Constitutive and Cytokine-Induced GITR Ligand Expression on Human Retinal Pigment Epithelium and Photoreceptors

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PURPOSE. The glucocorticoid-induced TNF-related receptor (GITR) plays a pivotal role in regulating the suppressive function of CD4+CD25+ regulatory T cells. GITR is also involved in the inhibition of T-cell receptor-induced apoptosis and the upregulation of inducible nitric oxide synthase (iNOS). GITRL expression on CD4+ T cells has been shown to correlate with the disease course of noninfectious uveitis. The current study was conducted to examine the expression of glucocorticoid-induced TNF-related receptor ligand (GITRL) and its regulation by ocular tissue.

METHODS. Immunohistochemistry and confocal immunofluorescence microscopy were performed on human ocular tissue to examine the in vivo protein expression of GITR. The regulation of GITR was investigated by culturing retinal pigment epithelium (RPE) with proinflammatory cytokines and performing immunocytochemistry and reverse transcription-polymerase chain reaction (RT-PCR). The in vivo mRNA expression of GITRL was studied by RT-PCR on RNA from microdissected tissue sections.

RESULTS. Both immunohistochemistry and confocal immunofluorescence microscopy demonstrated that GITRL was expressed constitutively on RPE and photoreceptor inner segments. Immunocytochemistry demonstrated that in vitro stimulated RPE cells expressed GITRL at the protein level, and RT-PCR showed that GITRL was upregulated at 24 hours by proinflammatory cytokines. Constitutive GITRL mRNA expression in vivo was confirmed by RT-PCR analysis of microdissected tissue.

CONCLUSIONS. GITR is expressed constitutively on RPE and in high levels on photoreceptor inner segments. The upregulation of GITR by proinflammatory cytokines suggests that GITR may play an important role in ocular immunity. The high level of constitutive GITR expression on photoreceptor inner segments suggests that photoreceptors participate in the regulation of ocular inflammation. (Invest Ophtalmol Vis Sci. 2004;45:3170–3176) DOI:10.1167/iovs.03-0919

The glucocorticoid-induced TNF-related receptor (GITR) is expressed constitutively at low levels on T cells and is upregulated after T cell receptor (TCR) stimulation.1,2 As its name implies, murine GITR is induced by dexamethasone and is a member of the TNF receptor superfamily. The initial reports characterizing GITR identified its role in activating NF-κB and inhibiting T cell receptor-induced apoptosis.1,3 Recently, GITR has received attention for its functional role in CD4+CD25+ regulatory T cells.4,5 These cells are a subpopulation of CD4+ T cells that constitutively express the IL-2 receptor α-chain (CD25). The CD4+CD25+ regulatory T cells have a potent ability to suppress the proliferation of CD4+ T cells in vitro and play pivotal roles in peripheral tolerance in vivo.6 Two independent groups discovered that the engagement of GITR by anti-GITR antibodies reduces the ability of CD4+CD25+ regulatory T cells to suppress the proliferation of CD4+ T cells.4,5 Other reports have expanded on these findings. Based on studies of mucosal inflammation, it has been suggested that GITR is a specific marker for both CD25+ and CD25− regulatory T cells.7 In the human system, it has been found that the intensity of GITR expression correlates with the suppressive capacity of CD4+CD25+ regulatory T-cell clones.8 In contrast to these observations, it also has been suggested that GITR may function as a negative regulator of T-cell proliferation. This is based on data from GITR−/− mice demonstrating that GITR−/− T cells proliferate more than wild-type T cells9 in response to TCR stimulation.

Although the human GITRL has been cloned independently by two different groups,2,10 less is known about the ligand than about its receptor. The human GITRL gene codes for a 20kDa protein that has sequence identity with TNF (21%), FasL (21%), Apo2 ligand (18%), and lymphotoxin-α (18%). GITRL mRNA is expressed constitutively by human umbilical vein endothelial cells (HUVECs) and this expression is upregulated by lipopolysaccharide. GITRL mRNA has also been found in several tissues, such as the small intestine, ovary, testis, and kidney, but GITRL expression is reported not to be found in resting or activated T cells.2,3 Numerous hypotheses have been proposed for GITRL’s functional roles. The previously mentioned studies with anti-GITR antibodies suggest that GITR plays an important role in controlling regulatory T cells.4,5 Furthermore, GITR has been shown to act on GITR to inhibit T-cell receptor-induced apoptosis.5 There may also be reverse signaling through GITR, as soluble GITR can act on GITRL to upregulate inducible nitric oxide synthase (iNOS) in the murine macrophage.10,11 Several types of ocular resident cells have been shown to be involved in regulating ocular immunity. For example, retinal pigment epithelium (RPE) and Müller cells can be induced to express major histocompatibility complex (MHC) class II and may act as antigen-presenting cells.12,13 Nonlymphoid ocular
Table 1. Summarization of Clinical Information of Eye Tissue

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*DPI: Death-to-preservation Interval.

