Retinal Microglial Activation and Chemotaxis by Docosahexaenoic Acid Hydroperoxide

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PURPOSE. Peroxynitrite generated during the early phase of experimental autoimmune uveoretinitis (EAU) causes peroxidation of docosahexaenoic acid (22:6), a principal unsaturated fatty acid of the photoreceptor membrane, to its hydroperoxide (22:6HP). During this phase, microglia migrate to the site of photoreceptors. The effect of 22:6HP on the migration of isolated retinal microglia was investigated.

METHODS. Retinal microglia were isolated and cultured from newborn Lewis rats and identified immunohistochemically by OX42 antibody staining. Chemotactic activity of the microglia toward 22:6HP was assayed and compared with 22:6 and other control cultures. The effect of 22:6HP on the organization of actin fibers and on the expression of Rac and Iba1 in the microglia was studied by confocal microscopy. The gene and protein expression of Rac and Iba1 in these microglia was analyzed by real-time PCR and Western blot.

RESULTS. Ninety-five percent of isolated microglia stained for OX42 and a chemotactic assay showed that 22:6HP was a potent chemotacticant for these cells. Exposure to hydroperoxide resulted in reorganization of F-actin with intense staining within the lamellipodia. Iba1 and Rac were upregulated in these activated cells and localized in the cell periphery and the lamellipodia.

CONCLUSIONS. 22:6HP can activate retinal microglia and is a potent chemotacticant for microglial migration in response to the activation of Rac and reorganization of actin cytoskeleton. In EAU, microglial migration toward the photoreceptors may be mediated by 22:6HP formed in the photoreceptors, and these migrating cells could help modulate the inflammation. (Invest Ophthalmol Vis Sci. 2006;47:3656–3663) DOI:10.1167/iovs.06-0221

Uveitis is a major cause of blindness, with visual loss primarily attributed to degeneration of the photoreceptors. Experimental studies have revealed that this photoreceptor degeneration is initiated by the presence of the potent oxidant peroxynitrite, which is generated in the early phase of uveitis. Peroxynitrite is known to react with 22:6, a principal polyunsaturated fatty acid of the photoreceptor membranes, and to convert the 22:6 to its hydroperoxide, 22:6HP.1 During this early phase of uveitis, the retinal microglia migrate toward the photoreceptors. However, the underlying mechanism of the migration of these cells is not clear.

The microglia, which normally lie dormant in adult retina, are rapidly activated in response to injury, infectious disease, inflammation, ischemia, or neurodegeneration.2 These activated microglia exhibit changes such as proliferation, migration, phagocytosis, and the production of bioactive molecules.3,4 Thus, in their resting state, microglia seem to monitor their surroundings, remaining poised to react quickly to an insult. Morphologically, the resting microglia are highly branched (ramified) cells with a small amount of perinuclear cytoplasm. In response to pathologic insult; however, these cells become hypertrophied with short processes, resulting in a bushy appearance. This morphologic change is accompanied by the expression of surface markers such as OX42, ED1, 5D4, and OX6.5–6

The activation and migration of microglia are associated with dynamic remodeling of the actin cytoskeleton. This remodeling, in turn, is regulated by the Rho family of small GTPases, including Rac, Rho, and Cdc4.2,7,8 Rac is one of the essential regulatory subunits of the activity of NADPH oxidase, the key enzyme in oxygen radical production.9 Iba1, the novel calcium-binding protein expressed specifically in the microglia, may also play an important role in Rac signaling of activated microglia. The Iba1 gene is located within the major histocompatibility complex class III region, which is known to accumulate large clusters of immunoresponsible genes and which may contain susceptible genes for immunorelated diseases.10 Because microglia are considered immunoresponsible cells, the Iba1 gene may play an important role in the activation and migration of microglia in early EAU. Studies have also indicated that Iba1 is a calcium-binding phosphoprotein and that it could be an intracellular signaling molecule unique to microglia.10 However, the putative chemotactic signal transmitted to the retinal microglia and the exact mechanism underlying the activation and their subsequent migration during EAU and other diseases are still largely unknown. The search for factors influencing microglial activation and motility is important for understanding the function of microglia in retinal disease and, in particular, the role of microglia in the photoreceptor damage that occurs in autoimmune uveitis.

In the present study, we attempted to determine whether the 22:6HP generated during EAU plays a role in the activation and migration of retinal microglia. We also investigated the signaling pathways involved in retinal microglial activation and migration.

