Neutrophil-dominant experimental autoimmune uveitis in CC-chemokine receptor 2 knockout mice

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ABSTRACT.

Purpose: Murine experimental autoimmune uveitis (EAU) is an animal model of human uveitis. It has been demonstrated that ocular-infiltrating macrophages are crucial for EAU induction, and monocyte chemoattractant protein-1 (MCP-1) was actually upregulated in the eye. CC chemokine receptor-2 (CCR2) is the receptor of MCP-1, and macrophages fail to recruit particular lesions in CCR2 knockout (KO) mice. To confirm the role of macrophages in EAU, we examined EAU in CCR2 KO mice.

Methods: CCR2 KO mice and wild-type (WT) mice that had the same genetic background were immunized with human interphotoreceptor retinoid-binding protein peptide 1–20 emulsified in complete Freund’s adjuvant. At multiple time-points, EAU severity was evaluated based on microscopic fundus observation and histological examination. To examine the phenotype of retinal-infiltrating cells, single cells were prepared from the eye and analysed by flow cytometry.

Results: In WT mice, EAU was induced at the peak of day 16 and marked macrophage infiltration was observed. Although macrophages failed to be recruited into the eye in CCR2 KO mice, severe uveitis was induced unexpectedly. Flow cytometry and histology revealed that most of the infiltrating cells were neutrophils. We also compared the intraocular chemokine concentrations between WT mice and KO mice. Two CXC chemokine (monokine induced by interferon-γ and interferon-γ-inducible protein-10) were upregulated in KO mice.

Conclusion: Interphotoreceptor retinoid-binding protein peptide immunization caused neutrophil-dominant uveitis in CCR2 KO mice. In the absence of macrophages, neutrophils can be alternatively recruited and can cause tissue damage.

Key words: autoimmunity – chemokines/monokines – eye (ocular) immunology disease – macrophages/monocytes

Introduction

Experimental autoimmune uveoretinitis (EAU) is an organ-specific, T cell-mediated autoimmune disease that can be induced in several animals by immunization with retinal Ag, such as interphotoreceptor retinoid-binding protein (IRBP) and S-antigens, emulsified with complete Freund’s adjuvant (CFA) (Nussenblatt et al. 1981). Immunized animals develop a disease that resembles human uveitis (Forrester 1991). In EAU, the blood retina barrier is broken, allowing leukocytes to move into the retina and cause tissue damage (Forrester 1990).

The accumulation of leukocytes at inflammatory sites is regulated by a family of small, discrete chemotactic proteins called chemokines. Several reports have described local chemokine expression in EAU (Crane et al. 2001; Foxman et al. 2002; Keino et al. 2003). Adamus et al. (2001) showed that the highest expression of RANTES, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α) in the eye was detected at the onset of anterior uveitis associated with rat experimental autoimmune encephalomyelitis (EAE). Also, chemokines have been shown to be involved in...
leukocyte recruitment in the eye in a study of patients in which MCP-1, IL-8, interferon-γ-inducible protein-10 (IP-10), RANTES and MIP-1α were significantly increased in the aqeous humour during the active stages of uveitis (Verma et al. 1997; Takase et al. 2006).

Two major chemokine subfamilies are distinguished by whether the first two conserved cysteine residues occur together (CC) or are separated by another amino acid (CXC). MCP-1 is one of the major CC chemokines and is produced by a wide variety of cell types in response to pro-inflammatory stimuli (Furutani et al. 1989). The CC chemokine receptors (CCR) are structurally related, seven transmembrane-spanning proteins that signal through heterotrimetric G-protein complexes. In mice, MCP-1 appears to bind solely to CC chemokine receptor-2 (CCR2) (Charo et al. 1994; Boring et al. 1996), although CCR2 also serves as a receptor for the other MCP subfamily members. CCR2 was mainly expressed on macrophages and monocytes. CCR2 knockout (KO) mice were originally reported by Boring et al. (1997) and Kuziel et al. (1997) independently and showed normal development and blood leukocyte differentiation. In response to thioglycollate, the recruitment of peritoneal macrophages decreased selectively (Boring et al. 1998).

