Experimental immunogenic rubeosis iridis

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We have developed a primate model of rubeosis iridis in monkeys systemically sensitized to crystalline beef insulin. After intravitreal insulin injection, the dose-related immunogenic inflammation includes cells, flare, fibrin, and blood in the anterior chamber. With more severe inflammation, posterior synechiae, iris bombe, and cataracts occur. Of particular importance, new blood vessels develop within the stroma and on the anterior surface of the iris. Following injection of small amounts of insulin, the anterior surface vessels may regress over time, and the iris regains its normal appearance and coloration. However, the new stromal vessels persist and are cuffied by inflammatory cells including plasma cells. After injection of large amounts of insulin, more extensive structural alterations develop as noted above in conjunction with persistent iris anterior surface and stromal neovascularization. The relationship of rubeosis iridis to clinical inflammatory syndromes and to previous laboratory studies is discussed. Stromal neovascularization was a consistent finding in this experimental model even when anterior surface vessels regressed. On the basis of these experimental data and a review of publications describing human pathology, we believe that a broadening of the classic definition of rubeosis iridis is warranted to include a recognition of the stromal component of the clinical and pathologic findings.

Key words: rubeosis iridis, iris neovascularization, primate, insulin, immunogenic inflammation, light microscopy, peroxidase, fluorescein angiography.

Rubeosis iridis was first described in 1928 by Salus, who related iris neovascularization to diabetes and named the condition "rubeosis iridis diabetica." Over the last half-century, rubeosis iridis has been identified as a serious complication of a variety of ocular diseases. In diabetes, iris neovascularization occurs most frequently in eyes with long-standing proliferative retinopathy and frequently results in neovascular glaucoma and blindness. Our knowledge of the pathogenesis and treatment of rubeosis iridis and neovascular glaucoma is severely limited. In a recent Symposium on Glaucoma, Hoskins stated that "a major obstacle to studying this process is the lack of an experimental animal model."

Various efforts to produce rubeosis iridis...
in the laboratory have met with limited success. In rabbits, Schultze attempted over a dozen different methods, including ligation of the optic nerve, laceration and diathermy of central retinal vessels, diathermy of vortex veins or the iris surface, and injection of autogenous blood into the vitreous. The only successful technique for producing rubeosis iridis was obliteration of both long posterior ciliary arteries. Anderson and Morin produced rubeosis iridis in rabbits by similar techniques involving profound interruption of globe vasculature with resulting anterior segment necrosis. These authors concluded that "rubeosis iridis, produced in association with anterior segment necrosis, is unsuitable as an experimental model for the study of hemorrhagic glaucoma."

We have reported previously a series of experiments on monkeys sensitized to exogenous beef insulin. After intravitreal injections of beef insulin, these monkeys develop a proliferative retinopathy with many features similar to diabetic retinopathy. This paper reports in more detail the development of rubeosis iridis in the monkey that is also induced by immunity to insulin.

Materials and methods

Twelve healthy, adult rhesus monkeys were sensitized systemically to crystalline beef insulin (U-100; Eli Lilly & Co., Indianapolis, Ind.) by three 1 cc. foot-pad injections at weekly intervals of an insulin-Freund's complete adjuvant mixture (25 units insulin/1 cc. total volume). Intravitreal injections were made under pentobarbital (Nembutal) anesthesia. The pupil was dilated with 1 percent cyclopentolate hydrochloride (Cyclogyl), and topical antibiotic (gentamicin sulfate, Garamycin) was applied to the globe. From 0.5 to 5 units of crystalline beef insulin in 0.05 ml. total volume (without adjuvant) were injected into the posterior vitreous of each eye. The injections were made under direct observation by indirect ophthalmoscopy and iris fluorescein angiography for periods of up to 7 months after injection. At selected time intervals, the eyes were fixed by vascular (intracardiac) perfusion or immersion fixation with 2 percent paraformaldehyde-2 percent glutaraldehyde in 0.1M sodium cacodylate (pH 7.4) for study by light and electron microscopy. In some of the animals, the ipsilateral common carotid artery was isolated prior to enucleation, and 500 mg. of horseradish peroxidase (Type II, Sigma Chemical Co., St. Louis, Mo.) in 5 cc. of sterile saline were slowly injected. Thirty minutes later, the eyes were removed and fixed by immersion. The next day, the tissues from these peroxidase eyes were sectioned at 75 to 100 μ with the Smith-Farquhar tissue chopper and incubated in peroxidase substrate (3-3'-diaminobenzidine) according to the method of Graham and Karnovsky. All the tissues were then processed by standard methods for electron microscopy, including postfixation in buffered osmium tetroxide, rapid alcohol dehydration, and embedding in Araldite.

