High Mobility Group Box Protein-1 in Experimental Autoimmune Uveoretinitis

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PURPOSE. To investigate whether high mobility group box protein (HMGB)-1, acting as a novel proinflammatory cytokine, is involved in experimental autoimmune uveoretinitis (EAU).

METHODS. HMGB-1 concentration was measured in aqueous humor, and serum was obtained from Lewis rats immunized with interphotoreceptor retinoid binding protein (IRBP) peptide (R14) and complete Freund adjuvant (CFA), rats immunized with CFA, and nontreated rats on day 14 after immunization. Immunofluorescence histochemistry was performed to examine the localization of HMGB-1 and the receptor for advanced glycation end products (RAGEs) in eyes obtained from nontreated rats or EAU-induced rats. Coexpression of CD68 (marker for macrophages) was investigated by double-immunofluorescence labeling.

RESULTS. The level of HMGB-1 in aqueous humor was significantly elevated in eyes with EAU, and HMGB-1 and tumor necrosis factor (TNF-α) levels correlated with active ocular inflammation. HMGB-1 was expressed in the iris, ciliary body, and retina of eyes from nontreated rats and EAU-induced rats. Furthermore, HMGB-1 and RAGE were found in inflammatory cells infiltrating the anterior chamber, vitreous cavity, and subretinal space in EAU-induced rats. Some HMGB-1– or RAGE–positive cells in eyes with EAU were CD68+ . Cultured macrophages expressing RAGE released TNF-α on stimulation with native HMGB-1.

CONCLUSIONS. HMGB-1 was elevated in the aqueous humor of eyes with EAU.flammatory cells infiltrating ocular tissue expressed HMGB-1 and RAGE. HMGB-1 has the capacity to stimulate TNF-α production in bone marrow–derived macrophages. These results support the possibility that extracellularly released HMGB-1 acts as a novel proinflammatory cytokine to promote and amplify ocular inflammation in autoimmune uveoretinitis. (Invest Ophthalmol Vis Sci. 2009; 50:2283–2290) DOI:10.1167/iovs.08-2709

High mobility group protein (HMGB)-1 is a highly conserved protein with 99% of amino acids conserved between rodents and humans. 1,2 HMGB-1 is a nuclear protein involved in nucleosome stabilization and gene transcription. 3 These functions are essential for survival because HMGB-1–deficient mice die of hypoglycemia within 24 hours of birth. 4 HMGB-1 is known to be present in almost all eukaryotic cells, and its presence has been confirmed in the rodent retina. 5 Although a recent study has suggested that the expression of HMGB-1 in photoreceptors is involved in the regulation of the circadian clock gene, 6 few data are available that describe the function of HMGB-1 in ocular tissues. In the developing nervous system, HMGB-1 promotes neurite outgrowth and extension by binding to the receptor for advanced glycation end products (RAGEs). 7,8

Recent evidence has identified HMGB-1 as a novel inflammatory cytokine and a late mediator of endotoxin lethality in mice and humans. 7,8 Furthermore, a cytokine-like role for extracellular HMGB-1 has been suggested. 9 HMGB-1 is released in two different ways, by passive release from necrotic cells and by active secretion from living cells. HMGB-1 passively released from necrotic cells induces a local inflammatory response. 8,9 Several proinflammatory stimuli have also been shown to trigger the active release of HMGB-1 from macrophages and monocytes. 7,9 Exposure of cultured monocytes, macrophages, neutrophils, or microvascular endothelial cells to HMGB-1 induces the release of various proinflammatory cytokines, including tumor necrosis factor (TNF-α), interleukin (IL)-1, interferon (IFN)-γ, IL-8, and monocyte chemoattractant protein-1. 7,9,11,12 Taken together, it is likely that HMGB-1 and several proinflammatory cytokines mutually induce their release from macrophages/monocytes, thus forming a proinflammatory loop.

Experimental autoimmune uveoretinitis (EAU) is an animal model that shares many clinical and histologic features with human uveitic disorders, such as Behçet disease, Vogt-Koyanagi-Harada disease, and sarcoidosis. 13–15 EAU is induced in rodents by immunization with a retinal antigen (S-antigen or interphotoreceptor-retinoid binding protein [IRBP]) or by adoptive transfer of retinal antigen–specific CD4+ Th1/Th17 cells. 16–20 TNF-α plays a central role in the pathogenesis of EAU and human uveitis (e.g., Behçet disease). 21–25 Because HMGB-1 acts as a potent stimulus of proinflammatory cytokines, including TNF-α, we investigated whether HMGB-1 is expressed and released in the ocular tissues and aqueous humor of eyes from nontreated and EAU-induced rats.