Macrophages play an important role in generating tissue damage in the course of experimental autoimmune diseases, such as collagen-induced arthritis (Dijkstra et al. 1987) and encephalomyelitis (Godiska et al. 1995). In uveoretinitis, it was reported that macrophages are involved in phagocytosis of rod outer segments (Butler & McMenamin 1996; Dick et al. 1996). A depletion study in rats showed that activated macrophages are major effectors of tissue damage in uveitis (Forrester et al. 1998; Pouvreau et al. 1998). To confirm the role of macrophages in EAU, we examined EAU in CCR2 KO mice. It was already shown that EAE might fail to be induced in CCR2 KO mice (Izikson et al. 2000), thus our original hypothesis was that EAU might also fail to be induced in CCR2 KO mice.

In this report, in contrast to our original hypothesis, we demonstrate that atypical neutrophil-dominant EAU was induced in CCR2 KO mice. Interferon-γ (IFN-γ)-producing autoreactive T cells were induced in CCR2 KO mice. Even in the absence of CCR2-dependent local macrophage infiltration, another type of ocular inflammation can occur and damage the eye.

**Materials and Methods**

**Mice**

Female, 8- to 10-week-old mice were used in all experiments. CCR2 KO mice were generated by homologous recombination as previously described (Boring et al. 1998). CCR2 KO mice and wild-type (WT) CCR2 (+/+) littermate controls generated by maternatal recombination as previously described (Boring et al. 1998). All treatments of the animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**EAU induction and evaluation**

Experimental autoimmune uveitis was induced in mice by the human IRBP peptide 1–20 sequence (GPTHFLQPSLVLMKAVLDD) that induces EAU (Avichzer et al. 2000). Mice were immunized subcutaneously in both footpads and base of tail with the peptide, in 0.2-ml emulsion in CFA (1:1, v/v) that had been supplemented with *Mycobacterium tuberculosis* strain H37RA to 2.5 mg/ml and 5 mg/ml gentamycin, 5 × 10⁻⁵ M 2-mercaptoethanol and 5 mg/ml HEPES buffer. They were incubated with or without IRBP₁₋₂₀ peptide (10 μg/ml) in 96-well culture plates (Costar, Cambridge, MA, USA) for 48 hr at 37°C. Supernatant was collected after 48 hr, and IFN-γ concentration was measured by quantitative capture ELISA, according to the manufacturer's instructions (BD Bioscience, San Jose, CA, USA). In brief, ELISA plates (Nunc-Immuno³ plate; VWR Scientific Products, Bridgeport, NJ, USA) were coated with rat anti-mouse IFN-γ mAb (R4-6A2; BD Bioscience). Recombinant mouse IFN-γ (BD Bioscience) was used to construct a standard curve, and biotinylated rat anti-mouse IFN-γ mAb (XMG1.2; BD Bioscience) was used as the detecting antibody. The plates were treated with alkaline phosphatase-conjugated ExtrAvidin (Sigma), substrat-ed colour by p-nitrophenyl phosphate (Sigma) and OD was measured at 405 nm by an MRX microplate reader (Synatek Laboratories, Chantilly, VA, USA). The values represent the arithmetic mean of triplicate cultures ± SD.
Isolation and counting of the ocular-infiltrating viable cells

To examine the phenotype of ocular-infiltrating cells, cells were prepared according to the procedure for the isolation of corneal-infiltrating cells, with some modifications (Sonoda et al. 2003). In brief, the tissue around the eyeball was removed, and the eyeball was dissected to remove the cornea and lens. The posterior segment of the eye was disrupted with scissors and then shaken in medium supplemented with 0.5 mg/ml of collagenase type D (Boehringer Mannheim, Germany) at 37°C for 40 min. As a basic medium, we used RPMI 1640 (Gibco Laboratories) with 10% foetal bovine serum (Gibco, Carlsbad, CA, USA). Streptavidin was purchased from Molecular Probes (Eugene, OR, USA). Biotin-conjugated IgG (clone A3-1) was purchased from IQ Products (Groningen, the Netherlands). Biotin-conjugated anti-F4/80 mAb double-stained cells were analysed as macrophages. We considered CD45 positive and F4/80 negative cells as neutrophils and confirmed that CD45 positive and F4/80 negative cells were also Gr-1 positive (Fig. 2A, lower panel).