As controls, a sensitized monkey was given single (one eye) or multiple (the other eye) intravitreal injections of 0.05 cc. of sterile saline. In order to test the effects of crystalline beef insulin directly on ocular tissues, another monkey which was not sensitized to insulin was given single (one eye) or three separate intravitreal (the other eye) injections of 10 units of crystalline beef insulin over a 10 day period.

Results

Small amounts of insulin. Within 3 days after a single intravitreal injection of a small amount of crystalline beef insulin (0.5 to 1.0 unit), the sensitized monkeys develop inflammation in the anterior segment. Flare, cells, and fibrin are present in the aqueous humor, and the iris is hypopemic. Over the subsequent week, there is vascular engorgement of the minor arterial circle, with a decrease in aqueous humor cells and flare. By 2 weeks, dilated, tortuous vessels appear on the anterior iris surface adjacent to the minor arterial circle and extend toward the pupil and the iris root (Fig. 1). Iris angiography performed from 3 days to approximately 3 weeks after injection of a small amount of insulin reveals a large number of iris blood vessels within the stroma and on the anterior surface that leak fluorescein profusely into the anterior chamber (Fig. 5, A). At 3 to 4 weeks, the blood vessels visible on the anterior iris surface gradually become less prominent, and over time, the iris largely recovers its normal appearance and
Fig. 1. Slit-lamp photograph of monkey iris 2 weeks after intravitreal injection of small amount of crystalline beef insulin. Numerous tortuous blood vessels are visible on the anterior iris surface that cause a reddened appearance.

Fig. 2. Same eye as in Fig. 1, at 6 weeks. The blood vessels on the anterior iris surface have regressed, and the iris has regained its normal coloration.

Fig. 3. One month after intravitreal injection of a large amount of insulin. Marked acute inflammation has resulted in posterior synechiae that occluded the pupil. Prominent iris bombe, a cataract, and rubeosis iridis are evident.

Fig. 4. At 7 months after intravitreal injection of a large amount of insulin, dense posterior synechiae bind the atrophic iris to the cataractous lens. Iris bombe has markedly shallowed the anterior chamber. Numerous new blood vessels are observed on the anterior iris surface. (See fluorescein angiogram, Fig. 9.)
Fig. 5. Fluorescein angiograms from monkey eye shown in Figs. 1 and 2. A, At 2 weeks after intravitreal insulin injection. The new iris vessels on the anterior surface and in the stroma leak fluorescein into the anterior chamber. B, At 6 weeks after injection (as shown in Fig. 2), the anterior vessels have regressed, but the stromal vessels persist. There is minimal fluorescein leakage at this time.

Fig. 6. Light micrograph of iris from same eye shown in Figs. 1, 2, and 5 at 6 weeks after intravitreal insulin injection. Numerous new blood vessels are present within the iris stroma. One blood vessel is present near the anterior surface (top, right). Chronic inflammatory cells including plasma cells are scattered throughout the stroma and frequently cuff the vessels. (x640.)
Fig. 7. Light micrograph of the iris 2 weeks after an intravitreal injection of a large amount of insulin. Dilated blood vessels within the stroma are cuffed by acute and chronic inflammatory cells that are also scattered throughout the stroma. One blood vessel on the anterior surface (above) is also surrounded by inflammatory cells even though it extends into the anterior chamber. (x256.)

