RESULTS. Vitreous injection of the C. pneumoniae antigen increased the size of CNV. Although lipopolysaccharide stimulation can induce multiple cytokines, cultured mouse RPE cells from C57BL/6 mice expressed IL-6 and VEGF, but not TNF-α mRNA, in response to C. pneumoniae antigen. RPE cells from either MyD88 KO mice or TLR2 KO mice did not respond to the C. pneumoniae antigen. TLR2 KO mice did not augment the size increase of experimental CNV by C. pneumoniae antigen in vivo.

CONCLUSIONS. C. pneumoniae can trigger inflammatory responses in the eye and promote experimental CNV in a TLR2-dependent manner. These data provide experimental evidence to imply persistent C. pneumoniae infection is a risk factor for AMD. (Invest Ophthalmol Vis Sci. 2010;51:4694 – 4702) DOI: 10.1167/iovs.09-4464

A
ged-related macular degeneration (AMD) is the leading cause of irreversible visual impairment in aged persons 60 years and older in Western countries.1,2 The pathogenesis of AMD is complex and has still not been well determined. Genetic factors,3 but also several other risk factors, have been proposed including sunlight exposure,4 smoking,5,6 and low levels of nutritional components such as antioxidants.4,7 Moreover, atherosclerosis, hypertension, and hyperlipidemia, which lead to cardiovascular diseases, are also considered to be risk factors of AMD.8,9

Chronic local inflammation from persistent infection has recently been identified as a candidate etiology for AMD. In particular, much interest has been focused on C. pneumoniae, a Gram-negative bacterium that causes respiratory disease such as bronchitis, pneumonia, and upper respiratory tract infections.10 It is actually one of the most common causes of pneumonia in young adults and children. Recently, C. pneumoniae has been associated with coronary heart disease and myocardial infarction in several seroprevalence studies.11,12 This microorganism has also been isolated from the coronary arteries of patients with acute coronary syndrome,13 and studies based on animal models have revealed that C. pneumoniae infection can significantly exacerbate atherosclerosis.14 –16

Choroidal neovascularization (CNV) is one of the harmful (micro)-vascular events directly related to AMD. Notably, several studies have demonstrated that chronic local inflammation at the choroid eventually leads to CNV.17–20 We thus generated the hypothesis that persistent C. pneumoniae infection in the choroid may induce chronic local inflammation, which may lead to CNV/AMD. In fact, the titer of a specific antibody against C. pneumoniae was increased in AMD patients,21 and the existence of C. pneumoniae was confirmed histologically in 4 of 9 patients with wet AMD.22

Innate immunity plays an important role in the host defense against C. pneumoniae.23 Innate immunity is conserved among multicellular organisms and recognizes certain molecular patterns specific to microorganisms, known as pathogen-associated molecular patterns (PAMPs), through germline-encoded pattern recognition receptors.24 Toll-like receptors (TLRs) are central transducers for the various PAMPs to invoke innate immunity.25 TLRs are type I transmembrane proteins with an extracellular domain composed of leucine-rich repeats and a cytoplasmic domain called the Toll/interleukin-1 receptor (TIR) domain.26 Activation of TLRs culminates in the production of proinflammatory cytokines, antimicrobial peptides, and costimulatory molecules that induce acute inflammation or subsequent activation of the adaptive immune system.

In this report, we provide evidence that C. pneumoniae can trigger inflammatory responses in the eye and promote CNV in an animal model. Our data imply that persistent C. pneumoniae infection is a risk factor for AMD.

MATERIALS AND METHODS

Mice

Female 8- to 10-week-old mice were used in all experiments. C57BL/6 mice were purchased from SLC Japan (Shizuoka, Japan). Myeloid dif
ferentiation factor (MyD) 88 KO mice (C57BL/6 background) were kindly provided by Kiyoshi Takeda (Osaka University, Osaka, Japan). TLR2 and TLR4 KO mice (C57BL/6 background) were obtained from Oriental Bio Service (Kyoto, Japan). All animals were housed in specific pathogen-free conditions at Kyushu University. All animals were treated humanely, and experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Reagents

Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 was purchased from List Biological Laboratories Inc. (Campbell, CA). *C. pneumoniae* antigen from TWAR strain CWL-029 cultured in HL cells was purchased from Meridian Life Science, Inc. (Saco, ME). Polymyxin B sulfate and fluorescein-labeled dextran (25,000 MWt) were purchased from Sigma-Aldrich (St. Louis, MO).