MATERIALS AND METHODS

Animals

Six- to 8-week-old female Lewis rats were purchased from Japan CLEA (Shizuoka, Japan). All rats were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. A mixture of ketamine hydrochloride and xylazine, administered by intraperitoneal injection, was used for anesthesia.

Reagents

Bovine IRBP peptide (R14) was synthesized with the use of Sawady (Tokyo, Japan) technology. Complete Freund adjuvant (CFA) was purchased from Difco Laboratories (Detroit, MI).
Induction and Scoring of EAU

Lewis rats received subcutaneous injection of R14 (0.5 μg) in 0.1 mL emulsion with CFA (1:1 vol/vol). As control, some rats were immunized with CFA alone (CFA-immunized rats). Eyes were examined daily after immunization by slit lamp biomicroscopy, and inflammation was assessed according to a standardized clinical scoring system. For histologic analysis, animals were killed on day 14 after immunization. Left eyes were removed and fixed in 10% neutral-buffered formalin. Sections were embedded in paraffin and stained with hematoxylin and eosin.

Immunohistochemistry

For immunohistochemistry, eyes were enucleated from nontreated rats and EAU-induced rats on day 14 after immunization. Whole eyes were fixed in 10% buffered formalin, sectioned into 6- to 8-μm thick slices, and mounted on glass slides. The frozen sections were dried at room temperature for 1 hour and then washed in 1× PBS. Sections were blocked with 5% goat serum diluted in 1× PBS for 30 minutes and incubated overnight with either rabbit anti–HMGB-1 polyclonal antibody (dilution 1:200; Shino-Test, Kanagawa, Japan) or rabbit anti–RAGE polyclonal antibody (dilution 1:200; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C. On the following day, sections were incubated with Alexa Fluor 555 goat anti–rabbit IgG (2 μg/mL; Molecular Probes, Eugene, OR) and cyanine monomers (2 μM; TO-PRO-3; Molecular Probes) at room temperature for 1 hour, mounted in antifade mounting medium, and examined under a confocal microscope (LSM510; Carl Zeiss Microimaging GmbH, Jena, Germany). Negative control samples were prepared in an identical fashion without incubation with anti–HMGB-1 or anti–RAGE antibody.

For immunohistochemical double labeling, sections were incubated overnight at room temperature with anti–CD68 antibody (dilution 1:20; Serotec, Oxford, UK) and either anti–HMGB-1 or anti–RAGE antibody. On the following day, sections were incubated with Alexa Fluor 555 goat anti–rabbit IgG (2 μg/mL) and Alexa Fluor 488 goat anti–mouse IgG (2 μg/mL; Molecular Probes) at room temperature for 1 hour, mounted in antifade mounting medium, and examined by confocal microscopy. Negative controls for immunohistochemical double labeling were again prepared in an identical fashion but without incubation with anti–HMGB-1 or anti–RAGE antibody. For statistical analysis, the number of HMGB-1–positive or RAGE-positive macrophages was calculated using ImageJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). Data represent the mean ± SD of three sections from each group (nontreated rats, CFA-immunized rats, and EAU-induced rats).

Assay for HMGB-1 and TNF-α in Aqueous Humor and Serum

Aqueous humor was collected from rats immunized with IRBP peptide and CFA, rats with immunized with CFA alone, and nontreated rats and was stored at −80°C until analysis. HMGB-1 was quantified by HMGB-1 ELISA kit (Shino-Test). TNF-α was measured by ELISA kit (R&D Systems, Minneapolis, MN). The kinetics of HMGB-1 in EAU was also examined in aqueous humor collected from rats immunized with IRBP peptide and CFA on days 10, 14, and 19 after immunization.

In Vitro Proliferation Assay

Pooled lymph node cells obtained from immunized rats on day 14 (1 × 10^6 cells/well) were cultured in 0.2 mL RPMI 1640 (Sigma Aldrich, St. Louis, MO) containing 10 mM HEPES, 0.1 mM nonessential amino acid, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin (all from Invitrogen Life Technologies, Carlsbad, CA), 1 × 10^-5 M 2-mercaptoethanol (2-ME; Sigma Aldrich), 10% FCS, R14 (10 μg/mL), and native HMGB-1 derived from bovine thymus (0.1 or 1.0 μg/mL; Shino-Test) for 72 hours. Cell proliferation was evaluated using a cell proliferation assay (bromodeoxyuridine; Roche Diagnostics, Mannheim, Germany).

