Amelioration of Experimental Autoimmune Uveoretinitis with Nuclear Factor-κB Inhibitor Dehydroxy Methyl Epoxyquinomicin in Mice

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Purpose. Experimental autoimmune uveoretinitis (EAU), a Th1/Th17 cell-mediated autoimmune disease induced in mice, serves as a model of human endogenous uveitis. In this model, proinflammatory cytokines and various stimuli activate the transcriptional factor, nuclear factor-κB (NF-κB), in the retina. The therapeutic effect of the NF-κB inhibitor, dehydroxy methyl epoxyquinomicin (DHMEQ), was examined on EAU.

Methods. EAU was induced in B10.BR mice by K2 peptide immunization. DHMEQ (40 mg/kg/d) was administered daily by intraperitoneal injection. Clinical severity and histopathologic severity were assessed. Translocation of NF-κB p65 into the nucleus in EAU retina was assessed. T cells were collected from draining lymph nodes of the K2-immunized mice to examine antigen (Ag)-specific T-cell active responses and cytokine production in vitro.

Results. Disease onset was significantly delayed in DHMEQ-treated mice (15.6 days) compared with untreated mice (12.6 days; $P < 0.01$). Histologic severity was significantly milder in DHMEQ-treated mice (score, 1.13) than in controls (score, 2.33; $P < 0.05$). DHMEQ suppressed the Ag-specific T-cell active responses and downregulated the productions of Th-1 type cytokines in vitro in a dose-dependent manner. Alternation was not observed in Th-2 type cytokines. Pretreatment of primed T cells or Ag-presenting cells with DHMEQ reduced T-cell activation and Th1/Th17 cytokine production. DHMEQ treatment suppressed the translocation of the NF-κB p65 subunit into the nuclei.

Conclusions. Systemic administration of DHMEQ suppressed NF-κB translocation in the retina, which might have reduced the inflammation of ocular tissues. DHMEQ-mediated regulation of NF-κB p65 could be a therapeutic target for the control of endogenous ocular inflammatory diseases. (*Invest Ophthalmol Vis Sci.* 2010;51:2077–2084) DOI:10.1167/iovs.09-4030

Experimental autoimmune uveoretinitis (EAU) induced in mice serves as an animal model for human endogenous uveitis, including sympathetic ophthalmia, birdshot retinochoroidopathy, sarcoidosis, Vogt-Koyanagi-Harada's disease, and Behçet's disease.[1] EAU can be induced by immunization with retinal antigens, such as interphotoreceptor retinoid-binding protein (IRBP) and S-Ag, or by the adoptive transfer of retinal Ag-specific T lymphocytes.[2–4] EAU induced in this manner represents a Th1/Th17 cell-mediated response to the immunizing retinal antigen. Massive inflammatory infiltration composed primarily of mononuclear cells causes rapid and irreversible destruction of photoreceptor cells.[5–8] It has been demonstrated that augmentation of the Th2 response and T-regulatory cytokine production, induction of regulatory T cells[9,10] or downregulation of the Th1 response can mitigate ocular inflammation resulting in mild EAU.[11–13]

The transcriptional factor nuclear factor-κB (NF-κB) activates genes encoding proinflammatory cytokines in cells of both innate and adaptive immunity. NF-κB is present in cytoplasm binding to the inhibitor of κB (IκB), which prevents NF-κB from entering the nucleus. When cells are stimulated, IκB is phosphorylated by a specific kinase, leading to ubiquitination and rapid degradation of IκB in proteasomes. A wide range of stimuli, including cytokines, activators of protein kinase C, viruses and reactive oxidative species, activates NF-κB.[14] Release of NF-κB from IκB results in the transfer of this activated form of NF-κB into the nuclei, where NF-κB acts on specific sequences in the promoter region of the target genes for proinflammatory cytokines, chemokines, and enzymes. These proteins generate mediators of inflammation such as tumor necrosis factor (TNF)-α, immune receptors, or adhesion molecules that play key roles in the initial recruitment of leukocytes to the sites of inflammation. Gene products regulated by NF-κB also activate NF-κB itself. Thus, the proinflammatory cytokine, interferon (IFN)-γ and TNF-α activate NF-κB and are activated by NF-κB. This type of positive regulatory loop appears to amplify and perpetuate local inflammatory responses. Therefore, effective regulation of NF-κB activation appears to be useful for control of local or systemic inflammation, and it is important for identifying agents that prevent NF-κB activation.[15]

A new NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), is a derivative of the weak antibiotic epoxyquinomicin-C, which is isolated from the culture broth of *Amycolaptosis* species.[16] Most NF-κB inhibitors prevent IκB phosphorylation, whereas DHMEQ inhibits the nuclear translocation of

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Submitted for publication May 25, 2009; revised September 11, 2009; accepted September 28, 2009.