**Vitreous Cavity Injection**

*C. pneumoniae* antigen (250 ng/mL, 2 μL) was injected into the vitreous cavity using fine, 32-gauge needles (Hamilton, Reno, NV) and 10-μL syringes (Hamilton). Because the total amount of ocular fluid was approximately 10 μL, the final concentration of *C. pneumoniae* antigen in the eye was approximately 50 ng/mL. The tip of the needle penetrated the sclera, choroids, and retina to reach the vitreous cavity, and maximum volumes of 2 μL per injection were introduced in each eye. We ensured that the antigen was injected into the vitreous cavity by carefully guiding, with the use of an operating microscope, the tip of the needle through a flattened cornea covered by a glass microscope slide. After inoculation of 2 μL solution, the intraocular pressure was sufficiently elevated to completely seal the retinal incision without any bleeding or detachment.

For neutralizing experiments, mixtures of *C. pneumoniae* antigen (500 ng/mL, 1 μL) and the following reagents (1 μL) were injected: anti-TLR2 mAb (1 mg/mL, clone T2.5, mouse IgG1; InvivoGen, San Diego, CA), control mouse IgG (1 mg/mL), anti-TLR4 mAb (1 mg/mL, clone MTS510, rat IgG2a; InvivoGen), and control rat IgG (1 mg/mL).

In the other control experiment, we injected zymosan (100 μg/mL, 2 μL, TLR2 agonist; InvivoGen), Pam2CSK4 (1 μg/mL, 2 μL, TLR2 agonist, InvivoGen), or LPS (10 ng/mL, 2 μL, TLR4 agonist) into the vitreous cavity as the control TLR agonists.

**Induction and Evaluation of CNV**

CNV was induced by photocoagulation and was evaluated as previously described. Briefly, laser photocoagulation (532-nm wavelength, 0.1-second duration, 75-μm spot size, 200-mW power) around the optic disc was administered to burn the posterior pole of the retina. One week later the mice were anesthetized and perfused with 1 mL phosphate-buffered saline containing 50 mg/mL fluorescein-labeled dextran for 1 minute, followed by removal of the eyes. The entire choroid was mounted flat on a glass slide. The total area of hyperfluorescence associated with each burn, corresponding to the total number of fibrovascular scars, was measured using image analyzing software (MacScope version 2.3; Mitani, Fukui, Japan).

**mRNA Quantification by Real-Time Reverse Transcriptase PCR**

RPE cells were prepared from eyes of each mice and cultured for approximately 2 weeks until they became confluent in 24-well plate in Dulbecco’s modified Eagle’s medium containing 20% heat-inactivated fetal calf serum supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, 1% l-glutamine, and 0.1 mM nonessential amino acids. Then the RPE cells either were left unstimulated or were stimulated with 1 μg/mL LPS or 1, 5, or 25 μg/mL *C. pneumoniae* antigen for 0.5, 1, 2, 4, 6, 8, or 12 hours. Total RNA was extracted using reagent (Trizol; Invitrogen Corp., Carlsbad, CA) according to the manufacturer’s instructions. Aliquots containing 1 μg total RNA were reverse transcribed with an RT-PCR kit (First-Strand cDNA Synthesis Kit; Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer’s instructions. The reverse-transcribed cDNA was then subjected to real-time PCR (SYBR Premix Ex Taq [Takara Bio Inc., Otsu, Japan].

