A New Target for Neovascular AMD

This molecule might enable earlier detection of neovascularization.

BY JAYAKRISHNA AMBATI, MD

Since the middle of the last decade, pharmacotherapy has transformed the management of neovascular age-related macular degeneration (AMD). Specifically, the ability to pharmacologically inhibit vascular endothelial growth factor (VEGF) has changed the prognosis of neovascular AMD from one of laser photocoagulation and observation of progressively worsening vision to the current paradigm of stabilization or improvement of vision for most patients with repeated intravitreal injections of an anti-VEGF agent.[1,2]

However, it is also clear that currently available anti-VEGF agents are not a panacea. Only one in three patients achieve high functioning vision, such as the 20/40 visual acuity that would make them legally eligible to drive a car, and one in six progress to legal blindness. Current unmet needs include a better understanding of the biology of the disease and its pathogenesis, and new and better potential targets for both diagnosis and therapy.

In addition, recent studies have shown that a major determinant of the trajectory of vision in patients who develop choroidal neovascularization (CNV) secondary to AMD is the initial presenting vision,[3] and there is accumulating evidence that delay in diagnosis or treatment of CNV lesions leads to worse vision than earlier treatment.[4-6]

Ideally, then, we would like to treat patients with CNV early—not at the stage when the new blood vessels have already breached Bruch's membrane and entered the retina, but rather when these vessels are still within the choroid, when the potential of the photoreceptors both anatomically and functionally has not been disturbed (Figure 1). To achieve this, we need a way to detect the disease and these new vessels at this earlier stage.

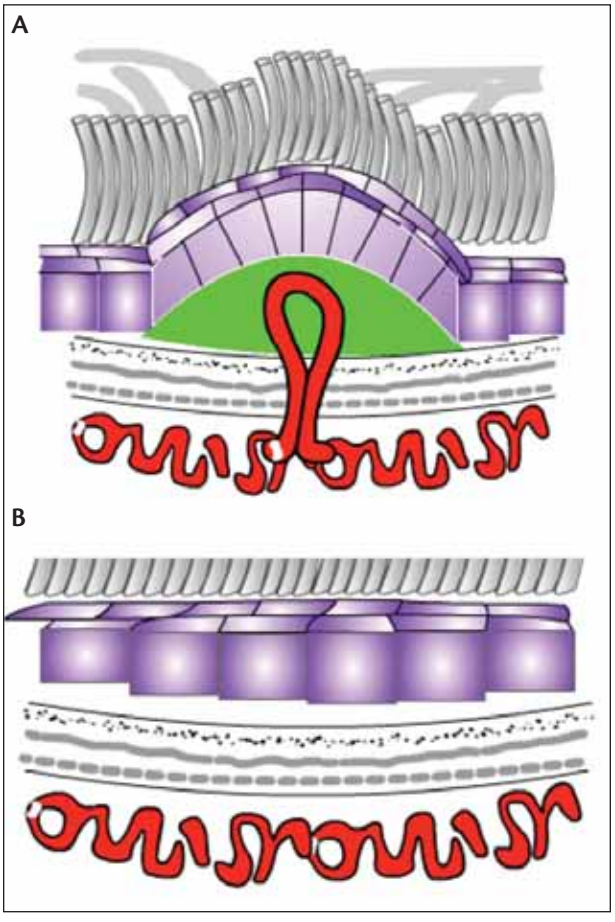

Figure 1. It would be preferable to treat patients with CNV early: not at the stage when the new blood vessels have already breached Bruch's membrane and entered the retina (A), but rather when the vessels are still within the choroid, when the anatomic and functional potential of the photoreceptors has not been disturbed (B).

