7-9-2019

CCR3 Inhibition for Ocular Angiogenesis and Macular Degeneration

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Provided are methods and compositions for the treatment or prevention of ocular angiogenesis and neovascularization. Administration of inhibitors of the CCR3 receptor or its ligands eotaxin (CCL11), eotaxin-2 (CCL24) or eotaxin-3 (CCL26) inhibits ocular angiogenesis.

11 Claims, 5 Drawing Sheets
Figure 3

A bar graph showing the CNV volume per laser lesion (µm³) with different concentrations of CCR3 Ab (µg). The concentrations tested are: Control, 0.01, 0.1, 1, 3, 10, and Rat IgG2a. The graph indicates a decrease in CNV volume as the concentration of CCR3 Ab increases from Control to 10 µg, with a notable increase at Rat IgG2a.
Figure 4

% of choroidal cells

Days after laser injury

- Eosinophils
- Mast cells
Figure 5

![Bar chart showing the Macrophage fraction of choroidal cells (in %) for PBS, Rat IgG2A, and CCR3 Ab. The chart shows a comparison between the three groups with error bars indicating variability.]
The present invention relates to the suppression of ocular angiogenesis by inhibiting the CCR3 receptor.

DESCRIPTION OF THE RELATED ART

The macula is the part of the retina which is responsible for central vision. Age-related macular degeneration is a chronic eye disease that occurs when tissue in the macula deteriorates. Macular affects central vision, but not peripheral vision. Macular degeneration is the leading cause of severe vision loss in people age 60 and older.

There are two forms of age-related macular degeneration: dry and wet. Dry macular degeneration is the most common type of macular degeneration and occurs when cells of the macula slowly begin to break down. Yellow deposits called “drusen” form under the retina between the retinal pigmented epithelium (RPE) and Bruch’s membrane, which supports the retina. The drusen deposits are debris associated with compromised cell metabolism in the RPE. Eventually there is a deterioration of the macular regions associated with the drusen deposits resulting in a loss of central vision.

Wet macular degeneration occurs when abnormal blood vessels grow behind the macula. These vessels are fragile and can leak fluid and blood, which result in scarring of the macula and raise the potential for rapid, severe damage. Bruch’s membrane breaks down, usually near drusen deposits. This is where new blood vessel growth, or neovascularization, occurs. Central vision can become distorted or lost entirely in a short period of time, sometimes within days. Wet macular degeneration is responsible for about 10 percent of the cases of age-related macular degeneration, but it accounts for about 90 percent of the cases of legal blindness.

SUMMARY OF THE INVENTION

The present invention relates to a method of inhibiting ocular angiogenesis. The method comprises exposing a choroidal cell to a CCR3-inhibitory effective amount of a compound which inhibits the activity of CCR3. The present invention also relates to a composition for the inhibition of ocular angiogenesis. The composition comprises a compound which inhibits the activity of CCR3.

Other systems, methods, features and advantages of the present invention will be or become apparent to one with skill in the art upon examination of the following drawings and detailed description. It is intended that all such additional systems, methods, features and advantages be included within this description, be within the scope of the present invention, and be protected by the accompanying claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the effect of laser injury on the number of CCR3 receptors on choroidal endothelial cells.
FIG. 2 shows the effect of CCR3 antibody on the proliferation of choroidal endothelial cells following laser injury.
FIG. 3 illustrates the dose-dependent effect of CCR3 antibody on choroidal neovascularization volume.
FIG. 4 shows lack of infiltration of eosinophils and mast cells into the choroid following laser injury.

FIG. 5 illustrates the lack of change of number of infiltrating macrophages in the choroid following laser injury and CCR3 antibody treatment.

DESCRIPTION OF PREFERRED EMBODIMENTS

Intraocular inflammation is not clinically apparent in age-related macular degeneration. However, there is evidence suggesting an influential role for inflammation in this condition. CCR3 is a promiscuous chemokine receptor that is predominantly expressed by eosinophils but also is found on other leukocytes and some endothelial and epithelial cells.

The invention relates to methods and compositions for the treatment or prevention of ocular angiogenesis and neovascularization. Administration of inhibitors of the CCR3 receptor or its ligands, for example eotaxin (CCL11), eotaxin-2 (CCL24) or eotaxin-3 (CCL26), inhibits ocular angiogenesis. Ocular angiogenesis includes choroidal angiogenesis and retinal angiogenesis. Compositions and methods for inhibiting CCR3, eotaxin (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26) for the treatment and/or prevention of neovascular disease are provided. Also provided are novel therapeutic targets and diagnostic markers for choroidal neovascularization.

