Abstract  Background: A new model of choroidal neovascularization (CNV) has been developed in the primate by implanting vascular endothelial growth factor (VEGF)-impregnated microspheres in the subretinal space.  Methods: CNV was induced in Macaca mulatta monkeys by implanting VEGF-impregnated gelatin microspheres in the subretinal space. Progression of CNV was followed for 24 weeks after surgery using fluorescein angiography. Eyes were enucleated at various time points, and lesions were evaluated for evidence of CNV by light microscopy and by immunohistochemical staining.  Results: CNV developed in 12 (92%) of 13 eyes. Fluorescein leakage was first observed in the 2nd postoperative week and was apparent for the following 12 weeks. CD31 staining for endothelial cells was first observed at day 7 and was evident for the following 8 weeks. Glial fibrillary acidic protein staining revealed a glial adhesion between the proliferative membrane and the retina at 6 weeks after implantation. Smooth muscle actin-positive cells were found a +2 weeks and remained prominent for at least the next 6 weeks. Cytokeratin-positive retinal pigment epithelial (RPE) cells, first identified in the proliferative membrane at day 3, predominated throughout the growth of the membrane. Macrophages (RAM-II positive) were present at day 3 but were no longer observed after day 7.  Conclusion: In monkeys, subretinal implantation of VEGF-impregnated gelatin microspheres leads to the development of CNV. Early, disciform and reparative stages of CNV were observed, similar to those seen in humans. This model will be useful for studying the pathogenesis of CNV and for evaluating potential treatment strategies.

Natural history of choroidal neovascularization induced by vascular endothelial growth factor in the primate

Introduction

Choroidal neovascularization (CNV) is a common feature of many ocular disorders, including ocular histoplasmosis, angiod streaks, and myopia, and is the major cause of severe visual loss in patients with age-related macular degeneration [7, 14, 29, 33]. The newly formed vessels originate from the choroid and enter the subretinal space, where they are recognized clinically by fluorescein leakage during angiography [7, 40]. The early pathogenesis of CNV is poorly understood since most human histological studies include, by necessity, only the late stages of the disease. A systematic study of the development of CNV, as well as an examination of possible treatment approaches, is possible only in an animal model. In the classic primate model of CNV, tissue damage is induced by laser photocoagulation, resulting in an inflammatory response, the release of angiogenic growth factors and neovascularization [15, 25, 31, 32]. Vascular endothelial growth factor (VEGF) is one of the most potent and specific angiogenic factors that is upregulated in both human and experimental CNV [6, 16, 44]. While there is strong evidence supporting a direct effect of VEGF in the induction of retinal neovascularization in...
rabbits and primates [27], the data supporting a similar role of VEGF in CNV induction is more circumstantial. We had previously shown that sustained release of basic fibroblast growth factor in the subretinal space leads to CNV in rabbits [17, 18]; however, we were unable to replicate these results in a primate model (results not shown), suggesting that there are species differences in responsiveness to some growth factors. In addition, basic fibroblast growth factor has been shown to be neither necessary nor sufficient for the development of retinal neovascularization in a transgenic mouse model [28]. To determine whether VEGF is sufficient to induce CNV and to avoid such species differences, we developed a novel primate model of CNV based on the promotion of neovascularization by VEGF-impregnated gelatin microspheres implanted in the subretinal space of Macaca mulatta monkeys. Our results show that this experimental model of CNV has features in common with the CNV that occurs in macular degeneration and other ocular diseases in humans.

Materials and methods
Preparation of VEGF-impregnated gelatin microspheres
VEGF-impregnated gelatin microspheres were prepared by forming a polion complex between gelatin and VEGF in a manner similar to that described for basic fibroblast growth factor [17]. Briefly, 5 mg of cross-linked gelatin microspheres (a gift from the Ophthalmology and Research Center for Biomedical Engineering, Kyoto University, Japan) was mixed with 100 µl of distilled water containing 10 µg of recombinant human VEGF (Pepro Tech, Rocky Hill, N.J.) and the mixture was incubated at 37°C for 1 h. After incubation, the mixture was diluted 1:10 with phosphate-buffered saline solution (PBS, pH 7.4), producing a 0.5% (wt/vol) microsphere suspension that was injected into the subretinal space. Control VEGF-free gelatin microspheres were prepared using the same procedure, without the addition of VEGF to the incubation mixture [11].

Injection of VEGF-microspheres in the subretinal space
Nineteen adult Macaca mulatta monkeys, each weighing 7–10 kg, were used for this study. The animals used in this study were maintained in animal care facilities fully accredited by the American Association of Laboratory Animal Science, and all animal experiments were carried out according to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. The monkeys were anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride and xylazine hydrochloride (4:1). One pupil of each animal was dilated with topical 1% tropicamide and to avoid such species differences, we developed a novel primate model of CNV based on the promotion of neovascularization by VEGF-impregnated gelatin microspheres implanted in the subretinal space of Macaca mulatta monkeys. Our results show that this experimental model of CNV has features in common with the CNV that occurs in macular degeneration and other ocular diseases in humans.

Results
Funduscopic examination
The retina in the area of injection became reattached within 3 days of surgery. None of the 19 eyes exhibited any significant complication. The media remained clear; no vitreous hemorrhage or vitreal opacification was observed throughout the study period, and no fluid accumulated in the subretinal space. Figure 1 shows a fundus photograph 2 weeks after implantation of the microspheres. The retina is attached and the implantation area is slightly de pigmented with hyperpigmentation at the bleb borders.