

Dysregulation of CXCR3 Expression on Peripheral Blood Leukocytes in Patients With Neovascular Age-Related Macular Degeneration

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PURPOSE. The chemokine receptor CXCR3 has been strongly related to inhibition of angiogenesis. The purpose of this study was to investigate the association between expression of CXCR3 on peripheral blood leukocytes and age-related wet macular degeneration. Furthermore, we measured the plasma concentration of chemokines CXCL9 to -11.

METHODS. The study group consisted of patients with age-related macular degeneration (AMD) attending our department. Patients referred for reasons other than AMD were enrolled as control subjects. The expression of CXCR3 on T cells and the plasma concentration of CXCL9 to -11 were measured using flow cytometry.

RESULTS. We looked at all CD8⁺ T cells expressing CXCR3 and found a significantly lower percentage of these cells in the neovascular AMD group compared to the age-matched control group ($P = 0.05$). When dividing the CD8⁺ cells into functional groups according to their expression of CXCR3, we found a significantly lower percentage of CD8⁺ CXCR3^{high} cells in the group with neovascular AMD compared to the control group ($P = 0.038$). We found a lower percentage of CD4⁺CD69⁺CXCR3⁺ T cells in the group of patients with neovascular AMD when compared to the age-matched control group ($P = 0.052$).

CONCLUSIONS. Our results point toward a systemic dysregulation of CXCR3 in patients with neovascular AMD. Since there is evidence to suggest that CXCR3 is able to alter the response of VEGF, the primary driver of choroidal neovascularization (CNV) formation, low levels of CXCR3 could potentially drive some patients toward a more angiogenic profile leading to CNV formation and growth. CXCR3-enhancing molecules could therefore be a possible target for treatment of AMD.

Keywords: AMD, chemokines, neovascularization, VEGF

The chemokine receptor CXCR3 plays an important role in chemokine ligand CXCL10-mediated inhibition of vascular endothelial growth factor (VEGF)-induced angiogenesis.¹ Angiogenesis is one of the cardinal pathogenic processes in neovascular age-related macular degeneration (AMD). In neovascular AMD the angiogenic signal seems to be driven primarily by VEGF.² Even though anti-VEGF treatment has drastically improved the outcome of AMD, development of new treatment is still needed.³

Age-related macular degeneration is the leading cause of visual impairment and legal blindness among elders in the western part of the world.⁴ A multitude of factors contribute to the development and progression of the disease. Among these are factors such as single nucleotide polymorphisms (SNP) in the complement factor H (*CFH*) gene, which have proven to have an important influence on the risk of developing AMD.⁵ It is now generally accepted that inflammation plays an important role in the development and progression of AMD. Para-

inflammation, low-grade chronic inflammation, has been proposed as an important factor for development of AMD.⁶ Among the different responses to chronic inflammation are accumulation of activated immune cells at sites of inflammation, scar formation, and angiogenesis.^{7,8} Angiogenesis in the form of choroidal neovascularization (CNV) is the cardinal feature of neovascular AMD. Left untreated, the development of a central scar in the retina in form of subretinal fibrosis might be the end stage of neovascular AMD.⁹

Angiogenesis is strictly regulated by levels of angiogenic and angiostatic factors.^{1,10} Alterations in expression of these factors can cause pathological conditions with either excessive growth of new vessels or premature termination of vessel growth.¹¹ Chemokines have been shown to possess both angiogenic and angiostatic properties.⁷ Chemokines are a subfamily of cytokines. Originally, chemokines were characterized by their ability to attract and activate leukocytes at sites of inflammation. Numerous chemokines and receptors have been shown to

influence the development and progression of AMD.^{6,12} Chemokines are divided into four groups according to a shared cysteine motif. One group of chemokines, the CXC chemokines, is characterized by the presence or absence of an ELR motif, a three-amino acid residue, preceding the first cysteine amino acid residue.¹¹ In general, chemokines that lack the ELR motif are interferon gamma inducible and inhibit angiogenesis. Chemokines containing the ELR motif are known to promote angiogenesis.^{11,13} CXCL10 belongs to the CXC group of chemokines that are ELR negative. CXCL10 signals through the chemokine receptor CXCR3. CXCR3 is highly expressed on T cells upon activation and is a known chemoattractant allowing cells to enter sites of inflammation.¹⁴ Furthermore, CXCR3 has been shown to possess antifibrotic abilities,¹⁵ and it has recently been demonstrated that CXCL10 via its signaling with CXCR3 has angiostatic properties.¹