**Figure 1.** *C. pneumoniae* antigen has an angiogenic effect in the eye. C57BL/6 mice were treated with laser-induced photocoagulation. Immediately after photocoagulation, 2 μL of PBS or *C. pneumoniae* antigen (250 ng/mL) was injected into the vitreous cavity. Seven days after laser treatment, the mice were perfused with fluorescein-labeled dextran and the eyes were removed to make choroidal flat mounts. (A) Laser-induced CNV was visualized in PBS or *C. pneumoniae* antigen treated mice. Representative CNV lesions of the choroidal flat mounts are shown. (B) Quantification of the size of CNV area. The bars show means (n = 10) of each group. *P < 0.05. Experiments were performed three times with similar results.
and Light Cycler (Roche Diagnostics GmbH). The primers used were 5'-AGCACCAAGCCTCTGAAAGATG-3' for VEGF, 5'-TGGAGTCAAGAGGAGTGGGCTAAG-3' and 5'-TCTGACCACAGTGAGGAATGTCCAC-3' for IL-6, 5'-AAAATTCGAGTGACAAGCCTGTAG-3' and 5'-CCCTTGAAGAGAACCTGGGAGTAG-3' for TNF-α, and 5'-GATGACCCAGATCATGTTTGA-3' and 5'-GGAGAGCATAGCCCTCGTAG-3' for β-actin. All estimated mRNA values were normalized to β-actin mRNA levels. Each experiment was repeated at least twice, and representative data are shown.

For quantification of TLR2, cultured RPE cells from C57BL/6 (B6) mice or TLR2 KO mice were stimulated by LPS (100 ng/mL) or C. pneumoniae (25 μg/mL) for 2 hours, and then total RNA was extracted. The primers used for TLR2 were purchased from Takara Bio Inc. (Ohsu, Japan; oligo names MA030880-F and MA030880-R). The set of TLR2 primers used amplified the targeted lesion in TLR2 KO mice; thus, the RNA from TLR2 KO mice was not amplified. All estimated mRNA values were normalized to β-actin mRNA levels. Each experiment was repeated twice, and representative data are shown.

ELISA

Supernatants were collected from the RPE cultures, and cytokine concentrations were measured using ELISA development kits (88-7064; eBiosciences, San Diego, CA) for the detection of IL-6 and VEGF. We also measured concentrations of IL-6 and VEGF in the intraocular fluid (mixture of aqueous humor and vitreous fluid). Twenty-four hours after C. pneumoniae antigen inoculation and photocoagulation, eyes were enucleated under deep anesthesia, the conjunctival tissue was removed, and the remaining eye tissues (cornea, iris, vitreous...
body, retina, choroids, and sclera) were homogenized (Biomasher; Nippi Inc., Tokyo, Japan). After centrifugation at 12,000 g for 30 minutes, supernatants were collected, and the concentrations of cytokine were measured using ELISA development kits (IL-6, 88–7064 [eBiosciences]; VEGF, Quantikine, PMMV00 [R&D Systems, Minneapolis, MN]).

Immunostaining of TLR2 in the Cultured RPE Cells

RPE cells from C57BL/6 mice were cultured until confluence in a two-well culture slide (BD 354629; Becton Dickinson, Franklin Lakes, NJ), stimulated with LPS (100 ng/mL) for 24 hours, and fixed with 4% paraformaldehyde for 5 minutes. Fixed cultured cells were rinsed with PBS, blocked using 5% skim milk in PBS for 1 hour at room temperature, and incubated in anti-mouse Alexa Fluor-conjugated TLR2 antibody (51–9021-82, 5 μg/mL; eBiosciences) at room temperature for 3 hours. Samples were counterstained with DAPI, mounted (Crystal/ Mount; Biomedia, Foster City, CA), and subjected to fluorescence microscopy (BZ-9000; Keyence, Osaka, Japan).

Statistical Analysis

Data were analyzed by ANOVA and Scheffe’s tests. Differences between experimental groups with \( P \leq 0.05 \) were considered significant.

RESULTS

Increase in the Size of Experimental CNV in C. pneumoniae-Treated Mice

To determine whether C. pneumoniae infection can affect the pathogenesis of AMD, we used a laser-induced CNV model. \(^{30}\) C. pneumoniae antigen was inoculated into the vitreous cavity of C57BL/6 mice at the day of laser treatment (day 0), and the

![Figure 3](https://iovs.arvojournals.org/pdfsaccess.ashx?url=/data/journals/iovs/932963/)

**Figure 3.** The expression of TNF-α and VEGF on primary-cultured RPE cells against C. pneumoniae antigen. TNF-α (stimulated for 1 hour) and VEGF (stimulated for 2 hours) mRNA were quantified by real-time RT-PCR and normalized to the corresponding amounts of β-actin mRNA. Data shown are mean ± SD of triplicate samples and are representative of three independent experiments. *\( P \leq 0.05 \). NS, not significant.