METHODS

Cell Cultures

All animals used for the cell cultures were maintained and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Newborn Lewis rats, 6 to 7 days old, were obtained from Charles River Laboratories (Wilmington, MA). Microglia were isolated from retinas according to the method described by Roque and Caldwell11 and Matsubara,12 with minor modifications. Briefly, the eyes were enucleated, and the retina was removed carefully without contamination. The retinas were briefly soaked in Dulbecco’s
modified Eagle’s medium (DMEM; Invitrogen-Gibco, Grand Island, NY) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM l-glutamine; briefly washed in Hank’s balanced salt solution; cut into small pieces; and digested with 0.5% trypsin in 0.53 mM EDTA for 30 minutes at 37°C. DMEM with 10% fetal calf serum (FCS) was added to terminate trypsinization. The retinas were manually dissociated by trituration and centrifuged. The cells were cultured in DMEM+10% FCS supplemented with 1 ng of granulocyte-macrophage colony-stimulating factor (GM-CSF, Sigma-Aldrich, St. Louis, MO) and allowed to grow to 37°C in 5% CO2 in 75-cm² flasks precoated with poly-l-lysine. The cells were grown to confluence, and the loosely adherent microglia were detached from the more adherent astrocytes by shaking the flask for 4 hours (200 rpm at 37°C) in an orbital shaker. The cell suspension was spun down at 100 rpm for 10 minutes, and the cell pellet was resuspended in DMEM+10% FCS. The purity of the microglia in cultures was determined by staining with the microglial marker OX42. The morphology of microglia in culture was carefully examined by phase-contrast and fluorescence microscopy.

**Immunocytochemical Analysis**

Indirect immunocytochemical analysis was used to confirm the identity of isolated retinal microglia. Purified microglia were plated onto poly-l-lysine–coated chamber slides at a density of 2 × 10⁶ cells/cm² and allowed to settle for 30 minutes. Nonadhesive cells were removed by washing with DMEM+10% FCS. The adherent microglial cells were allowed to grow for 24 hours at 37°C, washed with phosphate-buffered saline (PBS), air dried, fixed in 4% paraformaldehyde for 20 minutes, and blocked with 2% bovine serum albumin (BSA) and 2% goat serum for 30 minutes. A murine monoclonal antibody to rat complement receptor 3 (OX42, 1:50; Serotec, Oxford, UK) was used as the primary antibody. Cultures were also tested with anti-bovine glial fibrillary acidic protein (GFAP, 1:100; Dako, Carpinteria, CA) for staining of astrocytes. The negative-control cultures were incubated with PBS instead of the primary antibodies. A standard biotin-avidin immunocytochemical technique was performed using the avidin-biotin complex kit (ABC Kit; Vector, Burlingame, CA) before visualization with 3-amin-9-ethyl carbazole (AEC; Sigma-Aldrich). The cells were then counterstained with hematoxylin-eosin.

**Oxidation of Methyl Docosahexaenoate (22:6)**

Commercial methyl docosahexaenoate (Sigma-Aldrich) was used in its pure and oxidized forms. The oxidized methyl docosahexaenoate was prepared by air oxidizing the commercial methyl docosahexaenoate at room temperature for 5 to 7 days. An aliquot of the sample was dissolved in 1 mL of ethanol, and the UV absorption range (200 to 400 nm) was recorded using a spectrophotometer (model UV-1600; Shimadzu, Tokyo, Japan). The quantity of conjugated dienes formed in the sample was determined by measuring the absorbance at 233 nm, and the molar extinction coefficient of 25,200 was used for calculation, as described by Buege and Aust.15