Since formation of CNV seems to be mainly driven by VEGF factors influencing VEGF could have a potentially important role in the development and progression of AMD. The chemokine receptor CXCR3 has been shown to alter VEGF-induced angiogenesis when binding CXCL10.¹ Most studies on CXCR3 and angiogenesis are laboratory studies using cell cultures or murine models. Only a few studies have investigated CXCR3 and its ligands in AMD patients, and none have looked at CXCR3 expression on peripheral blood leukocytes in AMD patients. Therefore we decided to investigate the association between expression of CXCR3 on peripheral blood leukocytes and AMD. Furthermore, we measured the plasma concentrations of the CXCR3 ligands CXCL9 to -11.

METHODS

Participants

Patients with AMD attending the retinal clinic in our department were asked to participate in this case-control study, while individuals attending our department for other reasons were asked to participate as control subjects. Patients diagnosed with AMD were divided according to their disease stage into three groups: One was early patients with AMD defined by presence of drusen; another was patients with geographic atrophy (GA); and the third group consisted of patients with neovascular AMD. None of the patients had signs of polypoidal vasculopathy, retinal angiomatous proliferation, or chorioretinal anastomosis. Patients in the study group and in the control group were excluded if they were diagnosed with malignant disease or autoimmune diseases including type 1 diabetes or if they were in active treatment with immunosuppressant agents. Finally, all participants having a serum C-reactive protein (CRP) > 10 (as a sign of acute or chronic systemic inflammation) were excluded. All patients in the study group with neovascular AMD were treatment naïve to bevacizumab (Avastin; Roche, Basel, Switzerland) and aflibercept (Eylea; Bayer, Leverkusen, Germany) and had not received an intravitreal injection of ranibizumab (Lucentis; Genetech, San Francisco, CA, USA) for the 5 weeks prior to inclusion.

All participants underwent a structured interview focusing on current and previous medical conditions and current medication. All participants were asked about their smoking history and alcohol consumption habits. Patients were defined as current smokers, former smokers (more than 100 cigarettes during their lifetime), or never smokers.¹⁶ Alcohol consumption was graded according to the Danish National Board of Health's recommendations (maximum 7 and 14 units per week for women and men, respectively). Body mass index (BMI) was calculated from height and weight measured at the first visit.

Part of the study population involved in this study has been described in previous studies.¹⁷⁻¹⁹

Verbal and written informed consent was obtained from all participants prior to inclusion. The study was approved by the Regional Committee of Ethics in Research of the Region of Zealand (SJ-142) and was performed in accordance with the Declaration of Helsinki.

Ophthalmic Examination

All patients included in this study were examined with slit-lamp biomicroscopy and fundoscopy, color fundus photography (Carl Zeiss, Jena, Germany), spectral-domain optical coherence tomography (SD-OCT), and fundus autofluorescence imaging (Spectralis HRA-OCT; Heidelberg Engineering, Heidelberg, Germany). Visual acuity (VA) was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. Fluorescein and indocyanine green (FA/ICG) angiography was performed on patients suspected of having neovascular AMD in order to obtain the correct diagnosis.

Leukocyte Preparation and Flow Cytometry

Venous blood samples for flow cytometry and chemokine quantification were obtained from all patients included in the study. The phlebotomy was performed prior to angiography to avoid possible interference.²⁰ Within 4 hours of phlebotomy the blood samples were prepared for flow cytometry in the following steps. Red blood cell lysis was performed by adding 10% red blood cell lysis buffer (BioLegend, San Diego, CA, USA) to the whole blood followed by storage for 10 minutes in the dark at room temperature. After red blood cell lysis, the sample was washed three times in isotonic buffer (IsoFlow Sheath Fluid; Beckman Coulter, Brea, CA, USA) and centrifuged for 5 minutes at 500g. After washing, the cells were incubated at room temperature in the dark for 25 minutes with the following monoclonal anti-human antibodies: CD4 (IgG1, clone 13B8.2; Beckman Coulter), CD8 (IgG1, clone SFC121-Thy2D3; Beckman Coulter), CD25 (IgG2a, clone B1.49.9; Beckman Coulter), CD69 (IgG2b, clone TP1.55.3; Beckman Coulter), CXCR3 (IgG1, clone 49801; R&D Systems, Inc., Minneapolis, MN, USA). Corresponding negative isotype controls were used and set at 1%. Flow cytometry was performed on a Beckman Coulter FC 500 flow cytometer.