Disclosure: **D. Iwata**, None; **N. Kitaichi**, None; **A. Miyazaki**, None; **K. Iwabuchi**, None; **K. Yoshida**, None; **K. Namba**, None; **M. Ozaki**, None; **S. Ohno**, None; **K. Umezawa**, None; **K. Yamashita**, None; **S. Todo**, None; **S. Ishida**, None; **K. Onoé**, None

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p65 protein, an active component of NF-κB.[16,17] It has been reported that DHMEQ is effective in the treatment of breast cancer cells, prostate cancer cells, thyroid cancer cells, adult T-cell leukemia (ATL) cells, and myeloma in vitro and in vivo.[18–22] The potential for modulating cell activation suggests that DHMEQ provides therapeutic advantage in acute and chronic inflammatory conditions in which the activation of NF-κB plays a major role. Indeed we have reported that NF-κB plays an important role in the development of inflammation in the cornea and conjunctiva,[23,24] NF-κB p65 subunit is translocated to the nucleus in the retina of EAU mice, and treatment with the antioxidant pyrrolidine dithiocarbamate (PDTC) treatment suppresses uveal/retinal expression of NF-κB translocation.[25] DHMEQ selectively prevents the translocation of NF-κB into the nucleus and then prevents various transcriptional functions. Unlike many other NF-κB inhibitors, DHMEQ is a small molecule shown to be nontoxic in vivo and in vitro in mice.[26]

In the present study, DHMEQ was administered to EAU-susceptible mice in vivo to examine whether NF-κB p65 nuclear translocation contributes to the development of endogenous uveitis. DHMEQ was also added to the culture to evaluate the effect on Ag-specific T-cell proliferative responses in vitro. We then examined the distribution of NF-κB p65 in the retinas of EAU mice.

Materials and Methods

Experimental Animals

Six- to 8-week-old female B10.BR (H-2^k) mice were obtained from Japan SLC (Shizuoka, Japan). All studies were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Committee for Animal Use and Care of Hokkaido University (Sapporo, Japan).

Reagents

K2 peptide (ADKDVVVLTSSRTGGV; molecular weight, 1603.78) corresponding to the amino acid sequence 201–216 of bovine IRBP is the immunodominant retinal autoantigen of EAU in H-2^k mice.[27] K2 was synthesized by σ-Genosys Japan (Ishikari City, Hokkaido, Japan). Purified *Bordetella* pertussis toxin (PTX) was purchased from Sigma-Aldrich (St. Louis, MO). Complete Freund's adjuvant (CFA) and *Mycobacterium tuberculosis* strain H37Ra were purchased from Difco (Detroit, MI).

DHMEQ was synthesized as described previously.[28] It was dissolved in 100% dimethyl sulfoxide (DMSO) at 50 mg/mL and was stored in aliquots at −20°C. Before administration to B10.BR mice, it was diluted with the 0.5 mg/mL carboxymethyl cellulose dissolved in distilled water to a final DMSO concentration of 4%. Before use in cell culture, it was diluted with the medium described below to a final DMSO concentration of ≤0.05%, at which no direct effect of DMSO was observed on NF-κB activity[29]

Immunization

To analyze T-cell proliferative responses, K2 peptide (100 μg) was emulsified in CFA (1:1 vol/vol), and a total of 50 μL emulsion was injected subcutaneously. To induce EAU, K2 (160 μg) was emulsified in CFA (1:1 vol/vol) containing 5 mg/mL *M. tuberculosis* H37Ra. Two hundred microliters of the emulsion was injected subcutaneously. Concurrent with immunization, 0.1 μg PTX in 100 μL phosphate-buffered saline (PBS) was injected intraperitoneally (IP) as an additional adjuvant.[30]

DHMEQ Treatment

From the day of immunization, mice received intraperitoneal injections of DHMEQ (20 or 40 mg/kg) daily for 3 weeks. Control mice received 100 μL vehicle alone after immunization.

EAU Evaluation

The clinical severity of retinal inflammation was assessed every 3 or 4 days from day 7 after immunization. The severity of retinal inflammation was graded on a five-point scale, as described elsewhere.[31] Clinical scoring was based on vessel dilatation, number of white focal lesions in the vessels, extent of exudate of retinal vessels, hemorrhage, and retinal detachment.

At day 21 after immunization, the mice were euthanatized with an overdose of anesthesia, after which their eyes were enucleated and fixed in 4% phosphate-buffered glutaraldehyde for 1 hour. These eyes were transferred to 10% phosphate-buffered formaldehyde. Fixed tissues were stained with hematoxylin and eosin. Histologic severity was graded in a double-blind manner on a scale of 0 to 4, as reported.[1] The higher score of two eyes was adopted as the severity in each mouse.

Immunohistochemistry

At day 10 after immunization, eyes from DHMEQ (40 mg/kg)-treated and vehicle-treated EAU mice were fixed by intracardiac perfusion of 4% paraformaldehyde in 0.1 M PBS. The eyes were enucleated and immersed in the same fixative for 12 hours, followed by embedment and section. Sections were rinsed in PBS twice before incubation with normal goat serum. These were incubated with rabbit anti–p65 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Binding of primary antisera was localized using Cy-3–conjugated goat anti-–rabbit IgG (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA). Nuclei were stained with YO-PRO-1 (Molecular Probes, Eugene, OR) for 5 minutes Sections were examined by laser scanning confocal microscopy (MRC-1024 [Bio-Rad, Richmond, CA] and LSM 510 [Carl Zeiss, Oberkochen, Germany]). Within the internal granular layer of each retinal sample, two areas were randomly photographed, and the numbers of activated NF-κB–positive cells were counted by a researcher masked to the study protocol. Results of two areas were averaged for each sample in each group. Six eyes of three mice were counted in each group.[25]

