Mapping of the Neonatal Fc Receptor in the Rodent Eye

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Purpose. The neonatal Fc receptor (FcRn) has been known to modulate IgG transport and protect against IgG catabolism, resulting in extension of the serum half-life of IgG. The goal of this study was to localize FcRn receptor expression in the rat's eye.

Methods. The cornea, retina, conjunctiva, ciliary body and iris, retinal pigment epithelium and choroid, and lens were dissected from each rat's eye, and total RNA was purified. The first-strand cDNAs were synthesized and subjected to PCR reaction. For control samples, reverse transcriptase was omitted. A monoclonal antibody against the FcRn heavy chain was used to localize the distribution of the FcRn receptor in ocular tissues. Lymphatic vessels and blood vessels were stained with a rabbit anti-mouse lymphatic vessel endothelial receptor-1 polyclonal antibody and a rabbit anti-human von Willebrand factor polyclonal antibody, respectively.

Results. RT-PCR demonstrated expression of FcRn RNA in cornea, retina, conjunctiva, ciliary body and iris, and lens but absence of expression in the retinal pigment epithelium and choroid. Immunohistochemistry and double staining confirmed the expression of FcRn receptor to the conjunctival lymphatic vessels but not in the conjunctival blood vessels. In the ciliary body, the FcRn receptor was found to be expressed in both the nonpigmented ciliary epithelium and the ciliary blood vessels. The expression of FcRn receptor was confirmed in the retinal blood vessels, iris blood vessels, optic nerve vascular structures, corneal epithelium and endothelium, and lens epithelium.

Conclusions. The FcRn receptor is expressed in multiple ocular tissues. The blood–ocular barrier showed FcRn receptor expression, indicating that IgG transport from ocular tissues to the blood system may use this receptor. The role of the FcRn receptor in the anterior segment and the conjunctiva remains unclear. (*Invest Ophthalmol Vis Sci.* 2008;49:2025–2029) DOI: 10.1167/iovs.07-0871

The neonatal Fc receptor (FcRn, FcRp, or Fcgrt) is a heterodimer composed of major histocompatibility complex (MHC)-I, which binds to both albumin and the Fc portion of immunoglobulin G (IgG).[1,2] Unlike other Fcγ receptors that bind to the lower hinge region and top of the Cγ2 domain, the FcRn receptor binds to the interface between Cγ2 and Cγ3 of IgG.[2,3] The binding site of the FcRn receptor to the Fc fragment contains several histidine residues that account for a pH-dependent interaction.[2] This enables the FcRn receptor to transport IgG(s) into the blood by transcytosis, allowing intact IgG molecules to pass through cells and into the systemic circulation. This binding of FcRn to the Fc fragment also aids in increasing the circulating half life of IgG by allowing temporary sequestration in vessel endothelium and protection from catabolism and elimination. Several studies have detailed this transcytosis pathway. First, IgG is pinocytosed nonspecifically by the cell and trafficked to the acidic endosome, where (in the low-pH environment) it binds FcRn with high affinity. FcRn then diverts IgG from a degradative lysosomal fate and transports the IgG back to the cell surface, where (at a neutral pH) it releases IgG into the extracellular space.[1]

The FcRn receptor was first identified in rodents as the receptor that transfers maternal IgGs from mother to young via the neonatal intestine.[4] The mammary gland and placental endothelium also express the FcRn receptor which mediates the transfer of protective immunoglobulins to the newborn.[5,6] In addition, it is important to note that the FcRn receptor is expressed in the brain microvasculature and the choroid plexus epithelium.[7] Transcytosis of IgG from the brain to blood has been shown to use the FcRn receptor located on brain vascular endothelium.[8,9] FcRn receptors are also expressed in mammalian adult endothelial cells, hepatocytes, histiocytes, monocytes, intestinal macrophages, dendritic cells, bronchial epithelial cells, kidney epithelial cells, and adipose tissue.[5,10–14] However, FcRn receptor distribution in the eye has not yet been determined. Accordingly, the purpose of this study was to determine whether expression of the FcRn receptor occurs in various ocular tissues.

Methods

All animal studies conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Animal Care Committee at the National Institutes of Health.