To examine the CXCR3 expression on different subsets of T cells, the cells were first divided into two groups dependent on expression of CD4 and CD8. T cells expressing CD4 were then further divided into activated cells (cells expressing activation marker CD69) and regulatory cells (cells expressing CD25). CD8-positive T cells were gated further to isolate the subset expressing the activation marker CD69. The percentages of these different T-cell subsets expressing CXCR3 were then found. An example of the gating strategy is shown in Figures 1 and 2.

Quantification of CXCL9, CXCL10, and CXCL11

Blood samples were centrifuged for 15 minutes at 1000g, and plasma was isolated and stored at -80°C until analyses were performed. CXCL9, CXCL10, and CXCL11 were measured using a Cytometric Bead Array (BD Biosciences, Franklin Lakes, NJ, USA) following the manufacturer's recommendations.

Clinical Response

To assess the clinical relevance of peripheral expression of CXCR3 and CXCL9 to -11, we looked at treatment response after the initial three anti-VEGF injections of ranibizumab, since

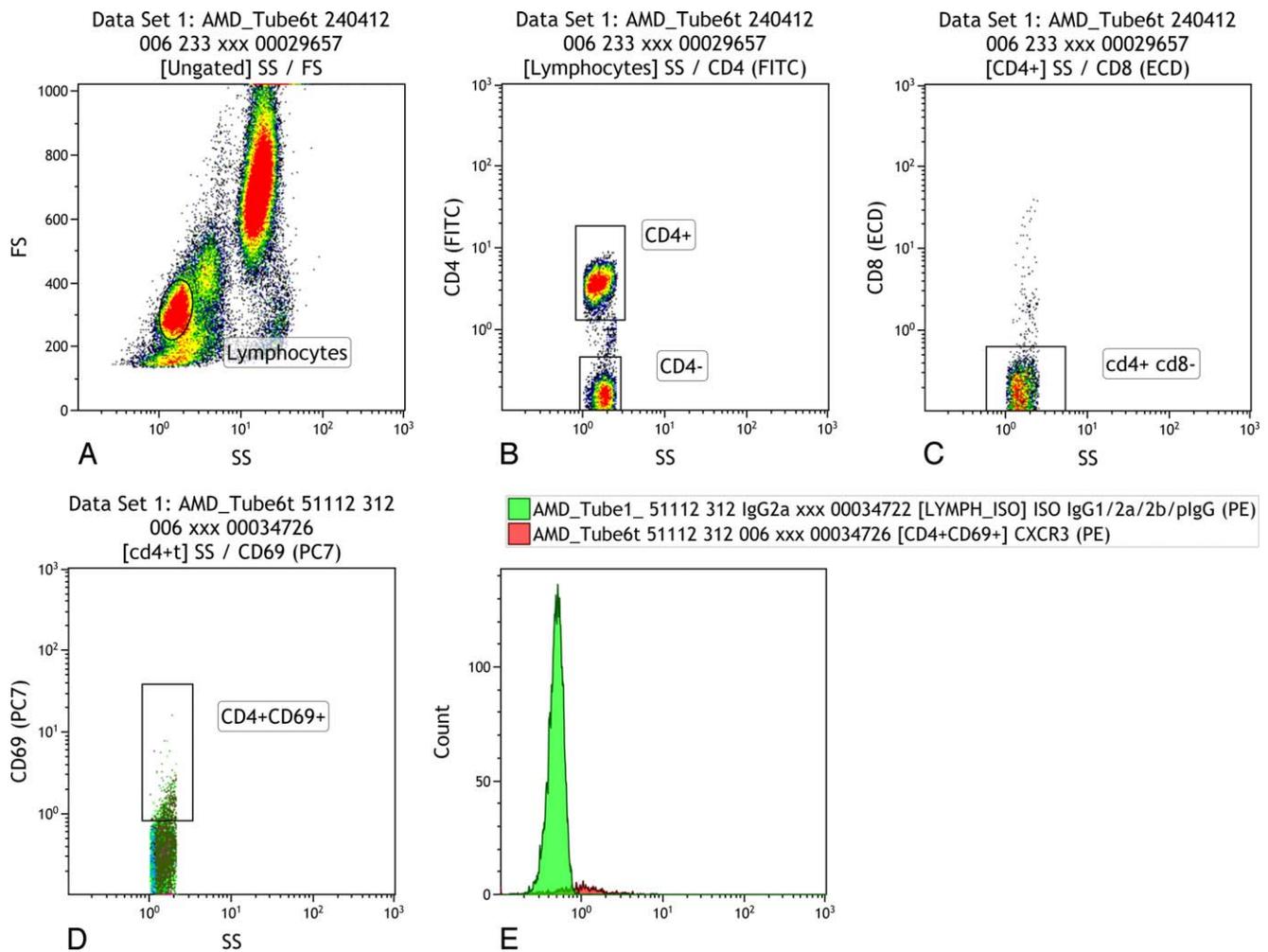


FIGURE 1. Gating strategy and quantification of CD4⁺ CD69⁺ T cells and their expression of CXCR3. (A) Lymphocytes were identified based on forward scatter and side scatter. (B) The lymphocytes were further gated based on their CD4 expression. (C) The subset of CD4⁺ T cells that were CD8 negative was found. (D) The subset of CD4⁺ CD8⁻ T cells that were CD69 positive was found. (E) The fraction of CXCR3⁺ cells within the CD4⁺ CD8⁻ CD69⁺ T cells was calculated. Histogram showing the percentage of CD4⁺ CD8⁻ CD69⁺ T cells positive for CXCR3 (red) and the negative isotype control set at 1% (green).