**Chemotactic Assay**

A chemotactic assay was performed with a 48-well chamber (Neuroprobe, Gaithersburg, MD). Briefly, the isolated microglia were suspended in DMEM containing 1% BSA (DMEM+1% BSA) at a density of 2 × 10⁶ cells/mL. In the first experiment, various dilutions of falkline (1, 3, 5, and 10 nM) were prepared in DMEM+1% BSA. Aliquots of 26 μL of these dilutions were distributed in quadruplicate in the lower wells. Controls consisted of DMEM+1% BSA alone. An 8-μm pore polycarbonate filter coated with poly-l-lysine was applied to favor microglial attachment. The filter separated the upper wells containing 50 μL of cell suspension. In a preliminary experiment, microglial chemotactic activity reached a maximum after 3 hours of incubation in the chemotactic chamber. Thus, all subsequent experiments were performed using a 3-hour incubation. The chamber was incubated at 37°C in a moist 5% CO2 atmosphere. After incubation, the nonmigrating cells adherent to the upper surface of the filter were gently scraped off. The filter was then fixed in methanol, stained with a differential histologic stain (Diff-Quik; Baxter Diagnostics, McGraw Park, IL), and dried on a glass slide. The number of migrated cells was counted at ×400 or ×1000 magnification, according to their density. At least five power fields were examined in each well, and the results were expressed as the mean cell number ± SD.

**Actin Staining**

Purified microglia were seeded onto poly-l-lysine–coated four-well chamber slides at a concentration of 4 × 10⁴ cells/mL (300 μL on each chamber) for approximately 1 hour, to allow the cells to adhere. A further 2 mL DMEM+10% FCS was added, and the cells were cultured overnight. The cells were washed and stimulated with either 22:6 (1 mg/mL) or 22:6HP (1 mg/mL) in serum-free medium at 37°C for 30, 60, and 120 minutes. Serum-free medium was used as the control. The cells were then washed in PBS and fixed for 20 minutes in 4% paraformaldehyde at room temperature, washed again in PBS, fixed in acetone for 5 minutes at room temperature, washed a third time in PBS, stained with tetramethylrhodamine isothiocyanate (TRITC)-labeled phalloidin (200 ng/mL; Sigma-Aldrich), and incubated at room temperature for 45 minutes. Finally, the slides were washed in PBS and distilled water and mounted in PBS/glycerol in ratio 1:1. The cells were then visualized by confocal microscopy (LSM510; Carl Zeiss Meditec, GmbH, Oberkochen, Germany).

**Translocation of Iba1 and F-actin**

To determine the translocation of Iba1 and F-actin to the region of membrane ruffling, cells were double-stained after a 60-minute stimulation with the same concentrations of 22:6HP and 22:6 used in actin staining. After fixation, cells were incubated with polyclonal antibody against Iba1 (Wako, Richmond, CA) and Alexa Fluor 488–labeled secondary antibody. F-actin was stained with TRITC-labeled phalloidin. Images were obtained with a confocal microscope.

**Analyses of Rac and Iba1 mRNA**

The quantitative real-time PCR was performed using an optical detection system (iCycler; Bio-Rad Laboratories, Hercules, CA). Isolated microglia were treated with 22:6HP and 22:6, as previously described. Total cellular RNA was isolated from the treated cells using the TRIzol reagent (Invitrogen, Carlsbad, CA), and the cDNA template was generated (Omniscript RT Kit; Qiagen, Valencia, CA). Each 25-μL PCR reaction contained a supermix (SYBR Green 1; Bio-Rad Laboratories), 0.5 μM of gene-specific primers, and cDNA template. The protocol for the reaction was three-step amplification with melting curve analysis, and the conditions were as follows: 1 minute at 95°C, 40 cycles of 10 seconds at 95°C, 30 seconds each at 57°C and 1 minute at 95°C, 1 minute at 55°C, and 80 cycles of 10 seconds each, starting at 55°C with an increase of 0.5°C for melting curve analysis at 94.5°C. Quantification analyses of Rac and Iba1 mRNA was normalized using the housekeeping gene GAPDH. The sequences of gene-specific primers used for Rac and Iba1 were as follows: Rac forward, 5'-GTAAACCTGCCTGCTGGT-3', and reverse, 5'-GCTTCTGTTCAAAACTGTTTCT-3', and Iba1 forward, 5'-GAAGCGATGTGCTGAGAAC-3', and reverse, 5'-CCTC-GAATTAGGCCACTCTCA-3'. The primers used for GAPDH were forward, 5'-TGACACCCACCAAGTGCTTA-3', and reverse, 5'-GAGTGACGG-GATGAGTCTTC-3'. PCR reactions for each gene were performed in triplicate on each cDNA template along with triplicate reactions of the housekeeping gene GAPDH. The specificity of PCR amplification prod-
RESULTS

Retinal Microglia Culture and Immunocytochemical Characterization

Retinal microglia were obtained from 14-day primary mixed glial cell cultures (Fig. 1) prepared from 6- to 7-day-old Lewis rats. The cells were collected by a shaking-off method when they were almost confluent. Cultures were then used for immunocytochemical characterization, chemotaxis, and further molecular studies, 1 day after reseeding. By this time, the microglia had recovered from the isolation process and had acquired a normal morphology. Immunocytochemical results demonstrated positive staining for OX42 in >95% of cells (Fig. 2A). Only a few cells stained positive for GFAP, indicating that the isolated cells were microglia and that they were not contaminated by astrocytes (Fig. 2B). In the absence of primary antibody, none of the cells showed staining (Fig. 2C).