Preparation of Peritoneal Macrophages

Peritoneal exudate cells were obtained from naïve female Lewis rats that had received 10 mL intraperitoneal 3% thioglycolate solution 3 days earlier. The cells were collected by lavage of the peritoneal cavity with PBS, resuspended in complete medium composed of Dulbecco modified Eagle medium (DMEM), 10 mM HEPES, 0.1 mM nonessential amino acid, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (all from Invitrogen-Gibco, Grand Island, NY) and were plated at 2 × 10^5 cells/well in 100 μL culture medium in 96 flat-bottom well plates for 2 hours at 37°C with 5% CO_2. Plates were then washed twice with culture medium to remove nonadherent cells. Adherent macrophage monolayers were stimulated with native HMGB-1 derived from bovine thymus (1 μg/mL; Shino-Test) in complete medium for 12, 24, 36, or 48 hours. In other experiments, adherent macrophages were cultured with or without native HMGB-1 (Shino-Test) for 12 hours. Some macrophages were cultured on a chamber slide (Laboratory-Tek, Nunc Nalge International, Rochester, NY) for immunohistochemistry.

Cytokine Assay in Culture Supernatants

Supernatants were collected from macrophage cultures stimulated with native HMGB-1, and TNF-α was measured by ELISA kit (R&D Systems).

Statistical Analysis

Results of experiments were analyzed with the Mann-Whitney U test. Means were considered to be significantly different for P < 0.05.

RESULTS

Extracellular Release of HMGB-1 into Aqueous Humor of Rats with EAU

We first determined the presence of extracellular HMGB-1 in the anterior chamber of nontreated rats, CFA-immunized rats, and EAU-induced rats. Aqueous humor was collected from nontreated rats, CFA-immunized rats, and EAU-induced rats on day 14 after immunization. Inflammation by biomicroscopic evaluation peaked on day 14 (Figs. 1A, 1B). Rats immunized with CFA alone and nontreated rats did not develop any clinical evidence of intraocular inflammation. HMGB-1 was detected in the aqueous humor of EAU-induced rats but not in the aqueous humor of nontreated rats or CFA-immunized rats (Fig. 1C).

Next, to examine the kinetics of HMGB-1 in the anterior chamber of EAU-induced eyes, we collected aqueous humor from EAU-induced rats on days 10, 14, and 19 after immunization and measured the level of HMGB-1. As shown in Figure 1D, though the HMGB-1 level was low on day 10, it rapidly increased during the peak phase of EAU (day 14) and was followed by a decrease during the resolution phase (day 19). No HMGB-1 was detected in the serum of EAU rats, CFA-immunized rats, or nontreated rats. The level of TNF-α in aqueous humor also rapidly increased during the peak phase in EAU (day 14) rats (Fig. 1E). These results indicate that HMGB-1 is elevated in inflamed eyes and that both HMGB-1 and TNF-α levels correlate with disease activity.

Expression of HMGB-1 in Ocular Tissues from Nontreated and EAU-Induced Rats

Immunohistochemical staining was performed to study the expression of HMGB-1 in ocular tissue from nontreated rats and EAU-induced rats. HMGB-1 was expressed in the cytoplasm and nuclei of epithelial and stromal cells in the iris and ciliary body (Figs. 2A, 2E) in nontreated and EAU-induced rats. HMGB-1 was
also expressed in the retina, including the ganglion cell layer, the inner nuclear layer, the outer nuclear layer, the inner and outer segments of photoreceptors, and retinal pigment epithelial cells, in nontreated and EAU-induced rats (Figs. 2B–D, 2G, 2H). In addition, HMGB-1 was detected in infiltrating inflammatory cells, particularly the cytoplasmic compartments, in the anterior chamber, vitreous cavity, and subretinal space of eyes with EAU (Figs. 2E, 2F, 2I). These results indicate that cytoplasmic translocation of HMGB-1 is induced in inflammatory cells infiltrating the ocular tissue in EAU.