![Figure 4](https://iovs.arvojournals.org/pdfsaccess.ashx?url=/data/journals/iovs/932963/)

**Figure 4.** MyD88 is essential for the reaction of RPE cells to C. pneumoniae antigen. RPE cells were prepared from eyes of C57BL/6 or Myd88 KO mice. The cells were then stimulated with C. pneumoniae antigen (25 μg/mL). Total RNA was extracted, and the amounts of IL-6 (stimulated for 6 hours) and VEGF (stimulated for 2 hours) mRNA were quantified by real-time RT-PCR and normalized to the corresponding amounts of β-actin mRNA. Data shown are mean ± SD of triplicate samples and are representative of two independent experiments. *\( P \leq 0.05 \).
appearance of CNV in choroidal flat mounts was visualized by fluorescence angiography on day 7. In contrast to the PBS control, much of the hyperfluorescent areas of new vessel formation were observed in C. pneumoniae antigen-inoculated mice (Fig. 1A). The areas of CNV were shown to be significantly larger in the C. pneumoniae antigen-inoculated mice than PBS-inoculated mice (Fig. 1B). These observations suggested an angiogenic effect of C. pneumoniae infection in this model.

In Vitro Production of IL-6 and VEGF by C. pneumoniae Antigen Stimulation in RPE Cells

Given that the C. pneumoniae antigen could augment laser-induced CNV, we anticipated that the inoculated antigen leads to a production of angiogenic or inflammatory factors. To confirm the mechanisms, we examined an in vitro system that resembled an in vivo infection. We focused on RPE cells because RPE cells are closely located at the chorioretinal interface damaged by aging in AMD patients and work as the first line of defense against external pathogens. As shown in Figure 2A, RPE cells were harvested from nontreated mice and cultured for more than 12 days. C. pneumoniae antigen was added, and the soluble factors were examined by ELISA and real-time PCR.

We initially examined the in vitro expression of IL-6 because IL-6 is known to be a multifunctional inflammatory cytokine promoting both angiogenesis and host defense against pathogens. When RPE cells were incubated with the C. pneumoniae antigen, quantitative real-time PCR and ELISA showed that mRNA expression of IL-6 was increased in a dose-dependent manner compatible with LPS stimulation (Figs. 2B, 2C). To rule out the possibility that this activity resulted from LPS contamination, the cells were also stimulated in the presence of polymyxin B, which neutralizes LPS activity, for 6 hours. Polymyxin B effectively inhibited the activity of LPS but not the activity of the C. pneumoniae antigen (Fig. 2D). C. pneumoniae can, therefore, directly stimulate RPE cells to produce IL-6.

**FIGURE 5.** RPE cells recognize C. pneumoniae antigen in a TLR2-dependent manner. RPE cells were prepared from eyes of C57BL/6, TLR2 KO, or TLR4 KO mice. The cells were then stimulated with LPS (1 μg/mL) or C. pneumoniae antigen (25 μg/mL). Total RNA was extracted, and the amounts of IL-6 (stimulated for 6 hours) and VEGF (stimulated for 2 hours) mRNA were quantified by real-time RT-PCR and normalized to the corresponding amounts of β-actin mRNA. Culture supernatant was subjected to ELISA, and the concentration of IL-6 (stimulated for 10 hours) was measured. Data shown are mean ± SD of triplicate samples and are representative of four independent experiments. *P < 0.05. NS, not significant; ND, not detectable.
We also compared VEGF (as an angiogenic factor) and TNF-α (as a proinflammatory factor) expression in RPE cells stimulated by either *C. pneumoniae* antigen or LPS. Preliminary time-course experiments revealed the maximum induction point of VEGF and TNF-α was 1 hour and 2 hours after stimulation, respectively (data not shown). In the modest experimental conditions, *C. pneumoniae* antigen induced VEGF that was comparable to LPS stimulation (Fig. 5A). However, the *C. pneumoniae* antigen completely failed to induce TNF-α (Fig. 3B). These results confirm that *C. pneumoniae* stimulation is distinct from LPS stimulation in RPE cells.