it has been suggested that VA after the initial three anti-VEGF injections (among others) is a useful predictor of treatment response.²¹ Patients in active treatment with ranibizumab were divided into three groups according to the change in VA between the first visit and the follow-up visit after the initial three intravitreal injections. The groups were (1) good responders—gain of more than 10 ETDRS letters; (2) intermediate responders—VA change (gain or loss) of fewer than 10 ETDRS letters; and (3) poor responders—loss of more than 10 ETDRS letters.

Statistical Analysis

The statistical software SPSS version 19 for Windows (IBM, Chicago, IL, USA) was used for statistical analysis. Kruskal-Wallis test and Mann-Whitney *U* test were used for not normally distributed continuous variables (CXCR3, CXCL9, CXCL10, CXCL11, age, and BMI), and data are given as medians and interquartile ranges (IQR). Categorical variables were analyzed using Pearson χ^2 test (sex, alcohol intake, smoking habits). A *P* level of 0.05 or less was considered significant.

RESULTS

Demographic Data and Clinical Characteristics

A total of 162 participants were included in the study. Among these were 131 diagnosed with AMD and 31 control individuals. The clinical and demographic data for participants are shown in Table 1. No significant difference in male-to-female ratio, age, smoking habits, alcohol consumption, or BMI was found between the groups.

CXCR3 Expression on T Cells

CXCR3 expression in the different groups can be seen in Table 2.

We looked at all CD8⁺ cells expressing CXCR3 and found a significantly lower percentage of these cells in the neovascular AMD group compared to the age-matched control group (*P* = 0.05). No significant difference was found in comparing the neovascular AMD group with the GA group (*P* = 0.39) or the early AMD group (*P* = 0.11), and no significant difference was found in comparing the GA group with the early AMD group (*P* = 0.81).