Determination of Immunologic Responses

We performed two types of T-cell activation assay. B10.BR mice were immunized with K2. Ten days after immunization, primed lymphocytes were obtained from draining lymph nodes (DLNs; axillar, cervical, inguinal). T-cell–enriched fractions were prepared by passing the dispersed cells from the DLNs of these K2-primed mice over nylon wool columns. Nylon wool nonadherent cells (5×10^5/well) were cultured with mitomycin-C (MMC)–treated splenocytes as antigen-presenting cells (APCs; 1×10^5/well) and various concentrations of K2 peptide in a 96-well flat-bottomed microtiter plate for 48 hours at 37°C. Cells were then pulse labeled with [^{3}H]thymidine (Perkin Elmer Japan, Tokyo, Japan) and incubated for 16 hours. Incorporation of [^{3}H]-thymidine was quantified with a direct β-counter (Packard, Meriden, CT), and data were presented as mean cpm (CPM) minus the background (medium alone; ΔCPM), as described elsewhere.[32,33] Cytokines produced in the culture supernatant were quantified as described.

In the first type of assay, T cells were purified from B10.BR mice primed with K2 and cultured with APCs and K2 peptide in the presence or absence of DHMEQ (1, 2, and 4 μg/mL).

In the second type of assay, K2-primed T cells or APCs from naive mice were cultured for 2 hours in the absence or presence of DHMEQ (10 μg/mL) and washed three times. These T cells from EAU mice, untreated or pretreated with DHMEQ, were cultured with DHMEQ-pretreated or untreated APCs in the presence of antigen. T-cell activation was determined as the first experiment.

Cytokine Assay

Cytokine levels in each sample were measured (Cytometric Bead Array kit; BD Biosciences, San Diego, CA). As described elsewhere,[34] 50-μL samples or known concentrations of standard samples (0–5000 pg/mL) were added to a mixture (50 μL each) of capture antibody-bead

