blood vessel length over time from less than one-half of normal at 14 days (56.4/98.26 mm/mm\textsuperscript{2}) to approximately one-third the normal amount at 12 months of age (33/98.26 mm/mm\textsuperscript{2}). The blood vessel lengths per unit area in the developing retinas of 4-,
Fig. 1. The four retinal quadrants are marked: superior temporal (ST), superior nasal (SN), inferior temporal (IT), and inferior nasal (IN). Central retina (C) is also indicated. Mag Bar = 0.5 mm². a, A whole mount retina from the right eye of a normal heterozygous animal (rd le/+ +) at 4.2 months of age with a mean blood vessel length of 113.1 mm/mm² ± 9.5. b, A whole mount retina from the right eye of a diseased homozygous animal (rd le/rd le) at 4.2 months of age with a mean blood vessel length of 36.5 mm/mm² ± 8.5 (littermate pair to figure 1a).
Fig. 2. Four quadrants of retinas from two age groups selected at the early and late phases of blood vessel loss. The peripheral (P) and central (C) retina are marked in each quadrant. Mag Bar = 0.25 mm. a, A normal heterozygous animal (rd le/+ +) at 1 month of age with a mean blood vessel length of 103.7 mm/mm² ± 10.3. b, A diseased homozygous animal (rd le/rd le) at 1 month of age (littermate pair to figure 2a) with a mean blood vessel length of 56.4 mm/mm² ± 3.75. c, A normal heterozygous animal (rd le/+ +) at 12 months of age with a mean blood vessel length of 104.2 mm/mm² ± 9.25. d, A diseased homozygous animal (rd le/rd le) at 12 months of age (littermate pair to figure 2c) with a mean blood vessel length of 33.0 mm/mm² ± 6.5.
Discussion. The arborization of blood vessels in the normal mouse is complete between 12–15 days of postnatal development. In this study we have shown that the blood vessels in the homozygous mouse at 14 days are approximately one-half the number when compared with the normal littermate and, therefore, the retinas of these animals are not fully vascularized at the time of blood vessel maturity (Fig. 3). In the homozygous mouse with retinal degeneration, photoreceptor degeneration becomes morphologically apparent between 10–13 days and the degenerative process is complete by 3 weeks. During this time there is a marked decrease in retinal thickness. Farber and Lolley showed a biochemical abnormality in cGMP metabolism at about 6–8 postnatal days or approximately 2 days before the rd visual cells began to degenerate. Due to the observed deficiency in number of the blood vessels at 14 postnatal days and the similarity of blood vessel lengths of undeveloped retinas at 4, 7, and 10 days, an attractive postulate might be that the blood vessel loss and/or lack of development is a secondary consequence of some aspect of a deranged retinal metabolism or to a release of metabolites during the degenerative process.

Several studies have found that monosodium glutamate (MSG) effects the retina at the critical stages of blood vessel development. Normal developing mouse retinal blood vessels migrate from the fiber layer to the inner plexiform layer by the 4th day and by the 8th day are in the outer plexiform layer. With administration of MSG on all postnatal days 1 to 10 or a single injection on post natal day 9, vascularization of the retina was inhibited and a retinal degeneration occurred. When MSG was administered on the 10th postnatal day or later, there was no effect on the retina or blood vessels. Release of metabolites from the retina during the degenerative process could have adverse effects similar to MSG on the development of the blood vasculature. Increased oxygen tension resulting from decreased retinal thickness also could play a role in the observed vascular abnormalities. Michaelson and Ashton have shown that the developing vasculature of the mouse is susceptible to hyperoxic conditions and that the blood vessels decrease in amount under these conditions. It may be that the blood vessel growth in the homozygote is effected by a hyperoxic condition created by the thinning of the retina and the resulting close proximity of the choriocapillaris to the neural retina.

