Exacerbation of Retinal Degeneration and Choroidal Neovascularization Induced by Subretinal Injection of Matrigel in CCL2/MCP-1-Deficient Mice

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Key Words

Neovascularization · Retinal degeneration · CCL2 · Matrigel · Macular degeneration, age-related

Abstract

This study presents a mouse model for human age-related macular degeneration (AMD) as characterized by subretinal deposit and choroidal neovascularization. Matrigel, a basement membrane extract, solidifies after implantation in tissue and can stimulate local angiogenesis. This study demonstrates the induction of neovascularization and focal retinal degeneration following subretinal Matrigel injection in mice. In senescent mice, the normal functioning of CC chemokine CCL2/MCP-1 and its receptor CCR2 confers protection against age-related retinal degeneration, a disease that shares many similar features with human AMD. Our data shows that CCL2-deficient mice develop more severe disease as compared to the wild-type controls. These findings suggest that Matrigel subretinal injection could be used to generate AMD-like pathological changes. The data support the previously proposed role of CCL2 in AMD pathogenesis.

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Age-related macular degeneration (AMD) is one of the leading causes of severe visual impairment among the elderly in the developed world [1]. Although the clinical

characteristics and histopathology are well described, the pathogenesis of this complex disease is not yet fully understood [2, 3]. Drusen development in the macula, a hallmark feature of early AMD, can be accompanied by choroidal neovascularization (CNV) or geographic atrophy. Currently, there are two types of animal models used in AMD studies. These models involve animals with CNV and retinal atrophy either induced by (1) physical or chemical stimulation by light, laser, or lipid diet [4–6] or (2) congenital [7] or genetic engineering [8–12]. This study presents the retinal degeneration and neovascularization induced by subretinal injection of Matrigel and compares the pathology in CCL2/monocyte chemoattractant protein 1 (MCP-1)-deficient (KO) mice and wild-type (WT) controls.

Two independent experiments were performed according to the tenets prescribed in the ARVO Statement for the Treatment of Animals in Ophthalmic and Vision Research. The 6- to 8-week-old KO and WT mice were bred from two original pairs generously provided by Dr. Bao Lu and Dr. Barrett Rollins (Children's Hospital, Harvard Medical School). The liquid Matrigel (75% v/v; BD Biosciences, Bedford, Mass., USA) was prepared using phosphate-buffered saline (PBS) at 4°C. A total of 11 KO and 11 WT mice were subretinally injected with Matrigel in both eyes under general anesthesia. After exposing the sclera, an incision was made between the limbus and the equator using a sharp No. 33 size needle. The Matrigel suspension (0.5 µl per eye) administered via a Hamilton

Fig. 1. Microphotographs showing Matrigel (*) in the subretinal space above degenerative RPE and photoreceptor cells. **a** Neovascular vessels (arrow, CNV) are induced in the Matrigel implant of a WT mouse. **b** Many small choroidal neovascular vessels (arrows) are observed in a CCL2 KO mouse. **c** An aggregation of macrophages (arrowhead) with small residue Matrigel is found in a WT mouse. **d** A focus of total photoreceptor cell loss mimics geographic AMD in a KO mouse. Hematoxylin and eosin. Original magnification $\times 200$.

microsyringe with a No. 33 blunt tip needle was slowly injected into the subretinal space along the previous needle tract. Following injection, the Matrigel polymerized to form a solid gel. Funduscopy was performed every other week and the mice were euthanized at week 4 (4 mice) and 12 (18 mice) postinjection. The eyes were harvested, fixed in 10% formalin, embedded in methacrylate and sectioned via the injection site for histological examination.

The two experiments reproduced similar data. Dilated funduscopy examination demonstrated subretinal or retinal lesions located in the far periphery. Vitreous hemorrhages were observed in 3/44 (2 KO, 1 WT) eyes. By week 12, the vitreous hemorrhages had dissolved in two of the three eyes. Histology of the eyes at 4 weeks postinjection demonstrated solid Matrigel located beneath the retina and above Bruch's membrane peripherally. At 12 weeks, Matrigel was observed either beneath or above the retinal pigment epithelium (RPE) below the neuroretinal layers.

RPE and/or photoreceptor degeneration, RPE migration, and various degrees of CNV were also observed (fig. 1a). Damage to the RPE cells and photoreceptors was observed in both groups. However, the retinal degeneration was exacerbated in the KO mice as compared to WT mice with the greatest differences found among the 12 weeks' postinjected mice. Furthermore, more KO (53.3%) than WT (30.7%) mice developed CNV (fig. 1b). Some of the lesions were accompanied by mild inflammation and macrophage infiltration (fig. 1c). Interestingly, fewer macrophages were found in the KO as compared to the WT mice (fig. 1d).

Matrigel is a solubilized basement membrane preparation extracted from Engelbreth-Holm-Swarm mouse sarcoma [13]. The major components of Matrigel are laminin, collagen IV, heparan sulfate proteoglycans, entactin and nidogen. Once the cold liquid Matrigel is introduced into tissue, it solidifies thus permitting host cell penetration as well as the formation of new blood vessels. A Matrigel-based in vivo angiogenesis assay has previously been standardized for the assessment of neovascularization [14, 15]. Our study demonstrates that CNV and photoreceptor degeneration can be induced by Matrigel.

CCL2, a member of the MCP group of CC chemokines, is produced by immune and nonimmune cells including the RPE in response to various stimuli such as

TNF- α , IL-1 β , and VEGF. CCL2 KO mice demonstrate impaired in vivo macrophage recruitment [16, 17], which may result in disabled degradation and removal of the subretinal Matrigel. Impaired deposit degradation may cause more severe lesions such as CNV and greater photoreceptor degeneration in these mice as compared to the WT. Ambati et al. [2] reported that KO of CCL2 or its cognate CC receptor CCR2 develop cardinal features of AMD including the accumulation of lipofuscin in, and drusen beneath, the RPE, photoreceptor atrophy, and CNV. Our data supports the hypothesis that macrophage dysfunction may play a role in AMD pathogenesis.

We propose that subretinal Matrigel injection can be used as a model for focal outer retinal degeneration and CNV. Since retinal degeneration and CNV are morphological features of AMD, this model may potentially be useful in studying the mechanisms, such as those involved in inflammatory and immune responses, underlying disease pathogenesis by providing more detailed information concerning the microenvironment infiltrating the Matrigel. Studies on macrophage activity, chemotaxis, and infiltration into the Matrigel would be interesting and should be considered for future investigation. A working model such as this may lead to advances in the screening of antiangiogenic or other therapies for AMD.

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