**Colocalization of Expression of HMGB-1 and CD68**

A rapid increase in number of macrophages in the inflamed ocular tissue of EAU-induced rats has been previously re-
To determine whether macrophages infiltrating the eyes of EAU-induced rats expressed HMGB-1, we used double-immunofluorescence staining to compare the localization of HMGB-1-positive cells with that of cells positive for the macrophage marker CD68. As Figures 3A–E show, HMGB-1 staining overlapped with CD68 staining in cells infiltrating the anterior chamber of the EAU-induced eyes. In addition, as shown in Figures 3F to 3J, staining for HMGB-1 and CD68 was found in cells infiltrating the subretinal spaces of EAU-induced eyes. Statistical analysis showed that the number of HMGB-1-positive macrophages in anterior and posterior segments significantly increased in EAU-induced eyes compared with CFA-immunized rats or nontreated rats (Fig. 3K).

**Expression of the HMGB-1 Receptor, RAGE, in the Ocular Tissue of Nontreated and EAU-Induced Rats**

We next examined the expression of the specific receptor for HMGB-1, RAGE, in ocular tissue and infiltrating cells. Anterior segments from nontreated and EAU-induced rats revealed RAGE expression in the cytoplasm of epithelial and stromal cells in the iris and ciliary body (Figs. 4A, 4B, 4E, 4F). Posterior segments also revealed RAGE-positive staining in the retina, including the ganglion cell layer, inner and outer nuclear layers, photoreceptor layers, and retinal pigment epithelial cells (Figs. 4C, 4D, 4G, 4H). Inflammatory cells infiltrating the anterior chamber (Figs. 4E, 4F), subretinal space (Figs. 4G, 4H), choroid (Figs. 4G, 4H), and vitreous cavity (Fig. 4I) were also RAGE positive. Furthermore, RAGE staining was found in infiltrating inflammatory cells, including CD68+ cells in the anterior (Figs. 5A–E) and posterior (Figs. 5F–J) segments of EAU eyes. Statistical analysis showed that the number of RAGE-positive macrophages in anterior and posterior segments significantly increased in EAU-induced eyes compared with CFA-immunized rats or nontreated rats (Fig. 5K). These data indicate that the receptor for HMGB-1 is also highly expressed in active EAU.

**Release of TNF-α by CD68+ Macrophages upon Stimulation with HMGB-1**

During EAU, there is extensive tissue destruction of ganglion cells and photoreceptors by activated bone marrow-derived macrophages.26 To investigate the functional role of HMGB-1 in macrophages derived from bone marrow, cultures of cells obtained from peritoneal exudates were stimulated with HMGB-1. First, CD68 expression and HMGB-1 receptor RAGE expression in those cultured cells were examined by confocal microscopy and image analysis software (Image J; developed

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**FIGURE 3.** Double-immunofluorescence staining for HMGB-1 and the macrophage marker CD68 in eyes of nontreated rats and EAU-induced rats. Localization of CD68 and HMGB-1 expression in inflammatory cells infiltrating the anterior chamber (A–E) and subretinal space (F–J). There was clear colocalization of CD68 (A, F, green) and HMGB-1 (B, G, red) immunoreactivity in cells within the anterior chamber (D, merged image; E, higher magnification) and subretinal space (I, merged image; J, higher magnification). Cell nuclei were stained with carbocyanine monomers (C, H, blue). The number of HMGB-1-positive macrophages (CD68+) in anterior and posterior segments significantly increased during the peak phase of disease (day 14) (K). *P < 0.05 versus nontreated rats or CFA-immunized rats. Data represent mean ± SD of three sections in each group. Arrows: HMGB-1 and CD68 double-positive cells. Scale bar, 100 μm.
by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). More than 93% of the cultured cells were CD68** and RAGE positive. Next, the cultured cells were stimulated with HMGB-1 for 12, 24, 36, and 48 hours, and the concentration of TNF-α released into the conditioned supernatant was measured by ELISA. TNF-α levels were highest at 12 hours after stimulation with 1.0 ng/mL HMGB-1 and decreased thereafter (Fig. 6A). This HMGB-1–stimulated release of TNF-α at 12 hours was dose dependent (Fig. 6B). Taken together, these data suggest that HMGB-1 represents a proinflammatory molecule capable of inducing the release of TNF-α from RAGE-expressing macrophages.