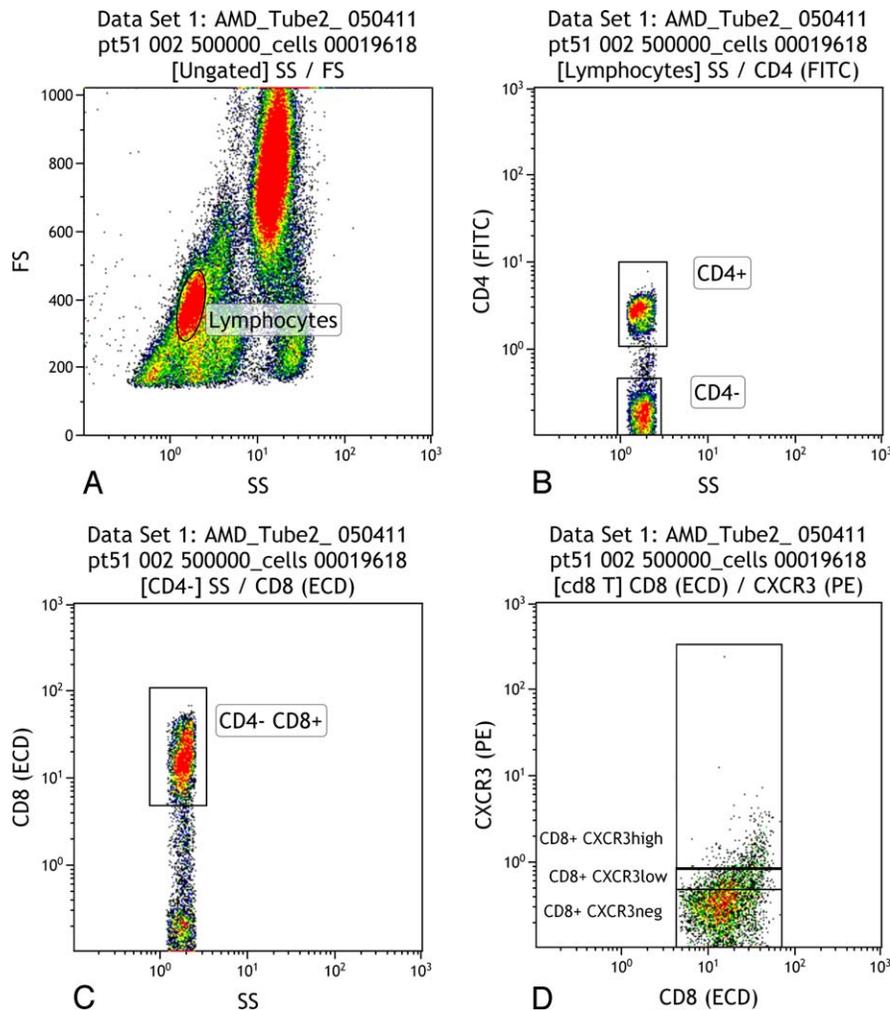


FIGURE 2. Gating strategy to quantify different subsets of CD8⁺ T cells based on their expression of CXCR3. (A) Lymphocytes were identified based on forward scatter and side scatter. (B) The lymphocytes were further gated based on CD4 and CD8 expression. (C) The subset of CD8-positive T cells that were CD4 negative was found. (D) The CD8⁺CD4⁻ T cells were divided into three groups based on their expression of CXCR3.

CD8 T cells can be divided into different functional groups according to expression level of CXCR3 (high, low, or no expression) (Fig. 2).²² We found that the neovascular AMD group had a significantly lower percentage of CD8⁺ CXCR3^{high} cells compared to the age-matched control group ($P = 0.038$). No significant difference was found in comparing the neovascular AMD group with the GA group ($P = 0.08$) or the early AMD

group ($P = 0.73$), and no significant difference was found in comparing the GA group with the early AMD group ($P = 0.21$).

Moreover, we found a larger percentage of CD8⁺ cells that were CXCR3^{negative} in the neovascular AMD group compared to the age-matched control group, a difference very close to being significant ($P = 0.052$). The CXCR3 expression in the different groups is shown in Table 2.