We measured intraocular cytokine concentrations in *C. pneumoniae*-inoculated eyes. Twenty-four hours after *C. pneumoniae* antigen inoculation and photocoagulation, eyes were enucleated. Three eyes were pooled (12 eyes were used per group; *n* = 4 groups) to obtain a high enough volume of intracellular fluid (mixture of aqueous humor and vitreous fluid) for ELISA. The concentrations of IL-6 and VEGF in PBS-inoculated mice were 14.40 ± 7.11 pg/mL and 60.01 ± 4.56 pg/mL. In contrast, both cytokines were significantly increased in *C. pneumoniae*-inoculated mice (IL-6, 52.06 ± 22.67 pg/mL [*P* = 0.023]; VEGF, 127.48 ± 18.19 pg/mL [*P* = 0.002]). *C. pneumoniae* antigen can augment IL-6 and VEGF production not only in vitro but also in vivo.

**Stimulation of RPE Cells by *C. pneumoniae***

*C. pneumoniae* stimulation of RPE cells culminated in the expression of IL-6/VEGF that was also produced by LPS, whose activation signal is transduced through the TLR4 receptor. Although the *C. pneumoniae* stimulation was distinct from LPS stimulation, it was still likely that the *C. pneumoniae* antigen stimulated RPE cells through TLRs that could directly recognize external infectious materials. Because the TLRs thus far identified used the specific adaptor protein MyD88,36 we decided to use MyD88 KO mice to determine whether the *C. pneumoniae* signal was mediated by TLRs.

When the expression of IL-6 and VEGF was evaluated in RPE cells derived from C57BL/6 or MyD88 KO mice, both *C. pneumoniae* antigen-medicated IL-6 and VEGF expression were significantly reduced in RPE cells from MyD88 KO mice (Figs. 4A, 4B). This result firmly indicates that *C. pneumoniae* antigen stimulates RPE cells through TLRs.

**Critical Role of TLR2 for *C. pneumoniae* Antigen Mediated CNV Enhancement**

To determine the specific TLRs responsible for *C. pneumoniae* stimulation, we assessed the IL-6/VEGF expression of RPE cells derived from TLR2 or TLR4 KO mice against the *C. pneumoniae* antigen. As shown previously in macrophages,36 the LPS-mediated inflammatory response is impaired in RPE cells from TLR4 KO mice (Fig. 5). In contrast, *C. pneumoniae* antigen-mediated IL-6/VEGF productions were normal in RPE cells of TLR4 KO mice but markedly reduced in RPE cells from TLR2 KO mice (Fig. 5).

To further confirm the role of TLR2 in vivo, we examined experimental CNV-forming ability in TLR2 KO mice. Compared with WT mice, the TLR2 KO mice lost CNV enhancement by *C. pneumoniae* antigen injection (Fig. 6). We also examined experimental CNV in either anti-TLR2 blocking mAb or anti-TLR4 blocking mAb. Anti-TLR2 mAb-treated mice lost CNV enhancement by *C. pneumoniae* antigen injection (Fig. 7A), but anti-TLR4 mAb-treated mice did not (Fig. 7B). Moreover, other TLR2 agonists (zymosan and Pam3CSK4) can also enhance the size of CNV but not of the TLR4 agonist (LPS; Fig. 7C).

![Figure 6. TLR2 mediates angiogenic effect by *C. pneumoniae* antigen in vivo. Control (C57BL/6), TLR2 KO, and TLR4 KO mice were treated to laser-induced photocoagulation. Immediately after photocoagulation, 2 μL PBS or *C. pneumoniae* antigen (250 ng/μL) was injected into the vitreous cavity. Seven days after laser treatment, the mice were perfused with fluorescein-labeled dextran and the eyes were removed to make choroidal flat mounts. (A) Laser-induced CNV staining was visualized in TLR2 KO or TLR4 KO mice treated with or without *C. pneumoniae* antigen. Representative CNV lesions of choroidal flat mounts are shown. (B) Quantification of the size of CNV area. The bars show means (±SEM). *P* < 0.05. NS, not significant.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932963/)

**Expression of TLR2 on Cultured RPE Cells**

Finally, we confirmed TLR2 expression on cultured RPE cells. According to quantitative real-time PCR, the intensity of TLR2 on unstimulated RPE cells was not very high but did express TLR2 because RPE cells of TLR2 KO mice showed no expression of TLR2 (Fig. 8A). Importantly, the intensity of TLR2 was augmented by 24-hour stimulation of LPS or *C. pneumoniae* antigen (Fig. 8A), and protein expression of TLR2 was confirmed in LPS-stimulated RPE cells by immunostaining (Fig. 8B, green).