RT-PCR Analysis for Detecting FcRn mRNA in Several Ocular Tissues

Two rat's eyes were enucleated, and under sterile conditions the ocular tissues were dissected and frozen immediately in liquid nitrogen. Total (t)RNA was isolated and purified from cornea, retina, conjunctiva, ciliary body and iris, retinal pigment epithelium and choroid, and the lens (TRIzol Plus RNA purification kit; Invitrogen, Carlsbad, CA), according to the manufacturer's protocol. The first-strand cDNAs were synthesized from 2 μg of total RNA of each tissue (SuperScript III first-strand synthesis system for RT-PCR; Invitrogen). For control samples, reverse transcriptase was omitted. The yielded first-strand cDNAs were stored at −80°C before use. Aliquots of cDNAs were subjected to PCR reactions using the following amplification primers for the FcRn sequence[15]: 5′-CTGTGGATGAAGCAACCTG-3′ and 5′-TCCACGTTTGACCTCTAGC-3′ (Integrated DNA Technologies, Coralville, IA). PCR reactions involved denaturation at 94°C for 5 minutes, followed by amplification for a total of 40 cycles at 95°C for 30 seconds, 60°C for 45 seconds, and 72°C for 42 seconds. Samples from each PCR product were electrophoresed on a 2% agarose gel, and the gel was stained with ethidium bromide for visualization under UV light.

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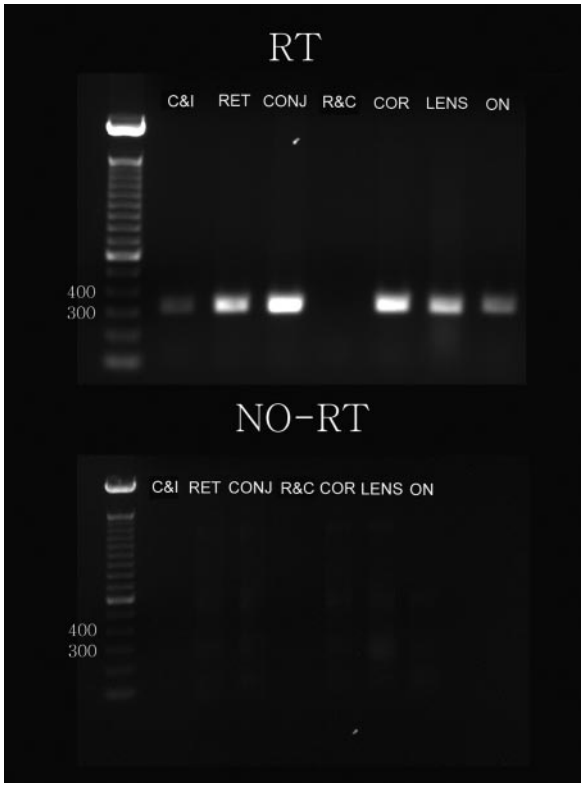

FIGURE 1. Expression of FcRn mRNA in a normal rat's eye. RT-PCR, using specific primers for FcRn, was performed on RNA extracts from isolated eye tissues, and RT-PCR products were analyzed on an ethidium bromide–stained agarose gel. RT and NO-RT represent reverse transcriptase and no reverse transcriptase, respectively. CandI, ciliary body and iris; RET, retina; CONJ, conjunctiva; RandC, retinal pigment epithelium and choroid; COR, cornea; LENS, lens; ON, optic nerve.

Anti-rFcRn Monoclonal Antibody 1G3

The existence of FcRn in the brain microvascular endothelium, choroid plexus epithelium,[7] and rat alveolar epithelium[15] has been demonstrated using an anti-FcRn mouse monoclonal antibody, 1G3, which identifies the rat FcRn heavy chain. This monoclonal 1G3 antibody was obtained from the mouse myeloma hybridoma cell line CRL-2434, as previously described.[15,16] The cells were grown in HL-1 medium (Cambrex, Walkersville, MD) supplemented with 4 mM L-glutamine (Invitrogen), 1 mM sodium pyruvate (Invitrogen), penicillin-streptomycin (Invitrogen), and 1% fetal bovine serum. Three days after incubation in the serum-free medium, the cell medium was centrifuged to remove the cells and harvest cell-free medium supernatant, which contained 1G3 antibody. The harvested medium supernatant was concentrated with a centrifugal filter (MWCO = 50 kDa; Millipore, Bedford, MA). The 1G3-containing concentrated supernatant solution was stored at −20°C.