22:6HP Induces Migration of Retinal Microglia

Retinal microglia exhibited strong migratory activity in response to the addition of 3 nM of fractalkine; therefore, fractalkine was considered a positive control for this experiment. An almost identical amount of chemotaxis was observed in cells treated with 22:6HP (Fig. 3A). Maximum migration was seen with 3 hours of incubation. The levels of microglial chemotaxis in cells treated with 22:6HP were two times greater than the levels obtained with 22:6 and with DMEM alone. A significant difference in migration was observed between cells treated with 22:6HP and those treated with 22:6 (P < 0.05). There was no significant difference between cells treated with 22:6 and the control cells treated with medium alone. After migration, the cells had a rounded morphology (Fig. 3B).

Role of 22:6HP in Actin Polymerization and Distribution in Retinal Microglia

Retinal microglia exhibited a migratory response in the presence of 22:6HP. Therefore, to study actin reorganization in response to 22:6HP, we stained the actin cytoskeleton of microglia with TRITC-labeled phalloidin, a stain specific for F-actin, after exposing them to 22:6HP for 30, 60, and 120 minutes. The 22:6HP-treated cells displayed pseudopodia, but the control cells were round. Our results demonstrated that 22:6HP caused a redistribution and polymerization of F-actin in retinal microglia within 60 minutes of stimulation (Fig. 4A). In the control cells stimulated with 22:6, the actin was concentrated around the nucleus (Fig. 4B), whereas the cells treated with 22:6HP displayed retraction and translocation of F-actin to the actin reorganization was checked by performing dissociation melting curve analysis. The threshold cycle difference between the experimental and control groups for Rac and Iba1 were calculated and normalized to GAPDH, and the relative x 0.6 x 10^7 method.15

Western blot analysis was performed to determine Rac and Iba1 protein levels. The cells were stimulated with 22:6 and with 22:6HP in DMEM without serum and washed with PBS. Protein was extracted from the cells, and the concentration was determined by a protein assay with BSA as the standard (Bio-Rad Laboratories). Equal amounts of protein samples were loaded and run on SDS-PAGE (15% Tris-HCl polyacrylamide Ready Gels; Bio-Rad Laboratories) at 120 V. After electrophoresis separation, proteins were transferred onto nitrocellulose membranes (Bio-Rad Laboratories) by a semidry transfer system (Trans-Blot, Bio-Rad Laboratories). The membranes were blocked in 5% milk in Tris-buffered saline–Tween-20 (TBST) for 1 hour and probed with a polyclonal antibody against Iba1 at 1:1000 dilution and against a monoclonal antibody against Rac overnight at 4°C. After a 30-minute incubation with the secondary antibody tagged with horseradish peroxide, signals were detected with a Western blot detection system (ECL; GE Healthcare, Cleveland, OH). Equal protein loading was confirmed by normalizing with GAPDH.
cells (Fig. 4A). Little difference was noted after 120 minutes of stimulation; therefore, for all further experiments, cells were stimulated for only 60 minutes.

**Colocalization of Iba1 with F-Actin in Membrane Ruffles**

To confirm the localization of Iba1 in membrane ruffles, retinal microglia were stimulated with 22:6HP for 60 minutes. The cells were then doubly stained with the anti-Iba1 antibody and rhodamine-conjugated phalloidin to visualize F-actin and observed with a confocal laser scanning microscope. The cells stimulated with 22:6HP rapidly induced the reorganization of actin to form lamellipodia and Iba1 vigorously accumulated in the lamellipodia and colocalized with F-actin (Figs. 5A–C), whereas stimulation with 22:6 exhibited contracted and round-ed-up cell bodies, with the Iba1 concentrated near the nucleus (Figs. 5D–F).