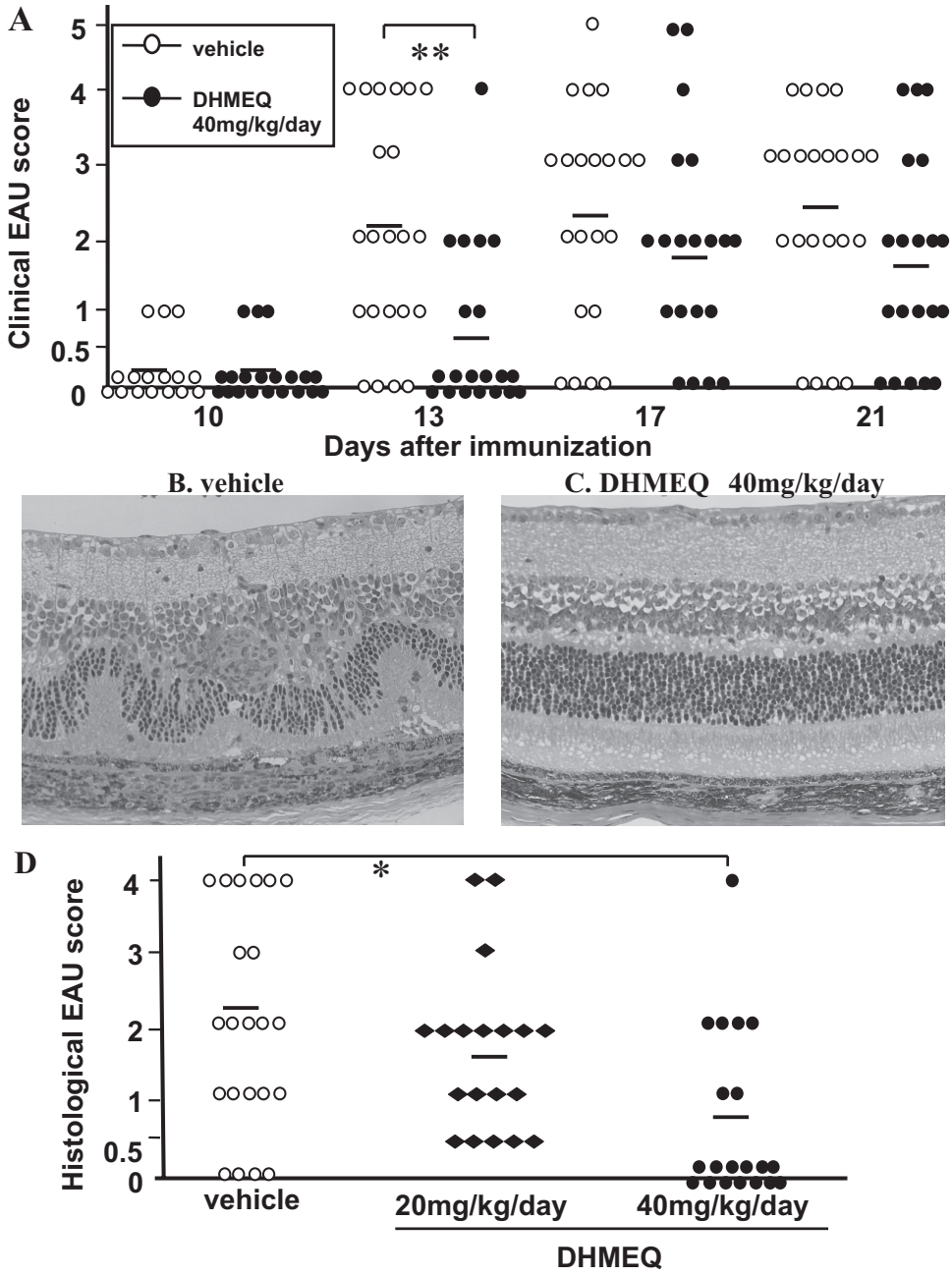

FIGURE 1. Clinical and histopathologic scores of EAU in DHMEQ-treated mice. (**A**) Clinical score of EAU in mice treated with DHMEQ or vehicle alone. EAU was induced in B10.BR mice. These mice were treated with DHMEQ (40 mg/kg; ●) or vehicle alone (○). Daily injections were given throughout the observation. Results are presented as the mean ± SEM clinical score for all eyes of each group of mice (10 mice per group). Significance was determined using the Mann-Whitney *U* test (**$P < 0.01$). (**B**, **C**) Histopathology and (**D**) histopathologic score of EAU mice treated with DHMEQ. Representative histopathology of mice treated with control (vehicle alone; **B**) or DHMEQ (40 mg/kg; **C**) is shown. (**B**) Inflammatory cells are present in the retina, vitreous, and choroid with retinal folds and granulomatous lesions in control mice, but (**C**) almost normal architecture of the retina in DHMEQ-treated mice is seen. (**D**) On day 21, the eyes from EAU mice were enucleated and scored by examining the histopathologic sections of these eyes. Results are presented as the histopathologic score of each eye, and the mean EAU score of each group is indicated by a bar. Significance was determined by the Mann-Whitney *U* test (*$P < 0.05$).

reagent and detector antibody-phycoerythrin (PE) reagent. The mixture was subsequently incubated for 3 hours at room temperature in the dark and was washed to remove unbound detector antibody-PE reagent. Data were acquired by flow cytometry (FACSCalibur; BD Biosciences) and analyzed on a computer (CBA software 1.1; BD Biosciences). Amounts of IFN-γ, TNF-α, IL-1α, -2, -4, -5, -6, -10, -17, and granulocyte macrophage colony-stimulating factor (GM-CSF) were quantified.

Statistical Analysis

Data are presented as mean ± SD in clinical and histopathologic scoring, T cell proliferation, cytokine production, and the proportion of activated NF-κB–positive cells. Statistical analysis was performed of EAU scoring and the proportion of activated NF-κB–positive cells using the nonparametric Mann-Whitney *U* test. Analyses of cell activation and cytokine production were carried out using one-way analysis of variance (ANOVA). $P < 0.05$ was considered significant.

RESULTS

Amelioration of EAU Treated with DHMEQ

To examine the suppressive effects of DHMEQ on uveoretinitis, B10.BR mice were treated with DHMEQ after immunization with K2 peptide. The experimental group of mice received 0.8 mg DHMEQ (40 mg/kg/d; intraperitoneally) throughout the course of the experiment. Control mice were administered the same volume of vehicle. The clinical EAU score was significantly lower on day 13 in DHMEQ-treated mice than in control subjects ($P < 0.05$). Disease onset of EAU in the DHMEQ-treated group occurred on day 15.6 ± 3.25 (Fig. 1A). EAU developed on day 12.6 ± 3.03 in the control group. Disease onset was significantly delayed in the DHMEQ-treated mice compared with onset in the control mice ($P < 0.01$).

To evaluate histologic scores, eyes were collected from mice 21 days after immunization. Representative hematoxylin