TABLE 1. Demographic Data and Clinical Characteristics of Patients in Whom CXCR3 Expression Was Measured

	Age-Matched Controls, <i>n</i> = 31	Early AMD, <i>n</i> = 30	GA, <i>n</i> = 12	nAMD, <i>n</i> = 89	<i>P</i> Value	Test
Male, %	59.1	50.0	33.3	42.7	0.778	Pearson χ^2
Female, %	40.9	50.0	66.7	57.3		
Median age (IQR)	74.0 (69.0–79.0)	77.5 (70.8–80.5)	75.5 (72.3–85.5)	75 (70.0–81.0)	0.473	Kruskal-Wallis
Current smokers, % yes	4.6	11.5	20.0	20.2	0.566	Pearson χ^2
Former smokers, % yes	40.9	46.2	50.0	41.7		
Never smokers, % yes	54.5	42.3	30.0	38.1		
Alcohol above recommended, % yes	18.2	13.3	16.7	15.7	0.981	Pearson χ^2
BMI median (IQR)	26.7 (24.2–30.6)	25.8 (22.3–28.4)	29.1 (23.0–30.7)	24.9 (22.8–28.1)	0.245	Kruskal-Wallis

nAMD, neovascular AMD.

TABLE 2. Median Frequency (%) of Cells Within the T-cell Subsets Expressing CXCR3 and Percentages of CD8⁺ Cells That Were CXCR3 High, Low, or Negative (Data Given as Medians and Interquartile Range)

	Age-Matched Controls, <i>n</i> = 31	Early AMD, <i>n</i> = 30	GA, <i>n</i> = 12	nAMD, <i>n</i> = 89	<i>P</i> Value*
CD4 ⁺ T cells					
CD4 ⁺ CXCR3 ⁺	14; 11.0–19.0	14; 8.0–19.7	10.8; 8.5–21.0	14.2; 7.3–24.3	0.898
CD4 ⁺ CD25 ⁺ CXCR3 ⁺	13.0; 10.0–20.1	11.7; 6.8–19.3	9.0; 5.9–21.0	14.0; 7.0–23.8	0.845
CD4 ⁺ CD69 ⁺ CXCR3 ⁺	35.8; 22.9–47.2	27.8; 22.2–37.4	25.0; 13.0–46.5	27.0; 17.5–43.4	0.052
CD8 ⁺ T cells					
CD8 ⁺ CXCR3 ⁺	17.5; 5.9–25.3	12.0; 4.8–15.3	12.0; 3.3–21.6	8.5; 4.2–18.3	0.05
CD8 ⁺ CD69 ⁺ CXCR3 ⁺	23.0; 8.3–32.0	17.4; 10.4–27.3	17.6; 3.4–28.9	13.3; 7.1–29.2	0.256
CD8 ⁺ CXCR3 ^{high}	3.4; 1.8–8.1	1.8; 0.7–4.6	3.6; 2.2–6.9	2.4; 1.1–3.8	0.038
CD8 ⁺ CXCR3 ^{low}	9.7; 6.5–16.2	12.3; 6.0–14.8	10.1; 6.3–16.6	7.0; 5.0–11.3	0.078
CD8 ⁺ CXCR3 ^{negative}	87.3; 73.7–91.2	85.2; 80.1–93.2	86.6; 75.6–89.1	90.7; 85.7–93.0	0.052

* Kruskal-Wallis was performed on all cell subset data. All reported *P* values are nAMD versus age-matched controls, Mann-Whitney *U* test since the other comparisons were nonsignificant.

We found a close to significantly lower percentage of CD4⁺CD69⁺CXCR3⁺ T cells in the group of patients with neovascular AMD when compared to the age-matched control group (*P* = 0.052).

Chemokine Plasma Level

The plasma concentrations of the chemokines CXCL9, CXCL10, and CXCL11 were measured in all patients included in the study. No significant differences were found between any of the AMD groups or between the age-matched control group and any of the AMD groups. The plasma levels of CXCL9 to -11 can be seen in Table 3.

Clinical Response to Anti-VEGF

To assess the clinical relevance of peripheral expression of CXCR3 on CD4⁺ and CD8⁺ T cells, patients were divided into three groups according to change of VA after the initial three injections with ranibizumab as outlined previously. We also correlated the peripheral concentration of CXCL9, CXCL10, and CXCL11 to change in VA. The VA was measured at the first visit in our department and at the follow-up visit after the third intravitreal injection of ranibizumab. Of the 46 patients in active treatment with ranibizumab, 17 were in the group of good responders defined by an improvement in VA of at least 10 ETDRS letters. Twenty patients were categorized as belonging to the second group, intermediate responders, defined by a change in VA of fewer than 10 ETDRS letters; nine patients were categorized as belonging to group of poor responders defined by a visual loss of at least 10 ETDRS letters.