**Elevated Antigen-Specific Proliferation in Draining Lymph Node Cells of EAU-Induced Rats with Supplementation of HMGB-1**

It has been reported that the release of HMGB-1 by dendritic cells controls T-cell activation by RAGE. Furthermore, recent studies have shown that RAGE expression on T cells contributes to antigen-specific cellular expansion in vivo and in vitro. To determine whether supplementation of HMGB-1 could elevate antigen-specific T-cell proliferation, draining lymph node cells obtained from rats with EAU on day 14 after immunization were stimulated in the presence or absence of HMGB-1. As shown in Figure 7, antigen-specific T-cell proliferation in draining lymph node cells was significantly increased in the presence of HMGB-1 (1.0 ng/mL). These results suggest that released HMGB-1 may elevate antigen-specific T-cell proliferation in the rat eye with EAU.

**DISCUSSION**

To our knowledge, this is the first report demonstrating extracellular and cytoplasmic expression of HMGB-1 and the specific receptor RAGE in the ocular tissue of eyes with autoimmune uveoretinitis. Growing evidence suggests an important role for extracellular HMGB-1 as a potent inflammatory mediator. Extracellular HMGB-1 evokes a strong inflammatory response; it stimulates the release of multiple proinflammatory cytokines such as TNF-α, IL-1, and IL-6 in monocytes and neutrophils and induces the expression of adhesion molecules such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on endothelial cells.

In the present study, to determine whether HMGB-1 plays a role in autoimmune uveoretinitis, HMGB-1 expression was examined in ocular tissues in EAU-induced rats. Our results demonstrated the presence of HMGB-1 in the aqueous humor of eyes with EAU. In addition, aqueous HMGB-1 levels increased, then decreased, in parallel with the severity of inflammation in EAU-induced rats. Ocular-infiltrating cells, including HMGB-1–positive cells, were also found to be CD68** in the cytoplasm. Positive staining for the HMGB-1 receptor RAGE was observed in infiltrating cells, including CD68** cells. Additionally, native HMGB-1 had the capacity to stimulate TNF-α production from bone marrow–derived macrophages. Taken together, these results support a role for HMGB-1 and the RAGE receptor in promoting and amplifying the inflammatory process in autoimmune uveoretinitis.

HMGB-1 is released into the extracellular space in two different ways, by passive release from necrotic cells and by active secretion from living cells. When the former occurs, local inflammatory responses are induced. The latter pathway may depend on stimulation by proinflammatory factors, resulting in the active release of HMGB-1 from macrophages and monocytes. Our data demonstrated that the concentration of HMGB-1 rapidly increased in the aqueous humor of EAU-induced rats, peaking in parallel with clinical inflammation on day 14 after immunization. Immunohistochemical analysis also showed that HMGB-1 was present in the cytoplasm of inflammatory cells infiltrating the anterior chamber in EAU-induced rats. However, HMGB-1 was not detected in the serum of EAU-induced rats. Therefore, it appears that HMGB-1 is elevated only locally in the EAU model, suggesting two possibilities: the secretion of HMGB-1 by intraocular inflammatory cells controls T-cell activation by RAGE. Furthermore, recent studies have shown that RAGE expression on T cells contributes to antigen-specific cellular expansion in vivo and in vitro. To determine whether supplementation of HMGB-1 could elevate antigen-specific T-cell proliferation, draining lymph node cells obtained from rats with EAU on day 14 after immunization were stimulated in the presence or absence of HMGB-1. As shown in Figure 7, antigen-specific T-cell proliferation in draining lymph node cells was significantly increased in the presence of HMGB-1 (1.0 ng/mL). These results suggest that released HMGB-1 may elevate antigen-specific T-cell proliferation in the rat eye with EAU.
cells, including activated macrophages in the anterior chamber, and the release of HMGB-1 into the anterior chamber by necrotic ocular tissue destroyed by infiltrating inflammatory cells. Recently, Arimura et al. have shown that the level of HMGB-1 in the vitreous cavity of eyes with endophthalmitis correlates with visual prognosis. Therefore, HMGB-1 may serve as a biomarker for ongoing ocular inflammation, though more studies are needed to validate such a role.