Flow cytometry was performed with EPICS XL (Becton Coulter, Mannheim, Germany). The numbers of intraocular-infiltrating macrophages, neutrophils and T lymphocytes were calculated from the percentage of each population in the gate of the precounted total number of viable cells using trypan blue dye exclusion. Appropriate liberal leucocyte gates and instrument variables were set according to forward and side scatter. We used “lymphocytes gate” and “neutrophils and macrophages gate” as we reported previously (Sonoda et al. 2003).

Ocular fluid preparation and protein assay

Eyes were enucleated under deep anaesthesia, the conjunctival tissue was removed and the remaining eye tissues were homogenized using a Biomasher (Nippi, Tokyo, Japan). After centrifugation at 12 000 g for 30 min, the supernatant was collected, and the concentrations of IP-10, the monokine induced by interferon-γ (MIG), keratinocyte-derived chemokine (KC), MIP-1α and MCP-1 were measured using a microbead-based ELISA system (Mouse Cytokine Twenty-plex Antibody Bead kit, cat. no. LMC0006; BioSource International, Camarillo, CA, USA) according to the manufacturer’s instructions, using a Luminex® Complete System 200 (Luminex®; Austin, TX, USA).

Subretinal transfer of immunized lymphocytes

The inguinal lymph nodes (LNs) were removed from KO or WT mice on day 16 postimmunization. T cells were enriched using IMMULAN™ columns (Biotext Laboratory, Houston, TX, USA) (the purity was more than 90%). Before all surgical procedures,

Antibodies and reagents

The Abs used for flow cytometry analysis were as follows: Fc Block® (anti-mouse FcRγ II/III mAb, 2.4G2), Cy-Chrome 5-conjugated anti-TCR/β mAb (H57-597) and fluorescein isothiocyanate (FITC)-conjugated anti-Gr-1 mAb (clone RB6-8C5) were purchased from BD Bioscience. FITC-conjugated anti-CD45 mAb (YW62.3) was purchased from IQ Products (Groningen, the Netherlands). Biotin-conjugated anti-F4/80 mAb (clone A3-1) was purchased from Caltag Lab (Burlingame, CA, USA). Streptavidin/R-PE was purchased from Molecular Probes (Eugene, OR, USA).

Flow cytometry and calculation of the number of neutrophils and macrophages

Intraocular-infiltrating cells were adjusted to the designated concentrations and then were stained by two colours with FITC-anti-CD45 mAb and biotin-conjugated anti-F4/80 mAb, followed by streptavidin/R-PE. Macrophages have been reported to be positive for CD45 and F4/80 surface molecules, and so CD45 and F4/80 mAb double-stained cells were analysed as macrophages. We considered CD45 positive and F4/80 negative cells as neutrophils and confirmed that CD45 positive and F4/80 negative cells were also Gr-1 positive (Fig. 2A, lower panel).

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recipient mice were anesthetized with an intraperitoneal injection of a mixture of 3 mg ketamine and 0.0075 mg xylazine. Using fine 32-ga. needles (cat. no. 0160832; Hamilton, Reno, NV, USA) and a 10-µl syringe (cat. no. 80330; Hamilton), 2 × 10^6 enriched T cells were inoculated into the subretinal space. The tip of the needle penetrated the sclera, choroid but not retina, then a volume of 2 µl per injection was introduced into the subretinal space. We were confident that the cells were being injected into the subretinal space, because the tip of the needle was carefully guided under a microscope through the flattened cornea covered by a glass microscope slide. After inoculation of 2 µl of solution, elevated intraocular pressure completely sealed the scleral and choroidal incision without bleeding or leaking of solution. Clinical scores for EAU were evaluated 6 days after cell transfer (as described above).

Statistics

Mann–Whitney nonparametric analysis was used to analyse differences between groups of mice. p < 0.05 was considered to be statistically significant.

Results

EAU was induced in CCR2 KO mice

It is well established that macrophages play an important role in generating tissue damage in the course of experimental autoimmune diseases (Dijkstra et al. 1987; Godiska et al. 1995). Macrophages are derived from blood monocytes that enter tissues according to the process of vascular endothelium specialization. The activation of macrophages is also prompted by locally produced stimuli such as the presence of cytokines, adhesion molecule binding or interaction with foreign infectious agents. To confirm the influence of macrophages in EAU, we studied the development of EAU in CCR2 KO mice. MCP-1 mediates the recruitment of monocytes in several inflammation models and diseases. As a result, mice lacking the MCP-1 receptor, CCR2, cannot recruit macrophages to the inflammatory site (Boring et al. 1997). Thus, our original hypothesis was that EAU would fail to be induced in CCR2 KO mice.