Any compound which inhibits the activity of CCR3 may be used in the present invention. Such compounds include inhibitory molecules which bind directly to the CCR3 receptor, antibodies which bind the CCR3 receptor or to the natural ligands of the CCR3 receptor, including eotaxin (CCL11), eotaxin-2 (CCL24) and eotaxin-3 (CCL26), RNA, DNA or RNA/DNA aptamers which specifically bind CCR3, eotaxin, eotaxin-2 or eotaxin-3, and siRNA or anti-sense oligonucleotides which inhibit the expression of CCR3, eotaxin, eotaxin-2 or eotaxin-3.

Numerous “small molecule” inhibitors for the CCR3 receptor have been developed and can be used in the present invention. In one aspect the CCR3 inhibitor is an organic molecule having a molecular weight less than 1000. In another aspect of the invention, the CCR3 inhibitor is an organic molecule having a molecular weight less than 500. The CCR3 receptor inhibitors include piperidine derivatives, piperidine amides and piperidine compounds such as those described in U.S. Pat. Nos. 6,984,651 and 6,903,115, and U.S. published applications 20050176708, 20050182094 and 20050182095; heterocyclic piperidines such as those described in U.S. Pat. No. 6,759,411; diphenyl-piperidin derivatives such as those described in U.S. Pat. No. 6,566,376; 2,5-substituted pyrimidine derivatives such as those described in U.S. Pat. No. 6,984,643; piperazimones such as those described in U.S. Pat. No. 6,974,869; bicyclic and tricyclic amines such as those described in U.S. Pat. No. 6,906,666; N-ureidoalkyl-piperidines such as those described in U.S. Pat. Nos. 6,949,546, 6,919,368, 6,906,066, 6,897,234, 6,875,776, 6,780,857, 6,627,629, 6,521,592 and 6,331,541; bicyclic diamines such as those described in U.S. Pat. No. 6,821,964; benzylcycloalkyl amines such as those described in U.S. Pat. No. 6,864,380; 2-substituted-4-nitrogen heterocycles such as those described in U.S. Pat. No. 6,706,735; ureido derivatives of poly-4-amino-2-carboxy-1-methylpyrrole compounds; cyclic and bridged nitrogen heterocycles such as those described in U.S. published application 20050234034; azetidine derivatives such as those described in U.S. published application 20050222118; substituted fused bicyclic amines such as those described in U.S. published application 20050197373; substituted spiro...
azabicycles such as those described in U.S. published application 20050197325; piperidine-substituted indoles or heteroaromatic derivatives thereof as described in U.S. published application 20050153979; piperidinyl and piperazinyl compounds substituted with bicyclo-heterocyclicalkyl groups such as those described in U.S. published application 20050095054; aryloxosulfonamide derivatives such as those described in U.S. published application 20040036779; (N-[2,3-dichlorobenzyl]morpholin-2-yl)methyl-N'[(2-methyl-1H-tetrazol-5-yl)methyl]urea (see, e.g., Nakamura et al., Immunol., 33:213-222, 2006; N-[[3R]-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl]-2-[1-[(3-methyl-1-oxopyridin-2-yl)carbonyl]piperidin-4-ylidene]acetamide (see, e.g., Suzuiki et al., Biochem. Biophys. Res. Commun., 339:2117-2123, 2006; N-[(3R)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl]-2-[1-[(3-hydroxy-3-methylpyrrolidin-2-yl)carbonyl]piperidin-4-ylidene]acetamide hexafluoride (see, e.g., Morokata et al., J. Pharmacol. Exp. Ther., Dec. 9, 2005 [Epub ahead of print]); bi(piperidine amide antagonists of CCR3 such as those described in Ting et al., Bioorg. Med. Chem. Lett., 15:3020-3023, 2005; (S)-methyl-2-naphthylamino-3-(4-nitrophenyl)propanate (see, e.g., Beasley et al., J. Allergy Clin. Immunol., 105: S466-S472, 2000; and the CCR3 antagonist compounds described in Fryer et al., J. Clin. Invest., 116:228-236, 2006.