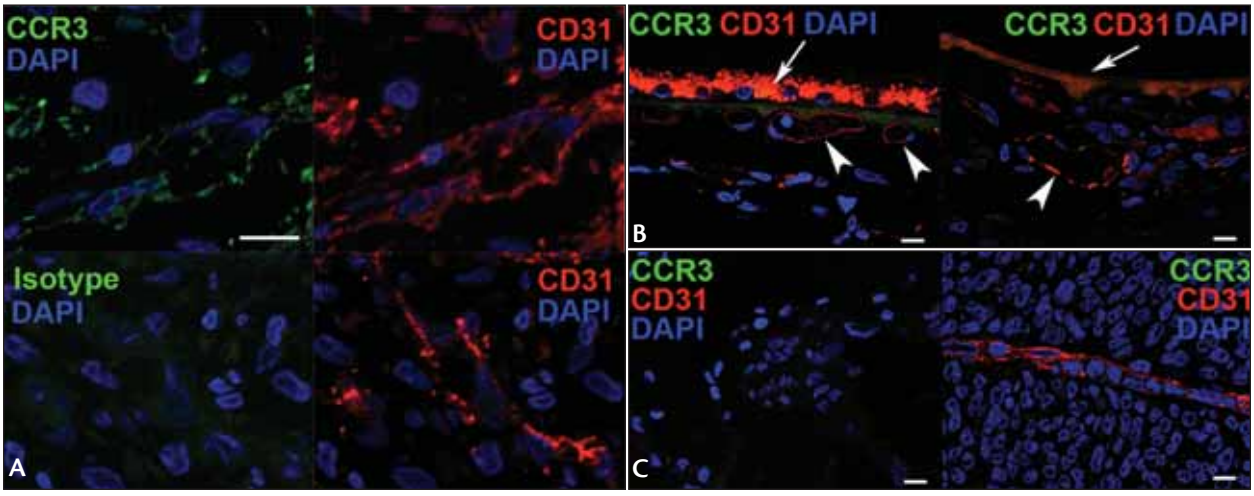

Figure 2. In these surgically excised CNV membranes, staining revealed the expression of CCR3 (A). This expression was not seen in tissue samples from age-similar patients who had no AMD or in patients with geographic atrophy (B), nor was CCR3 expressed in epiretinal membrane or other proliferating endothelial cells, such as in uveal melanoma (C).

A NEW TARGET

Recently our attention has been drawn to a molecule that may be capable of serving a dual capacity: identifying neovascularization earlier in its development and serving as a target for therapy.

CCR3 is a cell surface receptor found on a variety of types of cells that binds to numerous proteins, including a family of molecules called eotaxins. The eotaxins and CCR3 are understood to play a role in mediating allergic inflammation, including the release of mast cells, eosinophils, and other cells involved in allergy responses.

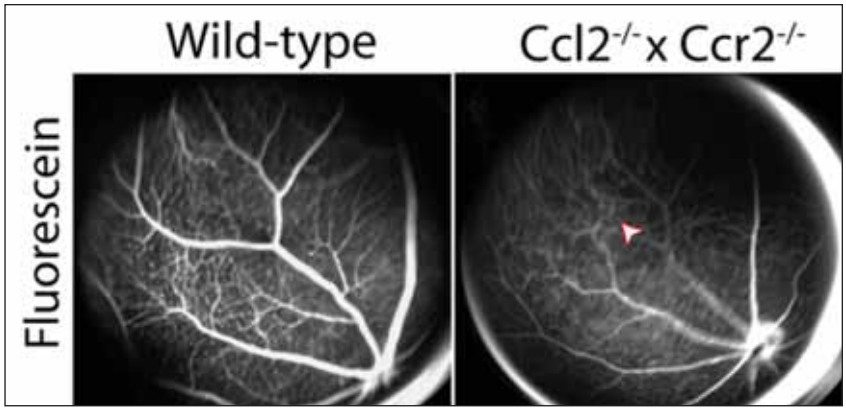

Figure 3. In the transgenic mice, accumulation of hyperfluorescent CCR3-targeted quantum dots in the choroid was observed, but not in wild-type mice.

In addition to this role, however, we have recently shown that these molecules are involved in promoting angiogenesis.[7] Our studies investigated the characteristics of CCR3 in several ways.