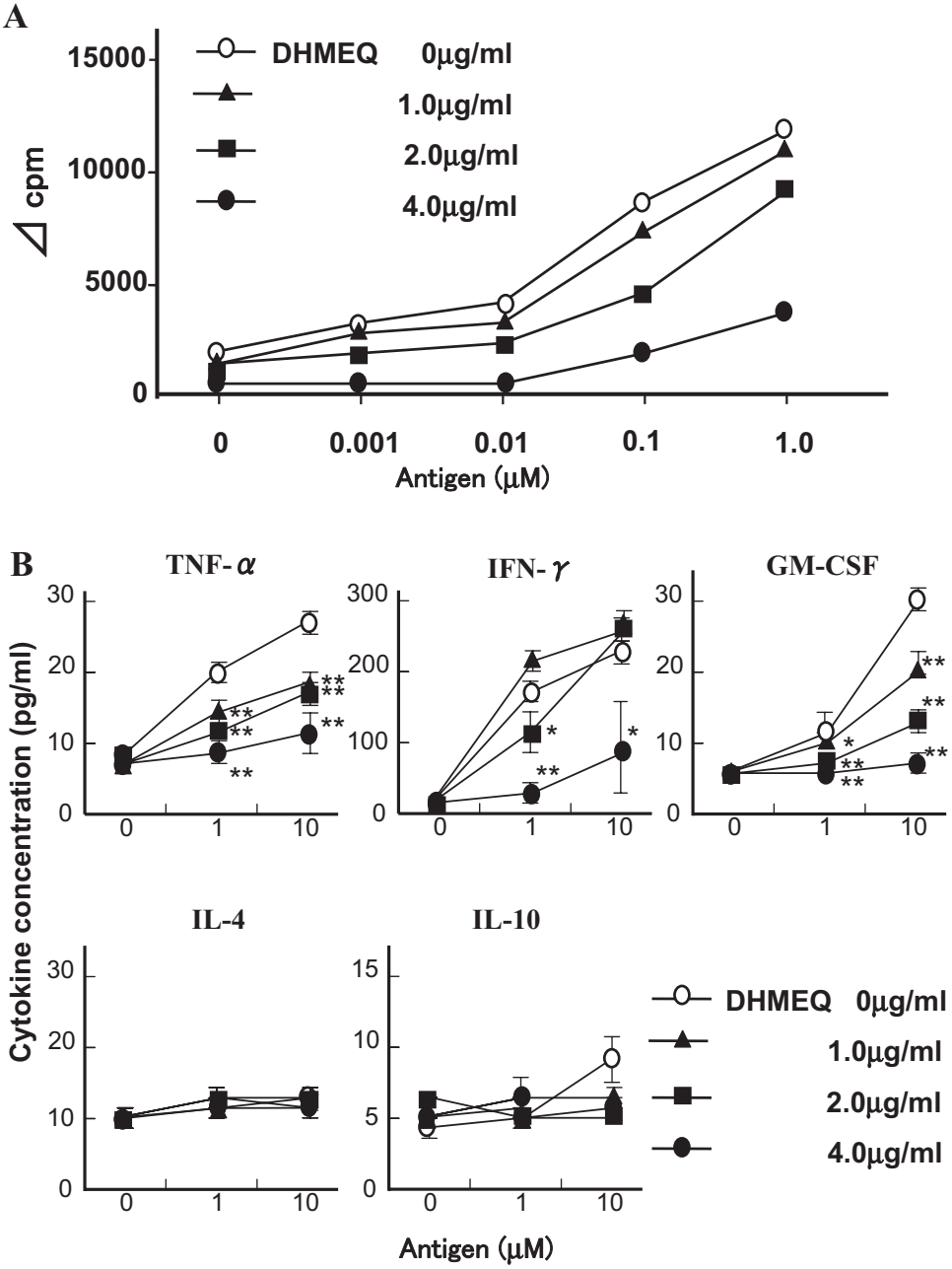

FIGURE 2. Antigen-specific T-cell proliferation and cytokine production under the influence of DHMEQ in vitro. (**A**) Incorporation of [^{3}H]-thymidine by K2-primed T cells. Lymphocytes were obtained from the DLNs of immunized mice 10 days after immunization. T cells enriched with a nylon-wool column were incubated with APCs in the presence of DHMEQ 0 μg/mL (○), 1 μg/mL (▲), 2 μg/mL (■), or 4 μg/mL (●). (**B**) Cytokine production by T cells in the culture supernatant. Cytokine production was quantified. Results are expressed as mean ± SD from two separate experiments with the same result. Significance was determined by one-way ANOVA (**$P < 0.01$; *$P < 0.05$).

and eosin histology of DHMEQ-treated and untreated mice is shown in Figures 1B and 1C. In control mice, inflammatory cells were found in the retina, vitreous, and choroids with retinal fold and granulomatous lesions (Fig. 1B). Retinas collected from mice treated with DHMEQ showed almost normal histology (Fig. 1C). Mean histologic scores were 1.13 ± 1.37, 1.66 ± 1.11, and 2.33 ± 1.52 in 40 and 20 mg/kg/d DHMEQ-treated and untreated mice, respectively (Fig. 1D). Thus, histologic severity was significantly milder in DHMEQ-treated mice than in controls ($P < 0.05$).

Downregulation of Antigen-Specific T-Cell Proliferation and Th1 Cytokine Production with DHMEQ In Vitro

We examined the direct effect of DHMEQ on the response of primed T cells to retinal antigen. DHMEQ (1.0–4.0 μg/mL) was added to the culture of primed T cells from EAU mice, antigen, and APCs. The antigen-specific T-cell proliferative response was suppressed in a dose-dependent manner according to the amount of DHMEQ added (Fig. 2A). Cytokine levels of TNF-α, IFN-γ, and GM-CSF in the culture supernatant were also significantly reduced in the presence of DHMEQ in a dose-dependent manner (Fig. 2B). Levels of the Th2-type cytokines IL-4 and IL-10 were not reduced by the addition of DHMEQ (Fig. 2B). These findings permit us to conclude that DHMEQ downregulates the proliferation of T cells and the production of Th1 cytokines, but not of Th2 cytokines, in the in vitro culture system.

Downregulation of Both T-Cell and APC Function Pretreated with DHMEQ In Vitro

Primed T cells or APCs were pretreated with DHMEQ to examine whether T cells or APCs are affected by DHMEQ treatment. T cells were enriched from the DLNs of immunized mice. Spleen cells were collected from naive mice as APCs.