No difference in expression of CXCR3 or difference in the concentrations of the chemokines CXCL9 to -11 was found between the three groups.

DISCUSSION

The purpose of this study was to examine the expression of CXCR3 on different subsets of T cells and to measure the plasma concentration of the chemokines CXCL9, CXCL10, and CXCL11 in patients with different stages of AMD.

The cardinal clinical finding in neovascular AMD is CNV formation. The only current treatment of AMD is intravitreal injections with anti-VEGF targeting the receptor for VEGF on endothelial cells in the choroid.^{2,4} Even though treatment with anti-VEGF has proven effective, there is a need for developing new treatments and finding new targets of treatment, since a

considerable proportion of patients with neovascular AMD lose vision in spite of treatment with anti-VEGF.³

The chemokine receptor CXCR3 has a great variety of functions and is expressed on cells throughout the body.^{11,23} CXCR3 has a range of functions in the inflammatory response and, among others, chemotaxis, the direction of immune cells to sites of inflammation. Furthermore, CXCR3 is involved in wound healing and scar formation.¹⁵

The chemokine CXCL10 belongs to the subfamily of CXC chemokines missing the ELR motif in the C-terminal end of the protein. These chemokines are known to contain antiangiogenic properties. The chemokine receptor for CXCL10 is CXCR3, which is highly expressed on activated T cells and on endothelial cells, where it has been strongly related to blocking formation of new blood vessels.^{11,24–26} Recently one study showed that the chemokine CXCL10, directly via its signaling with CXCR3, reduces the endothelial cells' ability to migrate and form tubes.¹ The molecular mechanism underlying the antiangiogenic mechanism of CXCR3 remains largely unknown. Whether the antiangiogenic mechanism of CXCR3 is related to the VEGF pathway has been discussed. One study on laser-induced CNV formation in mice did not find any differences in mRNA expression of VEGF and therefore suggested that the antiangiogenic mechanism of CXCR3 works independently of VEGF.²⁷ However, a recent study demonstrated that treatment of endothelial cells with VEGF induces an m-calpin-mediated growth while stimulation of CXCR3 expressed on these endothelial cells with its ligand CXCL10 significantly decreased this activity, suggesting that CXCR3 could have a counterproductive effect relative to VEGF.²⁴

CXCR3 has been detected in human and murine endothelial cells from choroidal vessels.²⁷ In a study using aged mice and mice with laser-induced CNV formation, Fujimura et al.²⁷ investigated the effect of CXCR3 on CNV formation. They found an increased expression of CXCR3 and its ligand CXCL10 in CNV tissue from mouse eyes with laser-induced CNV. Furthermore, they found that CXCR3-deficient mice developed larger CNVs and that leakage was greater compared to that in control eyes. Larger CNV and greater leakage were also found in laser-treated wild-type mice treated with intravitreal anti-CXCR3 or intravitreal neutralizing antibodies against CXCL10 when compared to control eyes.²⁷

Yates-Binder et al.¹ showed that in vitro growth of VEGF-stimulated human endothelial cells could be inhibited by CXCL10. When CXCR3 was blocked by addition of anti-CXCR3, the CXCL10-mediated growth inhibition was reversed. This demonstrated that CXCL10 was unable to override the VEGF signal when the CXCR3 receptor was blocked. At the

TABLE 3. Median Plasma Concentration of CXCL9, CXCL10, and CXCL11 (Data Given as Medians and Interquartile Range)

	Age-Matched Controls, <i>n</i> = 31	Early AMD, <i>n</i> = 30	GA, <i>n</i> = 12	nAMD, <i>n</i> = 89	<i>P</i> Value, Kruskall-Wallis Test
CXCL9	886.6; 527.9–1269.4	1109.9; 696.6–1597.6	832.1; 526.0–1501.4	886.7; 513.3–1765.1	0.896
CXCL10	183.7; 131.0–426.5	238.8; 177.5–352.1	192.5; 127.3–192.5	233.5; 166.1–314.4	0.469
CXCL11	665.8; 138.6–1116.1	637.0; 133.9–1240.4	577.0; 339.5–1303.2	461.8; 179.0–461.8	0.559

same time it was taken as proof of the antiangiogenic properties of CXCL10 as directly mediated by signaling with CXCR3.¹