**DISCUSSION**

In the present study, as a first step toward understanding the mechanisms of microorganism infection for influencing the pathology of AMD, we carried out intravitreal injection of the *C. pneumoniae* antigen, in vitro analysis of RPE cells, and in vivo analyses using MyD88 and TLR KO mice. We
demonstrated that intravitreous injection of the *C. pneumoniae* antigen increased the size of experimental CNV. Primary-cultured RPE cells expressed IL-6 and VEGF in response to the *C. pneumoniae* antigen. TLR2, but not TLR4, is essential for cytokine production from RPE cells in vitro and CNV augmentation induced by the *C. pneumoniae* antigen in vivo.

The exact cellular and molecular mechanisms that induce CNV remain to be elucidated, but chronic inflammation is at least one of the essential processes. Patel et al. showed that elevated levels of autoantibodies against retinal antigens appeared in the sera of AMD patients. Several reports have also demonstrated a role for the complement system, particularly factor H, in AMD. It is important to note that regional chronic inflammation is present in AMD patients. RPE cells have phagocytotic ability, and cultured RPE cells can take up microorganisms and several intercellular pathogens (c.g., *Toxoplasma gondii*) directly applied to RPE cells. Abnormal RPE activation caused by oxidative stress and amyloid beta depositing, promoting CNV formation, has also been reported.

We believe this is the first report to determine the function of TLRs against *C. pneumoniae* infection in the eye. *C. pneumoniae* antigen-mediated IL-6 and VEGF production was significantly reduced in MyD88 KO mice. These results strongly suggested that the *C. pneumoniae* antigen stimulated RPE cells through TLRs. RPE cells express TLR1–7, TLR1–9, and TLR1–10, and all have the potential to cause immune reactions in the retina. In addition, RPE cells can respond to LPS through TLR4. On stimulation, a homotypic interaction(s) between the TIR domain of the TLR and a cytosolic adaptor molecule(s) such as MyD88, harboring a TIR domain, leads to the activation of downstream signaling. Several groups have attempted to reveal the molecular downstream pathways for *C. pneumoniae* recognition by the TLRs. Results are controversial and may be dependent on specific cells and environments. It will be useful to elucidate the signaling details in RPE cells against *C. pneumoniae* in the future.

There is a possibility that regional cells other than RPE cells can contribute *C. pneumoniae*-inducing CNV argumentation. One possible candidate is accumulating macrophages. Grossniklaus et al. demonstrated local infiltrating macrophages could produce VEGF in AMD. Oh et al. showed the existence of activated macrophages in surgically removed fibrovascular subretinal membranes. We and other investigators also have shown a critical role of locally infiltrating macrophages in the eye, in the induction of experimental CNV. Moreover, microglia cells in the retina are another candidate-infecting cells because they have phagocytic potential and antigen presentation. Further studies will be required to elucidate this point.

It is important to note that *C. pneumoniae*-facilitated CNV augmentation is blunted by TLR2-blocking and that several factors and questions remain to be elucidated.
TLR2 agonists can enhance CNV without *C. pneumoniae*. A question may arise whether CNV enhancement is mediated by TLR2 but not necessarily by *C. pneumoniae*. Although *C. pneumoniae* tend to cause persistent intracellular infection related to cardiovascular diseases,11-13 and were actually detected in the eyes of patients with wet AMD,22 organisms other than *C. pneumoniae* may enhance CNV through TLR2. Further investigations are needed to clarify this point.

Our current hypothesis about the role of *C. pneumoniae* in AMD is as follows. Because of age-related changes at the chorioretinal interface, *C. pneumoniae* can make direct connect with the RPE cells in potentially infected patients. *C. pneumoniae*-infected RPE cells produce IL-6 and VEGF in the area and promote CNV formation in a TLR2-dependent manner. Our data provide the experimental evidence implying persistent *C. pneumoniae* infection is a risk factor for AMD. Preventive medicine for *C. pneumoniae* will be useful not only in cardiovascular diseases but also in AMD. Topical TLR-blocking therapy may be effective against harmful CNV development in elderly patients.

**Acknowledgments**

The authors thank Michiyo Takahara for her technical assistance.

**References**