Materials and Methods

Cell Lines, Reagents, Antibodies, and Tissue Preparation

ARPE19 cells originally were obtained from ATCC (Manassas, VA). Human retinal pigment epithelial cell (HRPE) cultures were prepared from donor eyes, as described elsewhere. All the HRPE cells demonstrated the presence of cytokeratin, an epithelial cell marker, indicating the purity of the cell culture. The human Müller cell line (MIO-M1) was originally provided by Astrid Limb (Institute of Ophthalmology and Moorfields Eye Hospital, London, UK). ARPE19 and HRPE cells were cultured in MEM with 10% FBS, 1% nonessential amino acids, 1% antibiotics, and 2 mM glutamine. Müller cells were cultured in DMEM containing l-glutamax I (Invitrogen-Gibco, Gaithersburg, MD) and 10% FBS (FBS; Invitrogen-Gibco). All cells were cultured at 37°C in 5% CO₂. Polyclonal goat anti-GITRL antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and R&D Systems (Minneapolis, MN). IL-1α, TNF-α, and IFN-γ were purchased from Biosource International (Camarillo, CA). All media and FBS were purchased as lipopolysaccharide (LPS)-free grade reagents. In addition, all culture media and sera were tested for potential LPS contamination by using a kit (Limu-nex; Pierce, Rockford, IL) with detection sensitivity at 0.1 U/mL. The LPS test was performed according to the manufacturer’s instruction.

Immunochemistry

ARPE19 cells were cultured on plastic chamber slides (Laboratory-Tek Permanox; Nalgene International Corp., Naperville, IL) for 24 hours and then stimulated with a cytokine mixture consisting of IL-1α (1 ng/mL), TNF-α (1 ng/mL), and IFN-γ (5 ng/mL) for another 24 to 48 hours. Six-micrometer frozen sections from normal eye donors were cut for immunohistochemistry. ARPE19 cells and frozen sections were fixed in acetone for 7 minutes. Cells were blocked with 0.3% H₂O₂ to quench endogenous peroxides and 10% animal serum to reduce non-specific binding. The cells were incubated with an anti-GITRL antibody specific against an intracellular domain of GITRL (Santa Cruz Biotechnology) at 1 to 4 µg/mL for 1 hour at room temperature followed by a biotinylated horse anti-goat secondary antibody incubation (Vector Laboratories, Burlingame, CA) at 1:200 dilution for 1 hour at room temperature. Similarly, the cells were incubated with a biotinylated anti-GITRL antibody specific against an extracellular domain of GITRL (R&D Systems) at 4 µg/mL for 1 hour at room temperature. Nonspecific goat IgG (Chemicon, Temecula, CA) and biotinylated goat anti-rabbit IgG (Vector Laboratories) were used as isotype controls at the same concentrations as the primary antibodies. An avidin-biotin-complex (Vectastain ABC) horseradish peroxidase (HRP) system (Vector Laboratories) was applied for detection of specifically bound anti-GITRL antibodies with 3,3’-diaminobenzidine tetrahydrochloride dihydrate (DAB; Sigma-Aldrich, St. Louis, MO) as a substrate. Cells were counterstained with 1% methyl green.

Confocal Immunofluorescence Microscopy

For immunofluorescence labeling, a modified protocol from Bhatti et al. was used. Briefly, frozen sections were fixed in acetone for 7 minutes. The tissue sections then were blocked with 5% normal goat serum diluted in ICC buffer (1× PBS [pH 7.3], containing 0.5% BSA and 0.2% Tween-20, 0.1% sodium azide) and incubated for 1 hour at room temperature with a biotinylated anti-GITRL antibody (R&D Systems) or a biotinylated goat anti-rabbit IgG (Vector Laboratories) as an isotype control. Each of these antibodies was used at 4 µg/mL and diluted in ICC buffer. Sections were washed repeatedly and incubated in the dark for 1 hour with the nuclear dye 4’,6’-diamino-2-phenylindole (DAPI; 1 µg/mL) and streptavidin-Cy5 (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) diluted 1:400 in ICC buffer. After repeated washing, sections were covered in mounting medium (Gem Mount; Biomed, Foster City, CA) and secured with a coverslip. For the GITRL blocking experiment, the same concentration of anti-GITRL antibody was first preincubated with or without recombinant human GITRL (R&D) at 40 µg/mL at 37°C for 30 minutes. The pretreated antibodies were then used to stain tissue sections as just described. Specimens were analyzed on a laser scanning confocal microscope (model SP2; Leica Microsystems, Exton, PA) equipped with Noramski optics. Immunolabeled and negative control sections were imaged under identical scanning conditions. Files were imported into image-analysis software (Photoshop; Adobe Systems, San Jose, CA) and converted to a digital format for analysis.