In accordance with this, we found a downregulation of the expression of CXCR3 on several subsets of peripheral T cells in patients with neovascular AMD. This could indicate a general dysregulation of the CXCL10/CXCR3 receptor/ligand system in patients with neovascular AMD and a subsequent disability to downregulate or inhibit the proangiogenic signal in the choroid, since Yates-Binder et al.¹ showed that dysregulation or blocking of CXCR3 leads to angiogenesis.

We did not find any significant differences in expression of CXCR3 between subgroups of AMD. However, looking at the results, there seems to be a lower expression level of CXCR3 on several T-cell subsets even though significance was not reached. This could be due to the relatively low number of patients included, especially in the GA and early AMD groups. Another possible explanation could be that even a limited change in expression of CXCR3 along with other contributing factors could have a large impact on angiogenesis in the eye while this change could be difficult to detect systemically. Finally, it could be hypothesized that some of the patients enrolled with early AMD eventually will develop neovascular AMD and therefore already will have dysregulation of CXCR3.

In addition to the direct effect of CXCR3-mediated angiostasis is the concept of immunoangiostasis.¹¹ In immunoangiostasis the biological function of CXCR3 is linked to cell-mediated immunity. This has been demonstrated in cancer research in which local Th1-mediated response to tumor antigen expression leads to a chemotactic gradient within the tumor tissue, attracting CXCR3-expressing Th1 cells and subsequent mononuclear cells into the tumor tissue. The mononuclear cells then promote vessel and tumor regression.^{11,28} We found a lower expression of CXCR3 on activated T-helper cells (CD4⁺CD69⁺ T cells) in patients with neovascular AMD compared to controls. Whether immunoangiostasis plays a role in the development of CNV remains to be resolved.

We found that patients with neovascular AMD have a significantly lower percentage of CD8⁺ CXCR3^{high} cells compared to age-matched controls. The CXCR3 expression on CD8 cells has been shown to play an important role in CD8⁺ cell development and in the CD8⁺ cells' ability to migrate. Cells with high expression of CXCR3 migrate more readily to sites of inflammation.^{22,29} In a flow-based adhesion assay with human hepatic endothelium, it was demonstrated CXCR3 expressed on the endothelium promotes adhesion of effector T cells to the endothelium and drives the trans-endothelial migration. This response could be stimulated by both exogenous and endogenous secreted CXCR3 ligands.³⁰ Our results indicate deficient T-cell chemotaxis in AMD.

The search for a possible biomarker to predict AMD at an early stage or even before ophthalmological signs are visible has been going on for the last 10 years. Especially, two biomarkers have been suggested: CCL3 and CXCL10.³¹ We did not find alterations in concentration of CXCL10 among the groups of AMD patients and the control group.

To find out whether the peripheral expression of CXCR3 or the plasma concentration of its ligands CXCL9 to -11 could

influence the treatment response to anti-VEGF therapy, we measured VA in patients in active treatment with ranibizumab. We did not find a correlation between either expression of CXCR3 and visual outcome or concentrations of the chemokines CXCL9 to -11 and visual outcome.

There is comprehensive evidence pointing toward CXCL10 as a strong inhibitor of angiogenesis via its signaling with the receptor CXCR3. Yates-Binder et al.¹ suggested the possible use of CXCL10 as a therapeutic target in diseases with uncontrolled angiogenesis. Our results point toward a systemic dysregulation of CXCR3 and indicate that patients with neovascular AMD have lower levels of T cells expressing CXCR3 than control individuals. Since evidence points toward CXCR3 as able to alter the response of VEGF, the primary driver of CNV formation, low levels of CXCR3 could potentially drive some patients toward a more angiogenic profile leading to CNV formation and growth. CXCR3-enhancing molecules could therefore be a possible target for treatment of AMD.

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