This finding of early blood vessel paucity in the rd homozygote is in contrast to albino dystrophic rat retinas (rdy/rdy) that show no differences in blood vessel length per area when compared with controls at 1 month of age (Matthes and Bok, unpublished data). Measurable thinning of the dystrophic rat retina occurs at a time well beyond the period of blood vessel maturation, whereas the rd mouse retina begins to thin at a time coincident with vascular maturation. This lends further support to the postulate that developing blood vessels in the degenerative mouse retina may be influenced by toxic agents such as excess oxygen and/or metabolites.

Key words: C57BL/6J-rd le Mouse, blood vessels, retinal degeneration

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Parameters Influencing the Expression of IgA Antibodies in Tears

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Rats were immunized repeatedly with dinitrophénylated type III pneumococcal vaccine by the intravenous (IV), subcutaneous (SC), gastrointestinal (GI), or ocular/topical (OT) routes at biweekly intervals. IgA anti-DNP antibodies were measured in serum, tears, saliva, bronchial, and intestinal washings, obtained 7 days after the third and sixth immunizations, using a solid phase radioimmunoassay. The GI route was most effective at eliciting and maintaining IgA antibody responses in tears. The OT group displayed markedly diminished IgA response frequencies and antibody levels in tears following prolonged immunization. These data show that repeated central mucosal (gastrointestinal associated lymphoid tissue) stimulation maintains a local IgA response in tears, while continued topical antigen stimulation does not. Isoelectric focusing was used to probe the spectral complexity of the IgA antibodies of individual animals undergoing GI and OT immunization. The reduction of spectral complexity and the decreased responses following OT immunization appear to reflect a diminution of IgA antibody producing cells in the lacrimal gland. The concomitant changes in spectral components and maintenance of responsiveness of the GI group suggests that central mucosal site stimulation provides the lacrimal compartment with a continuous but variable population of IgA antibody producing cells. Invest Ophthalmol Vis Sci 25:369–373, 1984

Certain compartments of the ocular area appear to be associated with the mucosal immune system. The presence of secretory component and the predominance of IgA in tears1,2 with a corresponding presence of secretory component producing epithelial cells3 and IgA plasma cells4,5 indicate that the lacrimal tissue is analogous to other glandular components of the secretory immune network. The remote-site induction of ocular antibody responses has been suggested by early studies showing induction of tear IgA antibodies following oral immunization6 and recent evidence on the natural occurrence of antibodies in tears with specificity to the oral microbe, Streptococcus mutans.7 Most recently, we have documented the effectiveness of gastrointestinal immunization in eliciting tear IgA antibody responses and shown IgA antibody spectrotype identities in tears and other secretions following gastrointestinal stimulation.8 These data and evidence on the preferential homing of IgA-bearing mesenteric lymph node cells to lacrimal glands8 indicate that the lacrimal glands are linked to the common mucosal immune system. In order to understand mechanisms underlying the expression of IgA antibodies in ocular compartments, the present study assesses the effect of immunization route and frequency on IgA antibody appearance in tears.

Materials and Methods. Fischer 344 CDF/CrlBR rats (female, 150 g, Charles River Laboratories, Wilmington, MA) were immunized with 2,000 μg of dinitrophénylated type III-pneumococcal (DNP-Pn) vaccine at 14-day intervals by one of four protocols: intravenous injection (IV), subcutaneous injection (SC), gastric intubation (GI) or by topical application of secretory component producing epithelial cells3 and IgA plasma cells4,5 indicate that the lacrimal tissue is analogous to other glandular components of the secretory immune network. The remote-site induction of ocular antibody responses has been suggested by early studies showing induction of tear IgA antibodies following oral immunization6 and recent evidence on the natural occurrence of antibodies in tears with specificity to the oral microbe, Streptococcus mutans.7 Most recently, we have documented the effectiveness of gastrointestinal immunization in eliciting tear IgA antibody responses and shown IgA antibody spectrotype identities in tears and other secretions following gastrointestinal stimulation.8 These data and evidence on the preferential homing of IgA-bearing mesenteric lymph node cells to lacrimal glands8 indicate that the lacrimal glands are linked to the common mucosal immune system. In order to understand mechanisms underlying the expression of IgA antibodies in ocular compartments, the present study assesses the effect of immunization route and frequency on IgA antibody appearance in tears.