Microdissection

A laser microdissection microscope (PixCell IIe; Arcturus, Mountain View, CA) was used to microdissect ocular cells from frozen or paraffin-embedded human eye tissue sections. Briefly, RPE and photoreceptor cells were identified under the microscope. The specific cells were captured and collected onto the membrane with a power setting of 40 mW, duration of 3.5 ms, and spot size of 7.5 µm. Alternatively, ocular resident cells have been shown to suppress the proliferation of autoimmune T cells and also may express proteins that can upregulate the immune response. We have demonstrated that GITR may play a functional role in noninfectious uveitis by showing that cell surface levels of GITR on human CD4 T cells correlate with the clinical course of patients with noninfectious uveitis. These findings about GITR stimulated our interest in GITRL. To our knowledge, GITRL has never been studied in ocular cells, and little is known about how its expression is regulated. We asked whether the eye expresses GITRL and sought to characterize when and where GITRL is expressed by the eye.
tissue sections were microdissected manually using a 30-gauge needle from frozen human eye tissue sections under light microscopy.

**Reverse Transcription–Polymerase Chain Reaction**

Total RNA from microdissected cells was extracted using an RNA isolation kit (PicoPure; Acturus) according to the manufacturer’s instructions. Each RNA sample was split evenly into two portions for RT-PCR to detect both GITRL and β-actin. Cell lines were stimulated with IL-1α, TNF-α, or IFN-γ, individually in three different concentrations—that is, IL-1α or TNF-α from 0.2 ng/mL (low), to 1.0 ng/mL (medium), to 5.0 ng/mL (high); IFN-γ from 1.0 ng/mL (low), to 5.0 ng/mL (medium), to 25 ng/mL (high); or in a cocktail of the three cytokines combined in low, medium, or high concentrations for the indicated amount of time. Total RNA from cultured cell lines was isolated with an RNA isolation kit (RNasey; Qiagen, Valencia, CA) according to the manufacturer’s instructions. Total RNA (1 μg) from cell lines was used for RT-PCR. Amplification of β-actin mRNA was used as an RNA-loading control. The RT-PCR was performed with a one-step RT-PCR kit (Promega, Madison, WI) according to the manufacturer’s instructions. The amplification procedure for RT-PCR was as follows: 48°C for 45 minutes, 94°C for 2 minutes followed by cycles of the following: 94°C for 30 seconds, 57°C for 1 minute, and 68°C for 2 minutes. The microdissected samples were amplified by 40 cycles. All other samples were amplified by 30 cycles unless otherwise indicated. The primers used for amplification were from different exons and their sequences were as follows: GITRL, 5′-CTCCTTCACTTGCTAATCT-3′ and 5′-TTCAACTGTCCTCTCATAT-3′; β-actin, 5′-CGTTGACATCGCAAG-3′ and 5′-CATAAAAAAGCTATGCAATAC-3′.

**RESULTS**

**Expression of GITRL on Normal Ocular Tissue**

To localize GITRL expression at the protein level in ocular tissue, we performed immunohistochemistry on frozen sections of normal human donor eyes with an avidin-biotin-complex (ABC) horseradish peroxidase (HRP) system. Figure 1A shows normal retina, choroid, and sclera that were stained by the isotype control (Fig. 1B) or a control with no primary antibody (data not shown). Although the lipofuscin pigment of the isotype control was nearly identical, indicating the specificity of each of these antibodies for GITRL (data not shown). To evaluate further where GITRL is expressed in the eye, each of these antibodies was also used to stain frozen sections of the anterior segment of the eye. There was no detectable staining of GITRL by either of these antibodies in the cornea, ciliary body, or iris (data not shown).