Additional compounds for inhibiting the CCR3 receptor include RNA, DNA or RNA/DNA aptamers directed against CCR3, eotaxin, eotaxin-2 or eotaxin-3. Exemplary methods for making aptamers are described in U.S. Pat. Nos. 5,270, 163, 5,840,867, 6,180,348 and 6,699,843.

Additional compounds for inhibiting the CCR3 receptor include anti-sense oligonucleotides or siRNAs directed against CCR3, eotaxin, eotaxin-2 or eotaxin-3. Exemplary methods for making antisense oligonucleotides are described (see e.g., Kohler et al., Nature, 256: 495-497 (1975) and Eur. J. Immunol. 6: 511-519 (1976); Milstein et al., Nature 266: 550-552 (1977); Koprowski et al., U.S. Pat. No. 4,172,124; Harlow, E. and D. Lane, 1988, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Cold Spring Harbor, N.Y.); Current Protocols In Molecular Biology, Vol. 2 (Supplement 27, Summer ‘94), Ausubel, F. M. et al., Eds., (John Wiley & Sons: New York, N.Y.); Chapter 11, (1991)). Generally, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as SP2/0) with antibody producing cells. The antibody producing cell, preferably those of the spleen or lymph nodes, are obtained from animals immunized with the antigen of interest. The fused cells (hybridomas) are isolated using selective culture conditions, and cloned by limiting dilution. Cells which produce antibodies with the desired specificity are selected by a suitable assay (e.g., ELISA).

Single chain antibodies, and chimeric, humanized or, primatized (CDR-grafted) antibodies, as well as chimeric or CDR-grafted single chain antibodies, comprising portions derived from different species, are also encompassed by the present invention and the term “antibody”. The various portions of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Cabilly et al., European Patent No. 0,125,023; B1; Boss et al., U.S. Pat. No. 4,816,397; Boss et al., European Patent No. 0,120,694 B1; Neuberger, M. S. et al., WO 86/01533; Neuberger, M. S.
mammalian species, including human, monkey, cow, sheep, pig, goat, horse, mouse, rat, dog, cat, rabbit, guinea pig, hamster and horse. Humans are preferred.

According to the method of the invention, one or more compounds can be administered to the host by an appropriate route, either alone or in combination with another drug. An effective amount of a compound (e.g., a small molecule CCR3 receptor antagonist which inhibits ligand binding, an antibody or a siRNA) is administered. An effective amount is an amount sufficient to achieve the desired therapeutic effect, under the conditions of administration, such as an amount sufficient for inhibition of a CCR3 receptor function, and thereby inhibition of ocular angiogenesis.

A variety of routes of administration are possible including, but not necessarily limited to oral, dietary, topical, parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops), and intraocular injection routes of administration, depending on the disease or condition to be treated. Intraocular injection routes include pericocular (subconjunctival/trans scleral), intravitreal, subretinal and intracameral modes of injection.

Formula of a compound to be administered will vary according to the route of administration selected (e.g., solution, emulsion, capsule). An appropriate composition comprising the compound to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer’s dextrose, dextrose and sodium chloride, lactated Ringer’s or fixed oils. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington’s Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). For inhalation, the compound is solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

EXAMPLE 1

Methods

Laser photocoagulation (532 nm, 200 mW, 100 ms, 75 µm) (OCULIGHT™ GL, Iridex Corporation) was performed (volume studies: 3/eye; protein analyses/flow cytometry: 12/eye) on both eyes of each animal to induce CNV (choroidal neovascularization). CNV volumes were measured by scanning laser confocal microscope (TCS SP, Leica) with 0.5% FITC-Griffonia simplicifolia Isolecitin B4 (Vector Laboratories). CNV was induced by laser injury in CD57BL/6J and Ccr−/− mice and volumes measured 7 days later by confocal evaluation of Griffonia simplicifolia Isolecitin B4 staining of RPE-choroid flatmounts. Neutralizing antibodies (Ab) against CCR3, eotaxin (CCL-11), eotaxin-2 (CCL-24), RANTES, MCP-3 or control goat IgG or rat IgG2a were injected into the vitreous humor following injury.