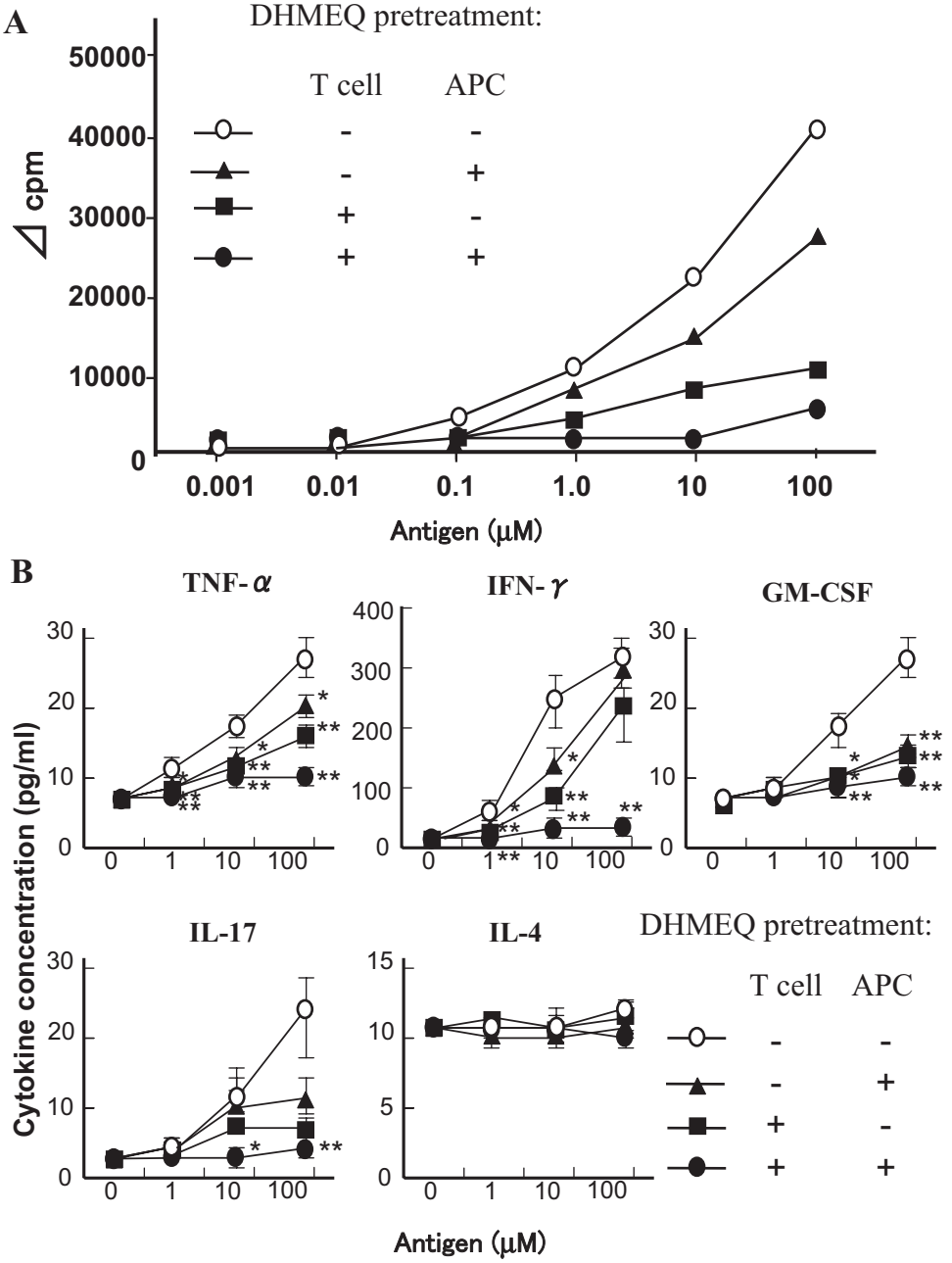

FIGURE 3. Antigen-specific T-cell activation and cytokine production after pretreatment with DHMEQ in vitro. (**A**) Incorporation of [^{3}H]-thymidine by K2-primed T cells pretreated or untreated with DHMEQ in the presence of antigen and DHMEQ-pretreated or untreated APCs, respectively. K2-primed T cells and APCs from naive mice were cultured for 60 minutes in the absence or presence of DHMEQ (10 μg/mL) and were washed extensively. DHMEQ-pretreated T cells and untreated APCs or untreated T cells and DHMEQ-pretreated APCs were cultured with peptide K2, and the proliferation was assayed. (**B**) Cytokine production by T cells in the culture supernatant. Cytokine production was quantified as described in the Figure 2 legend. Results are expressed as mean ± SD from two separate experiments with the same result. Significance was determined by one-way ANOVA (**$P < 0.01$; *$P < 0.05$).

Collected primed T cells, APCs, or both were pretreated with DHMEQ (10 μg/mL) before antigen-specific T-cell activation assay. Pretreatment of primed T cells or APCs with DHMEQ significantly reduced the T-cell activation (Fig. 3A) and production of IFN-γ, TNF-α, GM-CSF, and IL-17 (Fig. 3B). Almost no responses were detected in the culture of T cells and APCs, both pretreated with DHMEQ. IL-4 production was not reduced even when T cells and APCs were pretreated with DHMEQ in vitro.

Inhibition of Translocation of NF-κB into the Nucleus in the EAU Retina

NF-κB activation is involved in various inflammatory responses. We analyzed, by immunohistochemical methods, the expression of activated NF-κB in EAU retina. Retinas from the eyes of EAU mice on day 10 after immunization were obtained. To examine NF-κB expression in inflammatory ocular tissues, retinas from DHMEQ-treated and -untreated EAU mice were stained with an antibody against the NF-κB subunit p65.