The results were totally unexpected. In CCR2 KO mice, retinal vasculitis, exudates and optic disc swelling could be induced comparable to WT mice (Fig. 1A). The severity and the time-course of uveitis in CCR2 KO mice were almost identical with those of WT mice (Fig. 1B).

Neutrophils were accumulated in the eye in the EAU of CCR2 KO mice

To compare the phenotype of ocular-infiltrating cells in EAU of WT and KO mice, we isolated the ocular-infiltrating cells by collagenase treatment and analysed them by flow cytometry. Our previous data showed that unless macrophages were barely detected in naïve animals, F4/80 positive macrophages were clearly (approximately 40% of ocular-infiltrating cells) detected in the gated live cells in EAU in C57BL/6 mice (Sonoda et al. 2003). The ocular infiltration of macrophages began to be observed on day 9 after immunization, peaked at day 16 and then gradually reduced (Sonoda et al. 2003).

In CCR2 KO mice, the ocular infiltration of macrophages (CD45^+ F4/80^+ cells) was markedly reduced (Fig. 2A, upper panels). This is compatible with observations in other organs during the inflammation of CCR2 KO mice (Boring et al. 1997). However, the total numbers of ocular-infiltrating cells were not different between WT and KO mice (data not shown). Instead of macrophages,
CD45^+ F4/80^- cells were recruited to the eye of CCR2 KO mice (Fig. 2A, upper panel). We considered CD45^+ F4/80^- cells as neutrophils and confirmed that these cells were also Gr-1 positive (Fig. 2A, lower panel).

We also confirmed the accumulation of neutrophils in CCR2 KO mice by histological sections. Figure 2B shows the H/E staining of EAU sections in CCR2 KO mice. Marked cell accumulation was observed around the optic disc and retinal vessels. The view at higher magnifications (Fig. 2B inset of left panel) revealed that infiltrating cells were neutrophils, with clover-shaped nuclei. Some vessels were obstructed by the inflammatory cells (Fig. 2B, right panel). It is important to note that there was no granuloma formation in the EAU of CCR2 KO mice, which was frequently observed in WT EAU mice. We confirmed neutrophil-dominant ocular inflammation until day 21 in the histological section (data not shown). On the day 26, ocular inflammation came to settle down, many destroyed cells were prominent and hard to judge the type of infiltrating cells.

**Th1 cells were induced in the CCR2 KO mice**

It is well established that autoreactive Th1 cells mediate EAU (Rizzo et al. 1996), and EAU induction is correlated with T cell-derived IFN\( _{\gamma} \) production. Th1 responses play an important role in the pathogenesis of EAU, not only because they result in IFN\( _{\gamma} \) production and subsequently macrophage activation, but also because they stimulate Th1 cells to migrate to, and accumulate at, the site of inflammation, mediated by specific chemokines. The next step was to determine whether autoreactive T cells of the same type were actually induced in CCR2 KO mice or not during EAU induction. We compared the IRBP\(_{1-20}\)-reactive IFN\( _{\gamma} \) and IL-4 production from the lymph node cells between WT and CCR2 KO mice. There was no difference between the total number of regional lymph node cells between WT and KO animals (data not shown). Both groups produced IFN\( _{\gamma} \) predominantly (Fig. 3A) in vitro, and IL-4 production was below the detectable level (< 5 pg/ml).

We also compared the number of ocular-infiltrating T cells on day 16. Although the number of ocular-infiltrating macrophages was markedly reduced in CCR2 KO mice, the number of ocular-infiltrating T cells in CCR2 KO mice was identical with that in WT mice (Fig. 3B). Thus, CCR2 deficiency did not affect either the induction of pathogenic T cells or the recruitment of these T cells to the eye on day 16 postimmunization.

**The phenotype of EAU depended on the ocular environment rather than the source of autoreactive T cells**

The equivalent IFN\( _{\gamma} \) production and number of ocular-infiltrating T cells implied that there was no functional difference in the induced autoreactive T cells between WT mice and CCR2 KO mice. The recruitment of neutrophils in CCR2 KO mice might be an alternative local response under the macrophage-impaired condition, not by the functional changes of autoreactive T cells (including chemokine production). To clarify this issue, we evaluated the ability of immunized lymphocytes to transfer EAU when transplanted into the subretinal space of immunized mice. Column-enriched T cells from either immunized WT mice or CCR2 KO mice on day 16 postimmunization of IRBP\(_{1-20}\) were transferred into the subretinal space of either untreated WT mice or CCR2 KO mice, and then the score of EAU and the phenotype of ocular-infiltrating cells were examined.