Immunohistochemistry for FcRn Receptors

The rats' eyes were enucleated and frozen immediately in OCT compound and sectioned for 6 or 20 μm-thick slices. The frozen sections were dried at room temperature for 1 hour, fixed with 4% paraformaldehyde for 30 minutes, and then washed in 1× phosphate-buffered saline (PBS). Sections were blocked with 5% goat serum diluted in 1× PBS for 5 hours and incubated overnight in 2% goat serum concentrated 1G3-containing supernatant cell medium at 4°C. Sections were incubated in the Alexa Fluor 555 goat anti-mouse IgG (dilution 1:150; Invitrogen) and DAPI (4′,6′-diamino-2-phenylindole; dilution 1:1000; Invitrogen), at 4°C for 5 hours and mounted in antifade mounting media. Negative control samples were prepared in an identical fashion without incubation in the 1G3-containing supernatant cell medium.

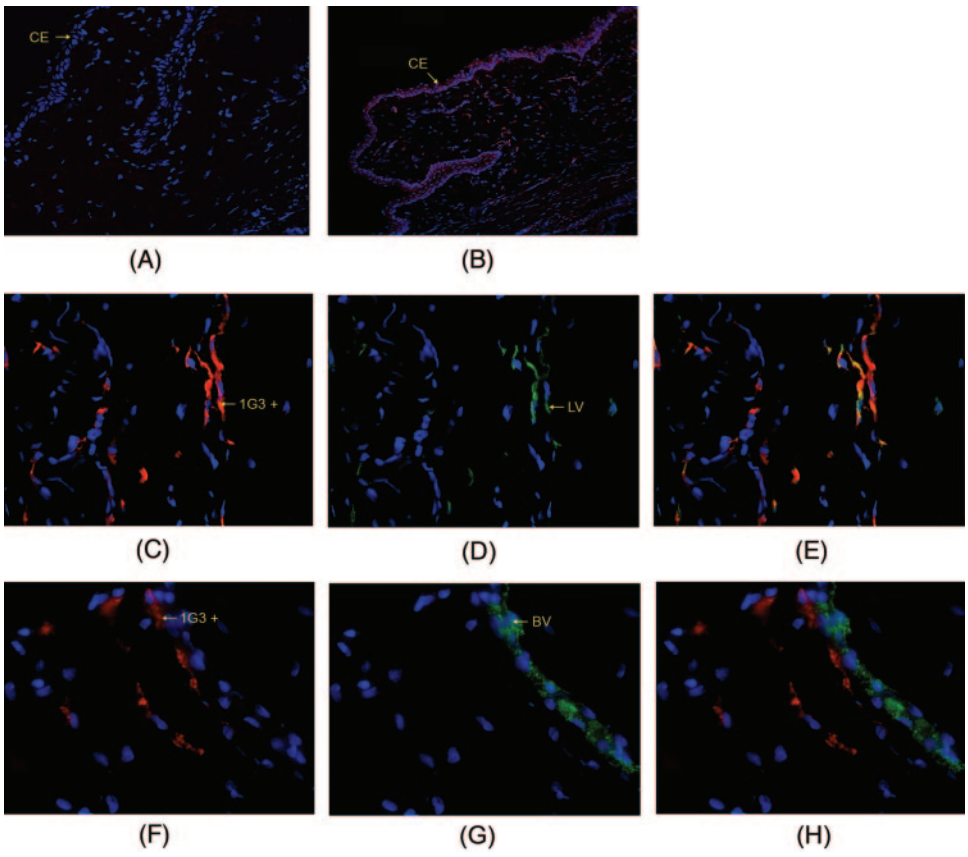

FIGURE 2. Immunohistochemical staining for rat FcRn receptor in frozen sections of rat conjunctival tissue. (**A**) Negative control without a 1G3 monoclonal antibody. (**B**) Anti-FcRn immunohistochemical staining of the conjunctival tissue. The second and third row images are double-stained for FcRn (**C**, **F**, *red*) and either lymphatic vessels (**D**, *green*) or blood vessels (**G**, *green*). (**E**) and (**H**) show merged images. A rabbit anti-mouse LYVE-1 polyclonal antibody and a rabbit anti-human von Willebrand factor polyclonal antibody were used for lymphatic vessel and blood vessel immunohistochemical staining, respectively. Cell nuclei were stained with DAPI (*blue*). CE, conjunctiva epithelium; LV, conjunctival lymphatic vessel; BV, conjunctival blood vessel.