**Localization of GITRL on Normal Human Retina**

Although the immunohistochemistry with the ABC system localized GITRL to the photoreceptors, it left two questions about the protein localization unanswered. The ABC system did not enable us to show definitive GITRL expression by the RPE, and it did not enable us to demonstrate whether GITRL was present on the inner or outer segments of the photoreceptors. To answer these questions, we used confocal immunofluorescence microscopy. Figure 1A shows a low-magnification view of GITRL staining of normal human retina from a 53-year-old female donor. Consistent with the ABC results, GITRL fluorescence was present on the photoreceptors when staining with the anti-GITRL antibody but was not present with the isotype control (Fig. 1B) or a control with no primary antibody (data not shown). Although the lipofuscin pigment of RPE autofluoresced over a broad range of wavelengths, one could distinguish the GITRL fluorescence (red) from lipofuscin autofluorescence (green) by looking at areas where the red fluorescence did not overlap with the lipofuscin autofluorescence. The low-magnification image had areas of GITRL fluorescence (Fig. 2A, arrows) in the RPE that were distinguishable from the autofluorescence of the lipofuscin pigment of RPE. At higher magnification of the RPE (Figs. 2C, 2D), GITRL staining was even more evident. Higher-magnification images also showed circular patterns of GITRL staining in the choroid, suggesting that GITRL was present on endothelial cells of the choriocapillaris (data not shown). This staining may serve as an internal positive control, because GITRL is expressed on HUVECs. At higher magnification of a retinal section with well-preserved photoreceptors (Figs. 2E, 2F), GITRL was localized to the inner segments of the photoreceptors.

To further confirm the specificity of GITRL staining, we used a recombinant human GITRL to block the anti-GITRL antibody binding to the eye tissue. As shown in Figure 3, whereas anti-GITRL antibody staining was still present within the inner segment of the photoreceptors and RPE (Figs. 3A–C), the recombinant human GITRL completely blocked binding of this antibody to photoreceptors and RPE (Figs. 3D–F). There was no staining if the primary antibody was not added (data not shown).

In total, we examined various parts of the eyes of six donors, ranging in age from 43 to 91 years, by either immuno-
histochemistry or confocal immunofluorescence microscopy. Table 1 summarizes data on the six donors. In all the donors examined, GITRL staining was observed in the inner segments of photoreceptors, RPE, and choroidal tissue. The anti-GITRL antibody stained the strongest in the inner segment of photoreceptors and the intensity of staining did not vary with the age of the donor. In contrast, anti-GITRL staining of the RPE seemed prominent in the young donor, but less abundant in sections from older donors.

Expression of GITRL on Cultured RPE

The constitutive expression of GITRL in vivo prompted us to investigate GITRL expression in vitro to study its regulation. We examined GITRL expression at the protein level in an RPE cell line (ARPE19) that was stimulated with a cytokine mixture consisting of IL-1α (1 ng/mL), TNF-α (1 ng/mL), and IFN-γ (5 ng/mL). This combination of cytokines was chosen because we have found it to have a significant effect on cultured RPE cells.24 The GITRL protein was detected quite readily by immunocytochemical analysis of RPE cells. As shown in Figure 4, GITRL was expressed by cultured RPE cells stimulated for 24 hours and 48 hours with the cytokine mixture. There clearly was more staining of the cells with the anti-GITRL antibody (left) than with the IgG isotype control (right). The stimulation effect of these cytokines is evident morphologically as the RPE cells take on an elongated, spindleshape morphology unlike the rounder morphology of typical, unstimulated RPE cells. Cytokine stimulation appeared to cause more GITRL staining after

Figure 2. Immunofluorescence localization of GITRL protein expression in tissues of the normal human retina. At low magnification, there appeared to be staining of the RPE (arrows) and there clearly was staining of the photoreceptors with the (A) anti-GITRL antibody but not with the (B) IgG isotype control. A higher-magnification view of the RPE clearly showed GITRL staining with the (C) anti-GITRL antibody but not with the (D) isotype control. In a sample with well-preserved photoreceptors (E, F), it became evident that the photoreceptor GITRL staining was localized to the inner segment.
48 hours of stimulation (lower right panel). LPS contamination in the culture medium could compromise these studies on GITRL expression in cultured cells. Therefore, in addition to using LPS-free media and sera, we used an assay (Limunex) to screen for potential LPS contamination. The results showed that there was no LPS contamination in any of the culture media (LPS < 0.1 U/mL).