As expected, the score of EAU was not different between the four combinations of donor/recipient...
Importantly, the ocular infiltration of neutrophils was observed in the CCR2 KO host, which transferred either WT or KO T cells. The macrophage-impaired condition in the eye can cause neutrophil-dominant uveitis after attack by or exposure to autogressive T cells. MIG, IP-10 and MCP-1 increased in the ocular fluid of IRBP1–20-immunized CCR2 KO mice

Several reports described local chemokine expression in both patients with uveitis (Verma et al. 1997; Adamus et al. 2001; Takase et al. 2006) and EAU (Crane et al. 2001; Foxman et al. 2002; Keino et al. 2003). Deficiency of the CCR2/MCP-1 system might affect the expression of ocular chemokines in the EAU system, so we measured the concentrations of several chemokines in the ocular fluid by the microbead-based Luminex® system, which allowed us to measure several chemokines at a time from a limited volume of samples.

There were no significant differences in the chemokine concentrations between WT mice and KO mice at day 11 of EAU (onset of uveitis). Interestingly, all five chemokines had the tendency to increase in CCR2 KO mice on day 17. The concentrations of MIG, IP-10 and MCP-1 were markedly increased in KO mice (Fig. 5).

MCP-1 KO mice in C57BL/6 background also induced neutrophil-dominant EAU

Our CCR2 KO mice were hybrid of C57BL/6 and 129/SvJae. Although we showed clear different EAU phenotype between CCR2KO mice and WT mice in exactly same genetic background, a question may arise mixed genetic background can affect our results. We currently cannot obtain C57BL/6-background CCR2KO mice, unfortunately. Instead, we performed EAU experiments using MCP-1 (the ligand of CCR2) KO mice in C57BL/6 background which were also impaired macrophage accumulation to the inflammatory site (Lu et al. 1998). MCP-1 KO mice can cause equivalent level of uveitis as observed in WT (C57BL/6 mice) (Fig. 6A). We confirmed MCP-1KO mice in C57BL/6 background also cause neutrophil-dominant uveitis comparable to CCR2KO mice (Fig. 6B). We thus conclude neutrophil-dominant uveitis is not because of specific genetic background.

Discussion

In this report, we have described that neutrophil-dominant EAU was induced in CCR2 KO mice. CCR2 is a prominent receptor for MCP-1, and thus the migration of macrophages to the local area was markedly impaired in CCR2 KO mice (Boring et al. 1997; Kuziel et al. 1997). It is known that the eye has resident antigen-presenting cells (APCs), such as retinal pigment epithelial cells and microglia cells. Without recruiting macrophages, these APCs might stimulate IRBP 1–20-specific T cells and cause an autoaggressive immune response in the eye. Our data imply that neutrophils can respond to and participate in the autoaggressive immune response initiated by T cells, instead of macrophages.

Dagkalis et al. (2009) have already shown the development of EAU and ocular macrophage infiltration in CCR2 KO mice. Our data were basically compatible to their results, and we also observed macrophage infiltration even in both CCR2 KO mice and MCP-1 KO mice (Figs 2A and 6B), but not much. Dagkalis et al. did not mention about the number of neutrophils in the EAU system, and our points are neutrophil-associated CXC-chemokines (MIG, IP-10) are actually upregulated and neutrophil-dominant uveitis is induced in CCR2 KO mice.
In our model, IRBP1–20-specific IFN-γ-producing T cells were actually induced in the draining LN on day 16 (Fig. 3A). Although CCR2 was mainly expressed on macrophages and monocytes, it is known that activated T cells can also express CCR2 (Zhang et al. 2009). However, the number of ocular-infiltrating T cells in the EAU of CCR2 KO mice was not significantly impaired compared to that of WT mice (Fig. 3B). The migration of autoreactive T cells into the eye must be mediated by a combination of chemokines–chemokine receptors other than MCP-1-CCR2.