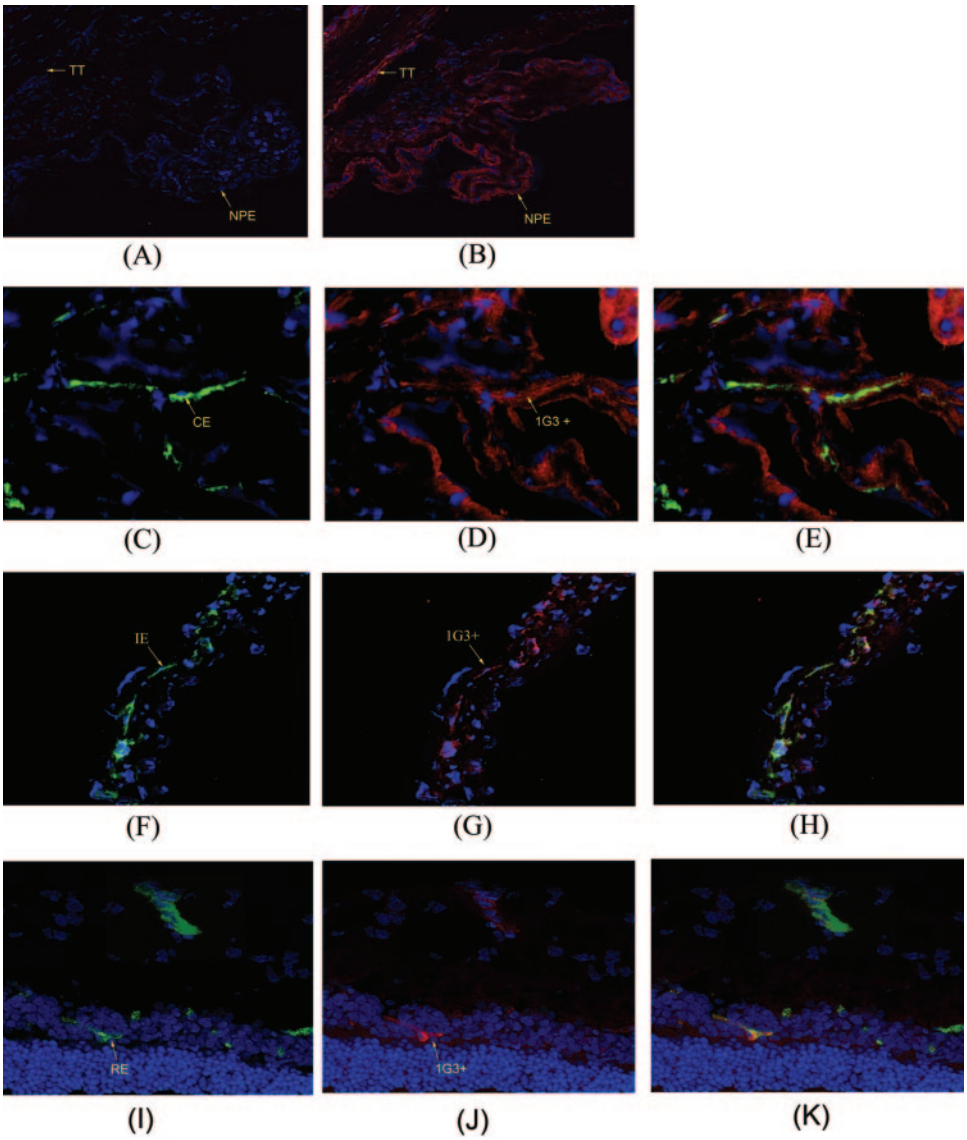

FIGURE 3. Immunohistochemical staining for rat FcRn in frozen sections of rat ciliary body, iris, and retina tissues. (**A**) Negative control without 1G3 monoclonal antibody. (**B**) Anti-FcRn immunohistochemical staining of the ciliary body. (**C**, **F**, **I**, *green*) and FcRn (**D**, **G**, **J**, *red*) are double-stained for blood vessels. (**E**, **H**, **K**) Merged images. Cell nuclei were stained with DAPI (*blue*). TT, trabecular tissue; NPE, nonpigmented epithelium; CE, ciliary vascular endothelium; IE, iris vascular endothelium; RE, retinal vascular endothelium.