Fig. 8. At 2 months after intravitreal injection of a large amount of insulin, the iris stroma is permeated by numerous, thin-walled blood vessels that extend up to the surface and even project into the anterior chamber (top). Perivascular cuffing is less prominent at this time, but chronic inflammatory cells persist in the iris stroma. (x64.)
Fig. 9. Fluorescein angiogram from same eye shown in Fig. 4 at 7 months after large amount of intravitreal insulin. Preinjection photo (above, left). The dye fills the new iris vessels which leak fluorescein over time into the shallowed anterior chamber.

Fig. 10. Electron micrograph of a new iris blood vessel that is filled with dense peroxidase reaction product (below). This tracer has penetrated the vessel wall, stains the basal lamina surrounding the endothelium, and fills the extravascular space of the iris stroma (above). (×1,300.)
color by gross and slit-lamp examination (Fig. 2). There is minimal evidence of anterior chamber inflammation at this time. Fluorescein angiography still demonstrates an increased number of blood vessels within the iris stroma, but the amount of fluorescein leakage is progressively and dramatically reduced (Fig. 5, B). Furthermore, despite the apparently normal external appearance of the iris and the minimal fluorescein leakage, histopathologic examination confirms the presence of new blood vessels within the iris stroma and near the anterior surface (Fig. 6). These vessels are often surrounded by plasma cells and lymphocytes and possess an endothelium that is irregular in outline.

**Larger amounts of insulin.** Following intravitreal injections of a larger amount of insulin (2 to 5 units), the initial anterior chamber inflammatory reaction is more marked. In addition to cells, flare, and fibrin, blood may be layered inferiorly in the anterior chamber or adherent to the corneal endothelium. Within 1 to 2 weeks, the minor arterial circle of the iris develops marked vascular engorgement. As was found with small amount of intravitreal insulin, dilated, sinuous vessels are visible on the iris surface bordering the minor arterial circle. Light microscopic examination reveals a large number of vessels within the iris stroma and on the anterior surface. There is prominent cuffing of these vessels by acute and chronic inflammatory cells, including lymphocytes and plasma cells, even in regions where the vascular profiles extend into the anterior chamber (Fig. 7). These abnormal new vessels leak fluorescein as well as horseradish peroxidase. With the electron microscope, peroxidase penetrates vessel walls, stains the basal lamina, and fills the extravascular spaces of the iris stroma (Fig. 10). The appearance of columns of peroxidase-reaction product within the endothelial intercellular spaces suggests a lack of the peroxidase-restrictive tight junctions (zonulae occludentes) that are present normally in iris vasculature.

In contrast to eyes given small amounts, with large amounts of intravitreal insulin, the iris vessels on the anterior surface persist and maintain a red coloration on gross examination. Histopathologic study 2 months after injection reveals multiple, large thin-walled vessels that permeate the iris stroma, extend to the anterior surface, and even project into the anterior chamber in some areas (Fig. 8). At this later stage, scattered inflammatory cells persist in the iris stroma, but perivascular cuffing of individual vessels is much less prominent. New blood vessels are found along the full radius of the iris and are present as well in the iris root.

In eyes with severe inflammatory reactions, posterior synechiae form and the lenses become cataractous (Fig. 3). Within a few weeks following injection, pupillary occlusion occurs, resulting in iris bombe. Fig. 4 illustrates a monkey eye 7 months after intravitreal injection of a large amount of insulin. Posterior synechiae occlude the pupillary aperture, resulting in long-standing iris bombe, and a cataract is present. The iris, which is partially atrophic, possesses a network of blood vessels that leak fluorescein into the shallowed anterior chamber (Fig. 9).