**Involvement of Rac GTPase in the Formation of Membrane Ruffles**

Rac was localized at the leading edge of the migrating microglia toward the pseudopodia, similar to F-actin and Iba1 in the 22:6 HP-treated cells (Fig. 5G). In the control cells Rac was localized mainly near the nucleus (Fig. 5H).

**22:6HP Induces Gene Expression of Rac and Iba1 in Retinal Microglia**

A significant increase was noted in the mRNA of Rac ($P < 0.01$) and Iba1 ($P < 0.01$) in the microglia stimulated with 22:6HP compared with the control (22:6) groups. Rac was upregulated 1.5-fold in the activated microglia, whereas Iba1 was upregulated twofold compared with control cultures stimulated with 22:6 (Fig. 6).

**Effect of 22:6HP on Rac and Iba1 Protein in Microglia**

Western blot analyses were performed to determine the effect of 22:6HP on Rac and Iba1 protein levels in activated retinal microglia. In the treated cells, prominent bands were detected at the expected molecular masses of approximately 21 and 17 kDa (Fig. 7). There was a marked increase in Iba1 and Rac in the treated cells compared with the control. These studies demonstrated that 22:6HP induced the production of Rac and Iba1 protein. In the control groups treated with 22:6, Iba1 was only minimally detectable.

**DISCUSSION**

The isolated retinal microglia showed significant chemotactic activity toward 22:6HP, indicating that the hydroperoxides can stimulate directed migration of retinal microglia. The migratory activity was brought about by the redistribution and polymerization of the actin filaments. F-actin was seen to be translocated to the ends of the pseudopodia. The small GTPase protein Rac and the calcium-binding protein Iba1 were colocalized with F-actin in the lamellipodia at the leading edge of the migrating microglia. An increase in the gene and protein expression of Rac and Iba1 was also noted in the 22:6HP-treated cells compared with the 22:6-treated control cultures. These results indicate that the hydroperoxides activate an Iba1-dependent Rac pathway in the retinal microglia.
It has been shown that peroxynitrite reacts with 22:6 during EAU and that it converts the 22:6 to its hydroperoxide, 22:6HP, primarily in the photoreceptors. The results of the present study show that 22:6HP could activate retinal microglia in their migration toward the hydroperoxides that are formed in the photoreceptors in EAU. Such activated and migrated cells could release inflammatory products with deleterious consequences for the photoreceptors during the amplification phase of EAU; or these migrated cells could phagocytose the damaged photoreceptors.

Activated microglia play a central role in the pathogenesis of many central nervous system (CNS)-associated inflammatory and neurodegenerative diseases through the production of mediators that may either perpetuate the inflammatory response or cause direct neuronal injury and death. These activated cells simultaneously remove the degenerating neurons and secrete proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β that are toxic to the neurons.

Potential microglial participation is also reported in various forms of retinal injury or disease, including diabetic retinopathy, glaucomatous optic nerve degeneration, light damage, and inherited retinal degeneration in the photoreceptors.

The relative multiple of change in mRNA expression was determined by the $2^{-\Delta\Delta CT}$ method. Data represent the mean ± SD of results in triplicate experiments. *$P < 0.01$ versus 22:6. The results showed an increase of 2- and 1.5-fold increases in the gene expression of Iba1 and Rac, respectively, compared with the control cultures treated with 22:6.
21 kDa

Expt

22:6HP

Rac

17 kDa

Control

Iba1

FIGURE 7. Protein expression of Iba1 and Rac was increased in 22:6HP-treated retinal microglia. Equal amount of total protein from 22:6 HP-activated cells and 22:6-treated cells were separated with 15% SDS-polyacrylamide gel. Protein bands were transferred to nitrocellulose membrane and probed with polyclonal anti-Iba1 and monoclonal anti-Rac as the primary antibodies and horseradish tagged goat anti-rabbit IgG as the secondary antibody. Iba1 was immunodetected at a molecular mass of 17 kDa and Rac at 21 kDa.