Immunohistochemistry of Lymph Vessels or Blood Vessels with FcRn Receptor

Rabbit anti-mouse lymphatic vessel endothelial receptor (LYVE)-1 polyclonal antibody (Cell Sciences, Inc., Canton, MA)[17] and rabbit anti-human von Willebrand factor (VWF) polyclonal antibody (DAKO, Carpinteria, CA)[18] were used for immunohistochemistry staining of lymphatic vessels and blood vessels, respectively. Double staining was performed using rabbit anti-mouse LYVE-1 polyclonal antibody (dilution 1:150) or rabbit anti-human VWF polyclonal antibody (dilution 1:150) in the 2% goat serum concentrated 1G3-containing supernatant cell medium, as a primary antibody solution. The primary antibodies, anti-FcRn and either anti-LYVE-1 or anti-VWF, were detected using Alexa Fluor 555 goat anti-mouse IgG (dilution 1:150) and Alexa Fluor 488 goat anti-rabbit IgG (dilution 1:150), respectively, with DAPI (dilution 1:1000).

Imaging Process

Immunohistochemistry staining in the conjunctiva, lens, and cornea was performed in 6-μm-thick cryosections, and photographs were taken using epifluorescence microscopy (DM5000B; Leica Microsystems Inc., Bannockburn, IL). Twenty-μm-thick cryosections were used for the immunohistochemistry studies in the retina, ciliary body, iris, and optic nerve bundle because of the need to detect vascular structures. Images were captured by laser scanning confocal microscopy (model SP2; Leica Microsystems, Exton, PA).

RESULTS

RT-PCR Analysis for Detecting FcRn mRNA in Rat Ocular Tissues

Total RNA from several portions of rat ocular tissue was tested for the presence of FcRn mRNA. As shown in Figure 1 (top), except for the retinal pigment epithelial and choroid tissue, ocular tissues, including the ciliary body and iris, retina, conjunctiva, cornea, lens, and optic nerve bundle, showed the presence of FcRn transcript at the predicted size, whereas samples treated without RT (bottom) showed no signal.

Immunohistochemical Localization of FcRn in Several Rat Ocular Tissues

Figure 2 shows the expression of FcRn receptor in the conjunctival tissue. The conjunctival epithelial cells expressed the FcRn receptor. Costaining with LYVE-1 and VWF