Flow cytometry was used to determine the numbers of eosinophils, mast cells and macrophages in the choroid, expression of CCR3 by various cell types in the eye, and the cell cycle state of choroidal endothelial cells (CECs) in vivo. Suspensions of cells isolated from mouse RPE/choroid by incubation with collagenase D (20 U/ml; Roche Diagnostics) treatment were incubated in Fe block (0.5 mg/ml; BD Pharmingen) for 15 min on ice. Rat antibody to mouse CCR3 (1:250; Santa Cruz) coupled with PE-donkey anti-
body to rat IgG (1:250; Jackson Immunoresearch) were used to quantitate cell surface receptor expression on choroidal endothelial cells, defined by CD31^+ VEGFR-2^ expression, gated by FITC-conjugated rat antibody to mouse CD31 (1:250; BD Biosciences) and PerCP-Cy-5.5-conjugated rat antibody to mouse CD11b (1:50; BD Biosciences). Macrophages, neutrophils, eosinophils and mast cells were defined as F4/80^+CD11c^-, Gr-1^+F4/80^-, CCR3^+CD3^+CD117^+CD49d^+ and CCR3^+CD3^+CD117^+CD49d^+ cells, respectively. DNA content for cell cycle was analyzed after incubation with propidium iodide (0.05 mg/ml; Molecular Probes) containing 0.1% TRITON™ X-100 and RNase A (0.1 mg/ml; Roche).

Because the probability of each laser lesion developing CNV is influenced by the group to which it belongs, the mouse, the eye, and the laser spot, the mean lesion volumes were compared using a linear mixed model with a split plot repeated measures design. The whole plot factor was the genetic group to which the animal belonged while the split plot factor was the eye. Statistical significance was determined at the 0.05 level. Post hoc comparison of means was constructed with a Bonferroni adjustment for multiple comparisons.

Results

As illustrated by FIG. 1, the number of CCR3 receptors on choroidal endothelial cells in vivo following laser injury (red) is significantly greater than the number before injury (green), indicating upregulation of CCR3 receptors on these cells. Eosinophils or mast cells are the principal cells in most systems that respond to CCR3. However, the number of eosinophils and mast cells in the choroid was unaffected by injury or CCR3 Ab (FIG. 4). CCR3 Ab did not inhibit choroidal macrophage infiltration following injury (FIG. 5), indicating that laser injury is not working by anti-inflammatory means. As illustrated by FIG. 3, CCR3 Ab suppressed CNV volume in C57BL/6J mice by nearly 60% in a dose-dependent and statistically significant manner compared to vehicle control (PBS) and control antibody (rat IgG2a). FIG. 2 demonstrates that CCR3 Ab blockade, but not control antibody (rat IgG2a), inhibited proliferation (S phase) of CECs (choroidal endothelial cells) in vivo following laser injury. Experiments in Ccr3^−/− mice confirmed these results. Of the CCR3 ligands, blockade of only eotaxin (45%) or eotaxin-2 (70%) suppressed CNV in C57BL/6J mice compared to control antibodies (all Ps<0.001). Experiments in Ccl11^−/− and Ccl24^−/− mice confirmed these results.

These findings demonstrate that CCR3 receptor promotes angiogenesis not via leukocyte modulation but rather by direct effects on CECs. Thus, CCL-11, CCL-24, and CCR3 are new targets for neovascular AMD (age-related macular degeneration).

All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various variations and modifications can be made therein without departing from the spirit and scope thereof. All such variations and modifications are intended to be included within the scope of this disclosure and the present invention and protected by the following claims.

1. A method of inhibiting ocular angiogenesis comprising exposing a choroidal cell to a CCR3-inhibitory effective amount of an antibody which inhibits the activity of CCR3.

2. The method of claim 1 wherein the choroidal cell is a choroidal endothelial cell.

3. The method of claim 1 wherein the choroidal cell is a choroidal endothelial cell.

4. The method of claim 3 wherein the antibody is orally administered to the mammal.

5. The method of claim 3 wherein the antibody is intravenously administered to the mammal.

6. The method of claim 3 wherein the antibody is intraocularly injected into the mammal.

7. The method of claim 1 wherein the antibody is an antibody or antibody fragment which specifically binds CCR3 and thereby inhibits the activity of CCR3.

8. The method of claim 1 wherein the antibody is an antibody or antibody fragment which specifically binds CCR3 and thereby inhibits the activity of CCR3.

9. The method of claim 1 wherein the antibody is an antibody or antibody fragment which specifically binds eotaxin and thereby inhibits the activity of CCR3.

10. The method of claim 1 wherein the antibody is an antibody or antibody fragment which specifically binds eotaxin-2 and thereby inhibits the activity of CCR3.

11. The method of claim 1 wherein the mammal is a human.