Considerable expression of activated NF-κB p65 was observed in the retinal cell nuclei of EAU mice treated with vehicle alone (Fig. 4A). Only a few NF-κB p65–positive nuclei were detected in DHMEQ (40 mg/kg)-treated EAU mice (Fig. 4B). The mean proportion of activated NF-κB–positive cells in the internal granular layer of control EAU mice was 9.1% ± 3.0%, whereas that in DHMEQ-treated EAU mice was 3.0% ± 1.7% (Fig. 4C). The proportion of activated NF-κB–positive cells was significantly lower in EAU mice treated with DHMEQ than in control mice ($P < 0.05$).

DISCUSSION

EAU, induced in several strains of susceptible animals by immunization with ocular antigen, is considered to be an animal

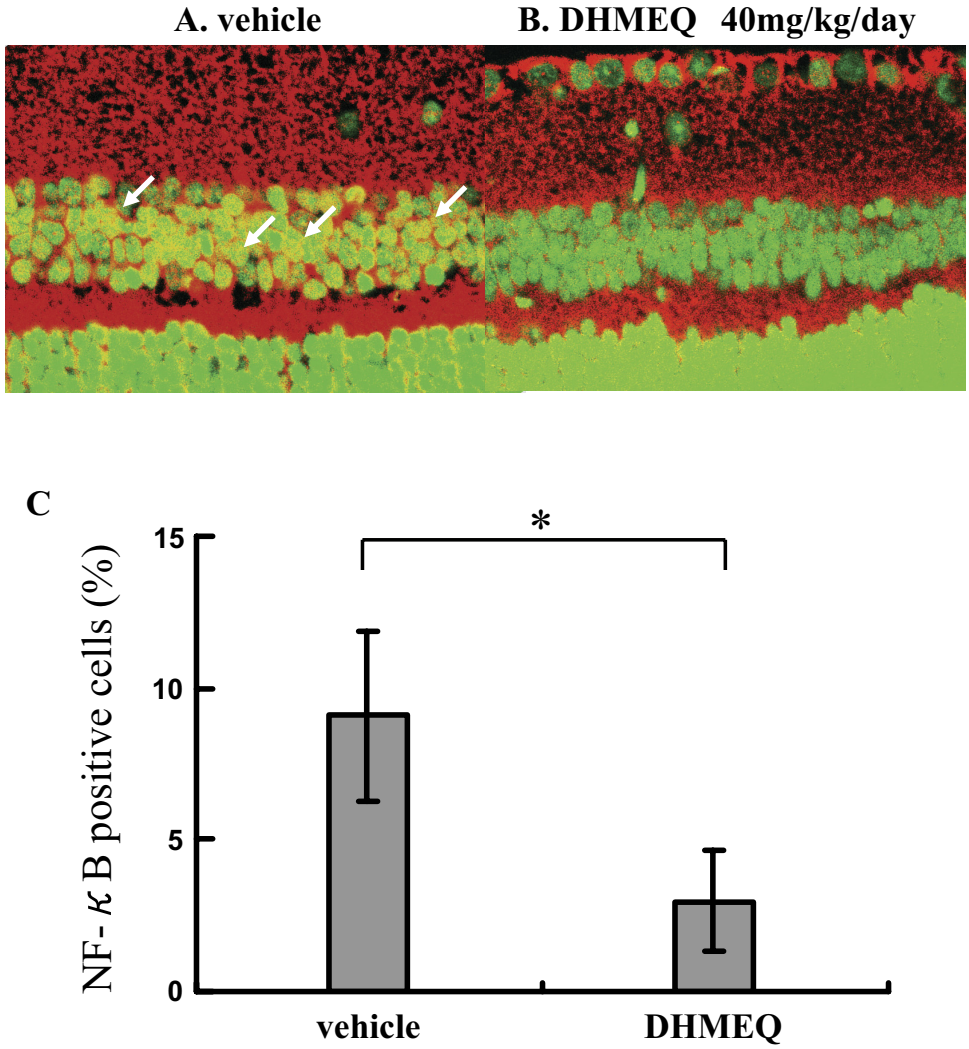

FIGURE 4. Localization of NF-κB p65 in the retinas of EAU mice treated with DHMEQ. YO-PRO-1 nuclear staining (*green*), and immunodetection of NF-κB p65 (*red*) in the retinas of control (vehicle-treated) EAU mice (**A**) and DHMEQ-treated EAU mice (**B**) is shown. *Arrows*: merged images of NF-κB (*red*) and YO-PRO-1 staining. (**C**) The proportion of activated NF-κB–positive cells was significantly lower in EAU mice treated with DHMEQ (40 mg/kg) than in control EAU mice (*$P < 0.05$). Significance was determined by the Mann-Whitney *U* test.

model for human endogenous uveitis. EAU is characterized by initial infiltration of mononuclear cells in retinal perivascular sites, followed by infiltration of phagocytes in the outer retina and uveal tract.[35,36] It has been demonstrated that EAU is immunologically induced by Th1/Th17 cells.[37–40] T cells collected from the DLNs of K2-immunized mice responded vigorously to the immunogen peptide and produced TNF-α, IFN-γ, IL-17, and other Th1-mediated cytokines.