**Control animals.** The control animal which was sensitized to insulin and received intravitreal saline injections did not develop any anterior or posterior segment inflammation. Similarly, no abnormalities were seen in the nonsensitized animal who was given single or multiple intravitreal insulin injections.

**Discussion**

We believe that we have developed a useful primate model of rubeosis iridis. After a single intravitreal injection of exogenous insulin in sensitized monkeys, the resulting immunogenic inflammation causes a variety of dose-related anterior segment changes ranging from cells and flare with hyphema to posterior synechiae and iris bombe. The value of this animal model lies in the capability for studying the develop-
ing pathology at selected stages in the disease process; in the potential for correlating clinical and laboratory methods of examination; and in the availability of material for evaluating therapeutic modalities.

It is not unexpected that immunogenic inflammation causes rubeosis iridis, since iris neovascularization has been reported in a variety of inflammatory syndromes. Some examples include chronic uveitis, radiation-induced uveitis, Eales' disease, and cranial arteritis. Severe ischemic syndromes such as anterior segment necrosis following retinal detachment surgery and carotid-occlusive disease also share underlying inflammatory mechanisms. In the successful laboratory studies of Schultze and Anderson and Morin, the anterior segment ischemia and necrosis also resulted in inflammation in the anterior segment. For example, Schultze reported that all his successful experiments followed a similar clinical course, including iritis, formation of extensive posterior synechiae, and by the third week, the development of new iris vessels. Anterior segment inflammation appears therefore to be a common finding in patients with rubeosis iridis and in experimental animals.

The fact that intraocular insulin can produce rubeosis iridis in sensitized monkeys raises the question of whether insulin therapy could initiate or potentiate inflammatory mechanisms in diabetic patients and result in anterior segment proliferative lesions. Commercial insulin, which is obtained principally from beef and pork pancreas glands, is an antigenic protein. Its low molecular weight and chemical structure contribute to its antigenic activity. Furthermore, fluorescent insulin-antibody binding has been demonstrated in the basement membranes of diabetic blood vessels in the eye.

The clinical presentations of diabetic patients with rubeosis iridis suggest an inflammatory syndrome similar to the spectrum of changes induced in our animal model. Inflammation has not been included as a principal part of the pathologic descriptions of diabetic rubeosis iridis. However, most specimens have been obtained from patients with long-standing or end-stage rubeosis iridis when the scarring phases of an inflammatory reaction would predominate. The formation of peripheral anterior synechiae in the chamber angle is recognized as a stage in diabetic rubeosis iridis and does suggest that inflammatory mechanisms are operative.

The classic pathologic definition of rubeosis iridis is a fibrovascular membrane on the anterior iris surface that grows over the trabecular meshwork. This definition implies that the iris stroma is not involved by neovascularization. It is difficult to accept the definition that the pathology of human rubeosis iridis is somehow restricted to the anterior iris surface. Our observations in the animal model as well as human pathologic material illustrate stromal involvement as an integral part of the disease process. In our experimental studies on the monkey iris, the clinical recognition of rubeosis iridis is based on an initial engorgement of the minor arterial circle and surrounding vessels on the anterior iris surface. However, fluorescein angiography demonstrates neovascularization throughout the iris. In addition, after intravitreal injections of small amounts of insulin, the vessels on the anterior surface regress, but the stromal vessels persist, with surrounding plasma cells. The hyperimmune status of the monkeys and the acceleration of the inflammatory course may result in differences from human rubeosis iridis. It would appear, however, that in both the human and our animal model, rubeosis iridis involves more than just a fibrovascular growth on the anterior iris surface. On the basis of these considerations, we propose that a broader definition of human rubeosis iridis is in order, that does recognize the stromal component of the clinical and pathologic findings.

A thorough study of this primate model should increase our basic knowledge of
anterior segment inflammation and neovascularization. It is also possible that this model could provide an inroad toward understanding mechanisms involved in diabetic rubeosis iridis and neovascular glaucoma.

REFERENCES