First, to characterize the expression of this molecule, we examined excised CNV membranes from patients with neovascular AMD who had not undergone treatment for the condition. In these surgically excised membranes, staining revealed the expression of CCR3. This expression, however, was not seen in tissue samples from age-similar patients who had no AMD or in patients with geographic atrophy, nor was CCR3 expressed in epiretinal membrane or other proliferating endothelial cells, such as in uveal melanoma (Figure 2). We concluded that the expression of CCR3 on endothelial cells in the choroid was specific to the context of neovascular AMD and was not seen in other conditions.

Second, we performed studies to examine the function of CCR3 in promoting neovascularization. Neovascularization comprises several steps, including proliferation and migration, and these abnormal new vessels are extremely permeable to plasma proteins. We investigated each of these characteristics in turn.

Using a mouse laser-induced model of CNV, we found that intravitreous administration of an antibody against CCR3 decreased CNV size in a dose-dependent fashion. The same was true with intravitreous administration of a small-molecule receptor antagonist against CCR3. Intravitreous administration of the CCR3-neutralizing antibody in this mouse CNV laser-injury model also reduced vessel leakage.

We also examined the effects of CCR3 on prolifera-

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tion in human cell culture. Primary human choroidal endothelial cells in culture, when exposed to CCR3-stimulating molecules, underwent proliferation. Angiogenesis also requires migration of choroidal endothelial cells, and again, in a dose-dependent fashion, we found that CCR3-stimulating proteins increased the migration of primary human choroidal endothelial cells.

IN VIVO IMAGING

Finally, based on our finding that CCR3 expression was related to CNV levels, we wanted to see if we could image the presence of CCR3 in the living eye to detect CNV that had not yet invaded the retina.

To do this, we performed in vivo imaging in a transgenic mouse model.[8] Quantum dots, highly fluorescent semiconductor nanocrystals, to which a CCR3 antibody fragment was conjugated, were injected intravenously. The transgenic mice, which spontaneously develop CNV, were compared with wild-type mice. Histologic examination showed that the choroidal new vessels were still confined to the choroid.

On intravenous fluorescein angiography, untreated wild-type and transgenic mice had normal filling patterns. When wild-type mice were injected with the CCR3-targeting quantum dots, angiography still showed normal filling patterns. In contrast, in the transgenic mice, accumulation of hyperfluorescent CCR3-targeted quantum dots in the choroid was observed (Figure 3). Quantum dots conjugated with a control antibody showed no fluorescence.

To confirm that the antibodies were targeting CCR3-expressing proliferating choroidal blood vessels, histology was performed on the same eyes that underwent the fluorescein imaging. Histology confirmed that CCR3 was expressed on these blood vessels, still within the choroid, in the same location where the quantum dots were localized.

CONCLUSIONS

Our investigations suggest that CCR3 is a signature of CNV, and that CCR3 has a direct effect in promoting various steps of angiogenesis, including the proliferation, migration, and permeability of choroidal endothelial cells. Our work also suggests that CCR3 may potentially be a suitable diagnostic and therapeutic target that might reduce vision loss through early identification and inhibition of CNV.

Notably, in regard to the therapeutic potential of this new target, at least in the laser-induced CNV mouse model used in our study, the CCR3 and VEGF pathways were not interwoven. That is, when CCR3 was blocked there was no change in the level of VEGF expression, and vice versa. This finding suggests that these two pathways may be independent, and therefore might be targeted with combination therapy. In addition, it is noteworthy that in this mouse model a CCR3-inhibiting antibody was modestly but significantly superior to a VEGF antibody in inhibiting CNV growth.

Currently, we are developing alternatives to quantum dots because of potential toxicity concerns related to their heavy metal constituents. A variety of organic near-infrared dyes seem to be capable of performing the same functions, including imaging CCR3 in vivo. We are also in the process of identifying patients who would be suitable as candidates for diagnostic trials, particularly those with high risk characteristics in eyes in which the fellow eye has already developed CNV, accompanied with information about genetic predisposition to progression of CNV, and with the potential for identifying the recurrence of CNV. ■

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