NF-κB plays active roles in cytoplasmic/nuclear signaling and rapid response to pathogenic stimulations.[41] NF-κB is a well-known key regulator of inflammation. DHMEQ derived from the antibiotic epoxyquinomicin-C inhibits NF-κB activation in vitro and in vivo,[42–44] with blockade of the nuclear translocation of NF-κB, which is postulated to be a potential mechanism of action.[17] We reported the amelioration of EAU in mice treated with antioxidant PDTC,[25] which attenuates NF-κB activation. PDTC inhibits IκB-ubiquitin ligase activity,[45] the degradation of IκBα, and the subsequent translocation of NF-κB subunits to the nucleus.[46] However, DHMEQ is unlike many other NF-κB inhibitors that target gene products of the NF-κB pathway, and it is also unlike proteasome inhibitors that prevent the degradation of pIκB. It was reported that DHMEQ selectively prevents the translocation of NF-κB into the nucleus and then prevents various transcriptional functions. It is also important that DHMEQ is a small molecule shown to be nontoxic in mice and rodents, unlike other NF-κB inhibitors.[26]

DHMEQ hardly inhibits the phosphorylation and degradation of IκB, but it inhibits the nuclear transport of p65 in TNF-α–stimulated COS-1 cells transfected with the DNA that encodes p65 combined with green fluorescent protein.[17] It does not affect the TNF-α–induced activation of JNK, the nuclear transport of Smad2, or the large T antigen. DHMEQ should therefore be considered a unique inhibition of NF-κB that acts at the level of nuclear translocation. This specificity is an advantage of DHMEQ over the inhibitors of the upstream molecules of the NF-κB pathway because kinase inhibitors or proteasome inhibitors may have disadvantages relating to specificity and undesired effects unrelated to the NF-κB pathway. Moreover, DHMEQ specifically inhibits NF-κB in different organs of various disease models without evidence of toxicity. It has been reported that DHMEQ protects animals from glomerulonephritis,[47] keloid fibroblasts,[48] and arthritis.[29] Further administration of DHMEQ leads to prolonged allograft survival in vivo.[49] As we previously reported, blocking of the angiotensin-II type I receptor (upstream of the NF-κB signaling pathway) also suppressed glucose-induced acute retinal inflammation in mice, and treatment with DHMEQ significantly decreased retinal adherent leukocytes, ICAM-1 expression, and VEGF expression.[50] However, it is unknown whether the novel NF-κB inhibitor DHMEQ is effective in chronic and severe autoimmune ocular inflammatory disorders. We demonstrated

herein that EAU was considerably ameliorated by systemic administration of DHMEQ.

It has been reported that DHMEQ has the potential to reduce the production of Th1-mediated cytokines.[51] We also demonstrated herein the inhibition of Th1 cytokine production by DHMEQ in vitro in the present study. Pretreatment with DHMEQ suppressed not only Th1 cytokines but also IL-17 production. It seems that the amelioration of EAU by DHMEQ could be attributed to the inhibition of T-cell active response to retinal antigen and the subsequent reduced production of Th1 and Th17 cytokines in vivo. The in vivo effect of DHMEQ on antigen-specific T-cell responses and cytokine production in EAU mice should be pursued in future studies.

It has recently been reported that DHMEQ blocks maturation of dendritic cells (DCs).[51] In the present study, DHMEQ-pretreated APCs showed reduced ability to stimulate antigen-specific T cells, which may be related to the reduced production of Th1/Th17 cytokines in vitro. These results suggested that DHMEQ inhibited not only antigen-primed T cells, but also APCs, perhaps DCs, in an ocular inflammatory model.

In our previous study,[25] disease onset of EAU was not delayed by antioxidant PDTC. This appeared to differ from the results of the present study. We also found that PDTC had no influence on the priming and expansion of retinal antigen-specific T cells in vivo.[25] The present study suggests that DHMEQ exerts an influence on the priming and clonal expansion of T cells through the inhibition of APC function in ocular autoimmune disorders.

In the present study, we demonstrated by immunohistochemical methods that NF-κB p65 was translocated to the nucleus in the inflamed retina. It was reported that susceptibility to EAU was correlated with the extent of TNF-α production by Müller cells under in vitro conditions.[52] NF-κB p65 was distributed in Müller cells whose nuclei were located in the internal granular layer of retina. The distribution of NF-κB p65 in EAU retina was successfully identified and reported in our previous publication.[25] It has been also reported that DHMEQ inhibits the subsequent translocation of NF-κB subunits to the nucleus.[28] We demonstrated herein that DHMEQ inhibited the translocation of p65 in retinal tissue under inflammation. The present result appeared to be consistent with those of previous studies conducted on other tissues. Because NF-κB activation leads to the production of TNF-α, NF-κB activation should be regulated to control ocular autoimmune conditions.

In conclusion, we demonstrated that DHMEQ regulated NF-κB activation in retina and suppressed EAU development. This inhibitory effect of DHMEQ may be related to the suppression of antigen-specific T-cell activation and the production of Th1/Th17 cytokines. Because DHMEQ is a powerful anti-inflammatory drug with few reported side effects, it may be a promising agent for the management of ocular inflammatory and autoimmune disorders.

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