In the adoptive transfer experiment, host-derived CCR2 determines the fate of ocular inflammation, either the macrophage-dominant or neutrophil-dominant phenotype of EAU. Subretinal transfer of autoreactive T cells derived from WT mice induced neutrophil-dominant ocular inflammation in the CCR2 KO host but induced macrophage-dominant inflammation in the WT host (Fig. 4). We assume that autoreactive T cells recruited to the eye are stimulated by the resident APCs and initiate the autoaggressive immune response. The candidates for resident APCs could be microglia cells, vascular endothelial cells and resident monocytes. After initial breakdown of the blood-ocular barrier induced by the autoaggressive T cells, the subsequent influx of bone marrow-derived cells might modulate the subsequent local inflammation. In that phase, CCR2 must come to influence the ocular inflammation. Under the unusual condition of impaired macrophage infiltration, alternative neutrophils come to the fore and must induce different ocular inflammations. In fact, CCR2 KO mice showed enhanced early accumulation and delayed clearance of neutrophils in the cornea (Oshima et al. 2006). Interestingly, Takeuchi et al. (2005) demonstrated that neutrophil-dominant EAU was also induced in CCR5 KO mice.

In contrast to EAU, Izikson et al. (2000) and Fife et al. (2000) individually reported that CCR2 KO mice did not develop EAE, the same experimental murine autoimmune disease. Although Gaupp et al. (2003) reported the susceptibility of EAE in the CCR2 KO mice, it is difficult to explain for this discrepancy at present.
One speculative explanation is that the pattern of producing chemokines derived from resident cells differs between the eye and the brain. After the initial autoaggressive response by IFN-γ-producing autoreactive T cells, inflammatory factors affect the local microenvironment, and resident cells might subsequently produce series of chemokines and cytokines. The constitutive lack of CCR2 in the iris, retina and choroid might lead to the dominant production of the CXC chemokines (MIG, IP-10) that mainly recruit neutrophils (Fig. 5). MCP-1, the ligand of CCR2, might compensatorily increase in the eye. The resident cells in the brain might not have this form of reaction.

The induction ability of autoreactive IFN-γ-producing T cells in CCR2 KO mice has also been discussed. Izikson et al. (2000) demonstrated that draining LN T cells from myelin oligodendrocyte glycoprotein (MOG) immunized CCR2 KO mice reduced antigen-specific proliferation and IFN-γ production on day 9 postimmunization. Also, Boring et al. (1997) showed an impaired type-1 cytokine response against the purified protein derivative of Mycobacterium bovis until 8 days after antigen challenge. In contrast, another report showed that MOG-specific Th1 T cells were normally induced in CCR2 KO mice and adoptively transferred EAE to naïve WT mice (Gaupp et al. 2003); they harvested draining LN cells on the peak day of acute clinical disease (on day 14–21 postimmunization). In our data, IRBPα2-specific IFN-γ-producing cells were actually induced in the draining LN on day 16 postimmunization (Fig. 3A). We think that CCR2 KO mice might have a low Th-1 response during the early phase of EAU, however, the reduced IFN-γ response was overcome during the state of persistent antigen stimulation.

So far, human uveitis has been broadly categorized into two types of inflammation: granulomatous and nongranulomatous uveitis. The former is macrophage-dominant inflammation and the latter is neutrophil-dominant inflammation. For example, the uveitis associated with Vogt-Koyanagi-Harada’s disease is a typical granulomatous uveitis, and the uveitis-associated Behçet’s disease is a typical nongranulomatous uveitis (Sakane et al. 1999). Although our experiments were performed in a highly artificial animal system, our data suggest that at least two mechanisms exist for inducing ocular inflammation. One is a macrophage-dominant mechanism and the other is a neutrophil-dominant mechanism. Under normal conditions, both macrophages and neutrophils can participate in ocular inflammation, but under the unusual condition of impaired macrophage infiltration, alternative neutrophils may come to the fore and induce unique ocular inflammation.

Not all chemokine–chemokine receptor blocking therapies may always be effective for the treatment of uveitis. It has been reported that neutralization of several anti-chemokine and chemokine receptors was effective for EAU suppression (Abu El-Asrar et al. 2007) and is thought to be of practical use against human uveitis. However, it is possible that a different kind of ocular inflammation could be initiated in some cases. Precise and careful examinations will be required to develop an effective therapy using a blocker of chemokines and chemokine receptors against human uveitis.

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