In Vivo GITRL mRNA Expression and Cytokine-Mediated Regulation of GITRL mRNA

We were interested in comparing GITRL mRNA expression with the protein expression. To observe in vivo GITRL mRNA expression, we microdissected RPE and photoreceptor cells from normal human retinas, isolated the RNA from these cells, and performed RT-PCR. We began with the microdissection of RPE from frozen and paraffin-embedded retina sections. The RNA from the frozen sections clearly showed that there was constitutive GITRL mRNA expression by the RPE (Fig. 5A). Next, we microdissected photoreceptors from frozen retina sections and again found that there was constitutive GITRL mRNA expression (Fig. 5B). These observations of in vivo GITRL mRNA expression further confirmed the results of the previous immunohistochemistry and confocal immunofluorescence microscopy experiments.

Whereas GITRL mRNA was found to be present constitutively in vivo, the in vitro immunocytochemistry data suggested that GITRL may be upregulated by proinflammatory cytokines (Fig. 4). To examine this further, we first used RT-PCR to examine the kinetics of GITRL mRNA expression on RPE cells over a 24-hour period. As shown in Figure 5C, there was constitutive GITRL mRNA expression in an RPE (APRE19) cell line. Similar GITRL mRNA expression was also found in another RPE cell line (HRPE1; data not shown). Semiquantitative RT-PCR demonstrated that this mRNA expression was upregulated at 24 hours after stimulation with the cytokine mixture consisting of IL-1α (1 ng/mL), TNF-α (1 ng/mL), and IFN-γ (5 ng/mL). We then investigated the effect of individual cytokines versus the mixture of cytokines in regulating GITRL expression at the mRNA level. As shown in Figure 5D, IL-1α, TNF-α, and IFN-γ all induced GITRL expression but at much lower levels. Only the mixture of the three cytokines showed a maximum effect in inducing GITRL expression. It was also interesting to note that the induction effect of individual cytokines seemed to reach a plateau at medium concentration levels, whereas the cytokine cocktail had the best induction at the highest concentration (Fig. 5D).

DISCUSSION

We have shown for the first time the constitutive and cytokine-regulated expression of GITRL by ocular tissue. This also represents the first demonstration of GITRL at the protein level in any tissue. We hypothesized that we would find GITRL expression on the RPE, as these cells have characteristics that would

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933229/)

**Figure 3.** Specificity of GITRL staining in normal human retina. Specific GITRL staining was further confirmed by a GITRL blocking experiment. Anti-GITRL antibody stained on retina (A), photoreceptors (B), and RPE cells (C), but a recombinant human GITRL protein completely blocked the antibody staining on all the tissues (D–F). These data are representative of results in three experiments.

![Figure 4](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933229/)

**Figure 4.** Immunocytochemical analysis of GITRL protein expression in ARPE19 cells. ARPE19 cells were cultured on plastic chambered slides and then stimulated for the indicated amount of time by a cytokine mixture consisting of IL-1α (1 ng/mL), TNF-α (1 ng/mL), and IFN-γ (5 ng/mL). The cells readily stained with the anti-GITRL antibody and did not stain significantly with the IgG isotype control.
GITRL mRNA expression in human RPE and photoreceptors with β-actin mRNA as a control. (A) GITRL RT-PCR analysis of total RNA isolated from microdissected normal RPE from frozen and paraffin-embedded sections of normal ocular tissue. In vivo expression of GITRL mRNA was observed in RPE from the frozen sections. (B) GITRL RT-PCR analysis of total RNA isolated from microdissected photoreceptors from frozen sections of normal ocular tissue. In vivo expression of GITRL mRNA was observed in photoreceptors. (C) Semiquantitative analysis of a time course of GITRL mRNA expression in ARPE19 cells. ARPE19 cells were stimulated for the indicated amount of time by a cytokine mixture consisting of IL-1 (1 ng/mL), TNF-α (1 ng/mL), and IFN-γ (5 ng/mL). Total RNA was isolated, and RT-PCR was performed for the indicated number of cycles. Expression of GITRL mRNA was induced by these cytokines after 24 hours of stimulation. (D) Comparison of individual cytokines and a cytokine cocktail in regulating GITRL expression. ARPE19 cells were cultured and stimulated for 24 hours as just described. The stimuli were medium alone (lane 0); IL-1: 0.2 ng/mL (lane 1), 1.0 ng/mL (lane 2), 5.0 ng/mL (lane 3); TNF-α: 0.2 ng/mL (lane 4), 1.0 ng/mL (lane 5), 5.0 ng/mL (lane 6); IFN-γ: 1.0 ng/mL (lane 7), 5.0 ng/mL (lane 8), 25 ng/mL (lane 9); or a mixture of the three cytokines at low (lane 10), medium (lane 11) and high (lane 12) concentrations. The data are representative of results in two experiments.