FIGURE 5. Double-immunofluorescence staining for RAGE and CD68 in eyes from nontreated rats and EAU-induced rats. Localization of CD68 and RAGE in inflammatory cells infiltrating the anterior chamber (A–E) and subretinal space (F–J). There was a clear colocalization of CD68 (A, F; green) and RAGE (B, G; red) immunoreactivity in cells within the anterior chamber (D, merged image; E, higher magnification) and the subretinal space (I, merged image; J, higher magnification). Cell nuclei were stained with carbocyanine monomers (C, H; blue). The number of RAGE-positive macrophages (CD68+/H11001) in anterior and posterior segments significantly increased during the peak phase of disease (day 14) (K). *P < 0.05 versus nontreated rats or CFA-immunized rats. Data represent mean ± SD of three sections in each group. Arrows: CD68 and RAGE double-positive cells. Scale bar, 100 μm.

FIGURE 6. Bone marrow–derived CD68+ macrophages released TNF-α on stimulation with HMGB-1. Cultured macrophages were stimulated with HMGB-1 for 12, 24, 36, and 48 hours, and the concentration of TNF-α released into conditioned supernatant was measured by ELISA. TNF-α levels were highest at 12 hours after stimulation with 1.0 μg/mL HMGB-1 and decreased thereafter (A). *P < 0.05 for 12 hours versus 24, 36, and 48 hours. When the cultured macrophages were stimulated with different doses of HMGB-1 for 12 hours, the level of TNF-α was significantly increased for a HMGB-1 dose of 1.0 μg/mL (B). *P < 0.05 versus 0 ng/mL. Data represent mean ± SD.
Because macrophages play a pivotal role in the pathogenesis of EAU, we examined whether macrophages infiltrating ocular structures expressed HMGB-1. Our results showed HMGB-1-positive staining in some CD68-expressing macrophages in the anterior chamber, the vitreous cavity, and the subretinal space. Additionally, we confirmed that macrophages expressing the HMGB-1 receptor RAGE released TNF-α by stimulation with HMGB-1. It is thus possible that HMGB-1 is secreted by infiltrating inflammatory cells, subsequently inducing infiltrating macrophages to release TNF-α and contribute to the pathogenesis of EAU.

Recent studies have suggested that the administration of HMGB-1 antibodies may confer significant protection in animals with sepsis, transient ischemia, and collagen-induced arthritis. It is conceivable that the same type of therapy may be beneficial in suppressing inflammation in the EAU model and in human uveitis.

In this study, we showed that some CD68+ macrophages infiltrating the anterior chamber and subretinal space expressed HMGB-1 and its receptor RAGE in EAU. Furthermore, TNF-α production by macrophages expressing RAGE was elevated with HMGB-1 stimulation in vitro. These results suggest the possibility that some macrophages expressing RAGE have the capacity to release TNF-α by HMGB-1 stimulation in the rat eye with EAU. However, recent studies have demonstrated that microvascular endothelial cells or human umbilical vein endothelial cells stimulated with HMGB-1 increased their expression of ICAM-1, VCAM-1, and RAGE receptor and increased their secretion of proinflammatory cytokines. In addition, TNF-α enhances RAGE expression by the activation of NF-κB in endothelium. Taken together, it is also likely that increased HMGB-1 within the eye with EAU may elicit proinflammatory responses on endothelial cells.

Our present study demonstrated that supplementation of HMGB-1 elevated antigen-specific proliferation in draining lymph node cells of rats with EAU. It has been reported that the release of HMGB-1 by dendritic cells controls T-cell activation through RAGE. Furthermore, recent studies have shown that RAGE expression on T cells contributes to antigen-specific cellular expansion in vivo and in vitro. Together, increased HMGB-1 in anterior and posterior segments may be involved in the activation of antigen-specific T cells within the uveitic eye. RAGE has been shown to regulate growth factors and cytokines such as TNF-α, IL-6, vascular endothelial growth factor, and adhesion molecules such as ICAM-1 and VCAM-1. In the present study, RAGE was present in the ganglion cell layer, the inner nuclear layer, and the RPE cells in nontreated eyes, consistent with previous reports. In EAU-induced eyes, we found RAGE to be highly expressed in inflammatory cells, including macrophages infiltrating the anterior chamber, vitreous cavity, retina, subretinal space, and choroid. Furthermore, HMGB-1 promoted the release of TNF-α from RAGE-expressing macrophages in culture. Thus, it is likely that elevated HMGB-1 induces RAGE-mediated activation of infiltrating macrophages and RPE and that this activation contributes to disease progression in EAU. In the future, targeting ligand/RAGE-mediated chronic activation before photoreceptor and vision loss may provide an alternative approach to current therapeutic interventions in human refractory uveitis.

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**References**