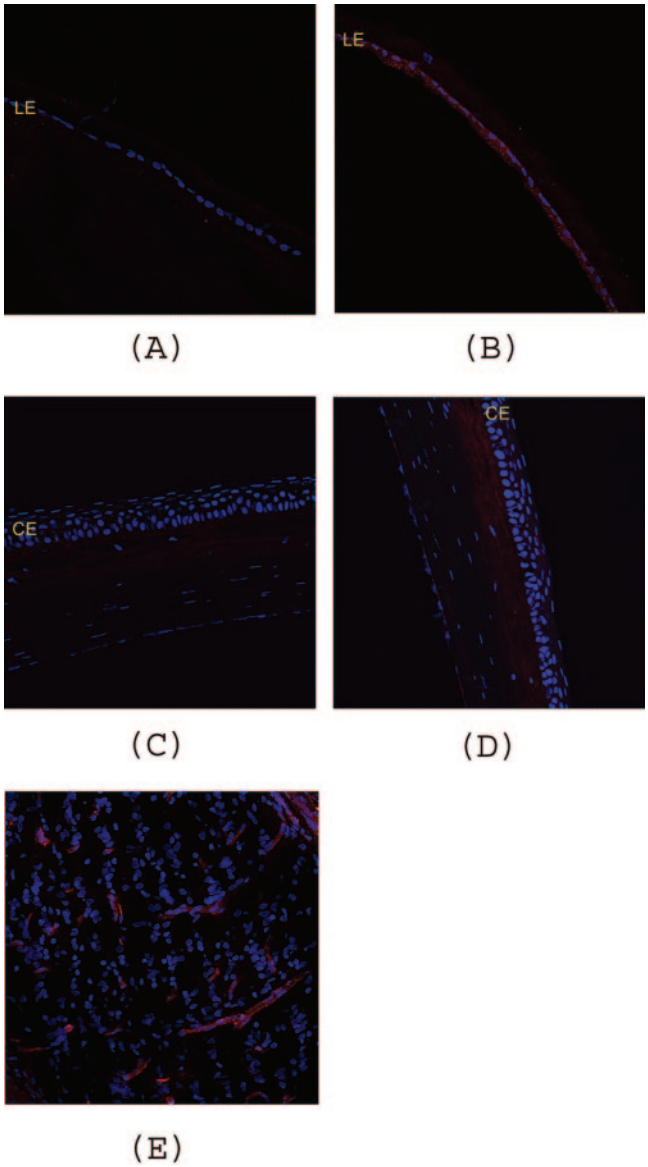

FIGURE 4. Immunohistochemical staining for rat FcRn in frozen sections of the lens, cornea, and optic nerve tissues. (**A**, **C**) Negative control subjects without 1G3 monoclonal antibody. (**B**, **D**, **E**) Anti-FcRn immunohistochemical staining of the lens, cornea, and optic nerve bundle, respectively. Cell nuclei were stained with DAPI (*blue*). LE, lens epithelium; CE, corneal epithelium; and ON, optic nerve.

markers indicated that the receptor localized to the lymphatic vessels (Figs. 2C–E), not the vascular endothelial cells (Figs. 2F–H). The ciliary body, specifically the nonpigmented ciliary epithelial cells (Figs. 3A, 3B) and the ciliary vascular structures (Figs. 3C–E), showed the expression of FcRn receptor. Within the iris, the vascular structures expressed the receptor, but the iris pigmented layer did not (Figs. 3F–H). In the retina, the inner retinal vascular structures demonstrated expression of the FcRn receptor (Figs. 3I–K), but the RPE demonstrated typical autofluorescence both in control and examined sections. Of interest, lens epithelium (Figs. 4A, 4B) and corneal endothelium and epithelium (Figs. 4C–I) express the receptor. Optic nerve vascular structures also highly express the FcRn receptor (Fig. 3E). Table 1 summarizes the results of the immunohistochemistry of the various tissues.

DISCUSSION

In the present study, both RT-PCR and immunohistochemistry data provide, for the first time, evidence of FcRn receptor expression in ocular tissues. This demonstration of the FcRn receptor expression has important implications for the concept of ocular endothelial barriers, which are localized in the vessels of the retina, optic nerve, ciliary muscle, and iris and are composed of complex tight junctions at capillary endothelial cells.[19,20] Other ocular epithelial barriers are found in the retinal pigment epithelium, the nonpigmented layer of the ciliary epithelium, and the posterior layer of the iris epithelium.[19] The blood–retinal barrier is present in the retina, the optic nerve, and the retinal pigment epithelium. The blood–aqueous barrier consists of the epithelial barriers in the ciliary body and the iridial endothelial cells. The blood–aqueous barrier in the anterior segment of the eye is known to maintain aqueous humor conditions.[21] Interestingly, the present study demonstrated expression of the FcRn receptor in the various components of these barriers.

It is thought that the FcRn selectively localizes on the abluminal membrane of brain microvessels where it mediates the transcytosis of IgG molecules from the brain to the blood across the blood–brain barrier.[7,8,9,21,22] Since the inner ocular tissues such as the retina are separated from the blood system by the blood–ocular barrier, one would not expect to detect a full-length antibody in the blood system only a short time after intravitreous injection. However, recent pharmacokinetic data from monkey and humans all indicate that intravitreous bevacizumab appears in the blood within hours after intravitreous injection (Cousins SW, et al. *IOVS* 2007;48:ARVO E-Abstract 22; Csaky KG, et al. *IOVS* 2007;48:ARVO E-Abstract 4936). Therefore, one might hypothesize that FcRn expressed at the blood–retinal barrier