Suggest they are capable of playing a role in the intraocular immune response. However, we were surprised to find strong constitutive expression of GITRL by the photoreceptor cells. Photoreceptors are highly specialized neurosensory cells and are not typically thought to interact with the immune system, other than being a site of uveitogenic antigens. Because the outer segment of a photoreceptor cell is laden with rhodopsin and devoted to sensing light, we were interested in determining whether the GITRL was expressed on the inner or outer segments of the photoreceptors. Our finding that expression was localized to the photoreceptor inner segment suggests that photoreceptor cells may perform other functions yet to be defined.

Recently, it has been reported that CD4+CD25 regulatory T cells are involved in neuroprotection. Depletion of CD4+CD25 regulatory T cells leads to increased survival of optic neuron function after exposure to glutamate toxicity or crush injuries. Because it is now recognized that engagement of GITR/GITRL can lead to the abrogation of the suppressive effect of those CD4+CD25 regulatory T cells, whether the constitutive expression of GITRL on the surface of photoreceptors is involved in the regulation of the local inflammatory response becomes an intriguing question.

The localization of GITRL expression only to certain ocular cells provides insight into the other potential functional roles of ocular GITRL. Ocular iNOS can be expressed in areas such as the photoreceptors, RPE, and Müller cells, and these are areas where we found GITRL. It has also been shown that signaling through GITRL increases iNOS expression and leads to nitric oxide production. Nitric oxide is thought to have multifaceted roles in the retina and can be beneficial or harmful. Although the roles of ocular nitric oxide are not completely understood, it has been reported that an inhibitor of iNOS can worsen the effector phase of experimental autoimmune uveitis in Lewis rats. If signaling through ocular GITRL induces iNOS at the right time, then this may be beneficial in an autoimmune disease scenario such as in uveitis.

On stimulation with individual cytokines or a cytokine mixture of IL-1α, TNF-α, and IFN-γ, we found that RPE cells upregulated GITRL mRNA expression at 24 hours. Immunocytochemistry experiments showed that the presence of this mRNA in cultured cells led to expression of the protein. Consistent with mRNA upregulation, GITRL protein expression appeared to be increased 48 hours after stimulation. These findings also imply that GITRL plays a dynamic role during ocular inflammation. IL-1α, TNF-α, and IFN-γ are present in the eye during inflammatory scenarios such as uveitis and may act synergistically. Our results clearly demonstrate a synergistic effect of proinflammatory cytokines in efficiently upregulating GITRL expression (Fig. 5D). Recently, it has been reported that IFN-γ acts synergistically with soluble GITR to increase iNOS expression. Cytokine-mediated regulation may be involved in GITRL’s potential functional roles in iNOS expression or neuroprotection. In addition to these potential roles, it should be emphasized that a significant part of the literature on GITRL and its receptor suggest that their role is to increase an immune response. Considering that the eye is an immune-privileged organ, it is provocative to consider that GITRL may play a proinflammatory role in the eye. With the reports of ocular expression of proteins such as intracellular adhesion molecule (ICAM)-1 and fractalkine, there is increasing evidence that some ocular resident cells express molecules that could increase an inflammatory response when needed. GITRL could be another proinflammatory protein used by ocular tissue to increase inflammation when necessary.

Among the donors we examined, we consistently observed strong GITRL expression in the inner segments of photoreceptors, whereas its expression in RPE cells appeared to decline in an age-dependent fashion. These observations are from a small sampling, however, and at this stage, the implication of this
variation among individual donors is not clear. Although we are limited by the shortage of availability of human eye tissue, especially those of a younger age and those from patients with inflammatory diseases. We are very interested in further investigating GITRL expression among those donors. It is also intriguing that based on our preliminary data there is no GITRL expression in the anterior chamber of the eye, including the cornea, ciliary body, or iris (data not shown). Further studies to confirm this finding and to investigate the implication and significance of this observation in relation to immune privilege are under way.

We found that GITRL was expressed in the eye at the mRNA and protein levels. This expression was primarily on photoreceptor inner segments and, to a lesser extent on RPE, and it was regulated by inflammatory cytokines. Ocular GITRL may have several important functions, and our observations suggest that it may play a critical role in ocular immunity. Our data also indicate that photoreceptors, in addition to their neurosensory role, may participate in the regulation of ocular inflammation.

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References