RCS rat,29,30 human retinitis pigmentosa and age-related macular degeneration,31,32 optic atrophy,33,34 EAU,35 retinal ischemia and reperfusion injury,35 and in retinal degeneration in rd mice.36,37 Activated microglia migrates from the inner retinal layers to the outer nuclear layer during the early phase of EAU. This migration occurs subsequent to the generation of oxygen free radicals in the photoreceptors.34 The activated microglia are known to release IL-1β, IL-3, IL-6, TNF-α, interferon-γ, tumor growth factor-β, vascular endothelial growth factor, lymphotixin, macrophage inflammatory protein-1α, matrix metalloproteinases, proteolytic enzymes, nitric oxide, and reactive oxygen species, all of which can cause photoreceptor degeneration.38 In addition, several of these agents can alter the expression of vascular cell adhesion molecules, and the latter can recruit lymphocytes and macrophages, thus causing further tissue damage.39 Moreover, the blood-retinal barrier is compromised and designated treatments. More important, it provides a system to observe a pure population of microglia and to form closely spaced actin bundles.52 In the present study, as shown with CNS microglia, our results clearly show the role of 22:6HP as chemotactant in early EAU.

The process of cell migration by activated microglia involves dynamic reorganization of the cytoskeleton, especially of the actin cytoskeleton, which in turn, leads to elongation of lamellipodia and filopodia.47 We analyzed the effect of 22:6HP on the actin polymerization and showed that 22:6HP caused redistribution and polymerization of F-actin. The cells treated with 22:6HP showed elongation of pseudopodia, whereas the 22:6-treated cells were round. The difference between 22:6HP- and 22:6-treated cells was pronounced (Fig. 4). F-actin was seen to be concentrated around the nuclei in the 22:6-treated cells. Our results clearly indicate that F-actin was involved in the migration of retinal microglia. Previous studies on activated brain microglia demonstrated a similar finding, wherein fractalkine and GM-CSF induce microglial migration and activation, as well as actin rearrangement and shape change.10,45 Similarly, the chemokines MCP-1, MIP-1α, MIP-1β, RANTES, IL-8, and IP-10 induce migration and changes in the distribution of F-actin in adult rat microglia and human microglial line CHME3, in vitro.43

Rac is a member of the Rho family of small G-proteins, and Rac activation is known to induce lamellipodia and membrane ruffles.48,49 Rac regulates reorganization of actin cytoskeleton and plays a central role in microglial activation.50 Such signaling molecules may also be involved in the activation of retinal microglia by 22:6HP during EAU. To explore this possibility, we immunolocalized Rac in the 22:6HP- and 22:6-stimulated microglia. Our results showed that Rac was localized at the cell periphery and translocated to the lamellipodia at the leading edge of the 22:6HP-treated cells; but in the 22:6-treated cells, Rac was localized in the nuclei. A similar finding was observed in brain microglia activated with GM-CSF.51

Iba1, the novel calcium-binding protein specific to microglia and macrophages, is an essential molecule in Rac signaling of activated microglia. Previous findings have shown that it translocates together with filamentous actin toward the lamellipodia in activated microglia.51 Therefore, in this study we also colocalized F-actin and Iba1 in 22:6HP-activated retinal microglia to see whether Iba1 is involved in the activation and migration of retinal microglia. Iba1 was seen to colocalize with F-actin and to accumulate at the cell periphery and toward the lamellipodia, indicating that it has a major role in the activation of retinal microglia by 22:6HP. Earlier reports indicated that Iba1 physically binds to F-actin and cross-links actin filaments to form closely spaced actin bundles.52 In the present study, Iba1 may be playing a similar role.

Gene expression of Rac and Iba1 was upregulated significantly in the cells treated with 22:6HP. Reports from earlier studies on brain microglia activation demonstrated a similar result. Iba1 expression levels increased in activated microglia after facial nerve axotomy,53 ischemia,54 and several brain diseases,55,56 thereby implicating it in the activated phenotypes of microglia. We also observed an increase in the protein expression of both Rac and Iba1 in activated microglia. These data clearly suggest that Rac and Iba1 may play a crucial role in the activation and migration of retinal microglia during uveitis.

The current experimental results indicate the existence of an Iba1-dependent Rac activation pathway that produces actin reorganization and membrane ruffling in retinal microglia activated by hydroperoxides of docosahexaenoic acid. Such hydroperoxides are known to be generated in the early phase of
EAU. An understanding of molecular signaling in retinal microglial activation is important in the development of potent inhibitors of microglial activation mediated by 22:6 HP, and thus in preventing retinal degeneration in uveitis. However, further investigation is needed to determine the precise molecular mechanisms underlying retinal microglia activation during the early phase of EAU and the role of other cytokines and chemokines in retinal microglial migration.

References