TABLE 1. The Distribution of FcRn Receptor in Rats' Eyes

	Ocular Tissue	Reaction in Rats' Eyes Aged 8–12 Weeks
Cornea	Epithelium	Positive
	Stroma	Negative
	Keratocytes	Negative
	Endothelium	Positive
Conjunctiva	Epithelium	Positive
	Stroma	Positive reaction on specific cells
	Lymphatic vessels	Positive
	Blood vessels	Negative
Sclera	Fibroblast	Positive
	Stroma	Negative
Ciliary body	Nonpigmented epithelium	Positive
	Muscle fibers	Negative
	Blood vessels	Positive
Iris	Muscle fibers	Negative
	Stroma	Negative
	Blood vessels	Positive
Lens	Capsule	Negative
	Epithelium	Positive
	Cortex	Negative
	Nucleus	Negative
Retina	Müller cells	Negative
	Nerve fiber layer	Negative
	Photoreceptor layer	Negative
	Retinal pigment epithelium	Negative
	Blood vessels	Positive
	Retinal cellular layers	Negative
Optic nerve bundle	Blood vessels	Positive
	Stroma	Negative
	Bundle wall	Positive

functions in a way similar to that at the blood–brain barrier, transporting IgG from the retina into the systemic circulation. Whether the FcRn receptor plays a role in transport of IgGs from the ocular tissue to the blood system remains to be determined. However, recent widespread use of intravitreous therapeutic IgGs, including rituximab for the treatment of intraocular lymphoma and bevacizumab for choroidal neovascularization, suggests that further investigation of the role of FcRn in IgG pharmacokinetics is critical.

The FcRn receptor was detected in the lymphatic vessels but not in the blood vessels of the conjunctiva. The FcRn receptor modulates IgG transport and protects IgG in the blood from catabolism.[3,23] Therefore, it may be that the function of the FcRn receptor in the conjunctival lymphatic vessels is to act as an efflux receptor for the efficient elimination of antigen-antibody IgG complexes from the conjunctival space. Since the conjunctival blood vessels do not form a tight junction barrier, IgG from the blood system can enter into the conjunctival tissue interstitium by pinocytosis and/or convective transport through paracellular pores in the vascular endothelial layer. Extravasated IgG is then eliminated from the conjunctival tissue into the lymphatic vessels via convective transport with lymph fluid.[24] The antigen–antibody complex may be more efficiently eliminated via lymphatic vessels if convective transport into the lymph fluid is supplemented by FcRn receptor-mediated transcytosis.

In the cornea, the FcRn receptor was expressed in the corneal epithelium and the endothelium, the same locations in which Fcγ receptors are expressed.[25] In this case, the FcRn receptor may deliver antibodies to the cornea when corneal stromal antigen deposition occurs. FcRn receptor expression was also detected in the nonpigmented ciliary epithelium. The expression locations of FcRn receptor in the anterior segment are again consistent with that of Fcγ receptors. Tripathi et al.[25] suggested that the expression of Fcγ receptors, as well as the recent demonstration of class II MHC molecules in the anterior segment of the eye, is involved in antigen presentation, in addition to regulation, maintenance, and defense of the aqueous outflow pathway. In the aqueous humor, low levels of immunoglobulins can be expected because of the integrity of the blood–aqueous barrier and an active drainage mechanism that prevents accumulation of the immunoglobulins.[26] How macromolecules enter the aqueous humor has not been clearly demonstrated. Raviola[19] proposed that proteins enter the aqueous humor via iris root diffusion while Uusitalo et al.[27] speculated that macromolecules pass between adjoining nonpigmented cells in certain regions of the ciliary body. Despite similar molecular weights, IgG (150 kDa) was detected in the aqueous humor; however, IgA (160 kDa) was not.[26] The discrepancy between IgG and IgA penetration from the serum into the aqueous humor may be explained by the presence of the FcRn receptors, which are selective for IgGs.

In conclusion, this study demonstrated FcRn receptor expression in several different ocular tissues. The expression of FcRn receptor at the blood–ocular barrier suggests transportation of IgG across complex tight junctions in the eye may occur via FcRn-mediated transcytosis.

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