Immunology Letters 206 (2019) 49–53

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Distinctly regulated functions and mobilization of CD11c-positive cells elicited by TLR3- and IPS-1 signaling in the cornea

Mayumi Ueta[a,*], Junji Hamuro[b], Suzuko Ohsako[a], Shigeru Kinoshita[a]

[a] Department of Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
[b] Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

ARTICLE INFO

Keywords:
TLR3
IPS-1
CD11c
Cornea

ABSTRACT

The human ocular surface epithelium expresses TLR3, which recognizes double-stranded (ds) RNA mimicking polyinosine-polycytidylic acid (polyI:C). Its stimulation induces the secretion of the inflammatory cytokines such as interleukin (IL)-6, IL-8, and type I interferon. The cytoplasmic helicase proteins RIG-I and MDA5 are also expressed on the ocular surface. We investigated the function of TLR3 in the cornea of CD11c$^-$ YFP$^+$ and TLR3 KO CD11c$^-$ YFP$^+$ mice. We also examined the function of IPS-1, an adaptor molecule common to RIG-I and/or MDA5, in IPS-1 KO CD11c$^-$ YFP$^+$ mice. In the central corneal epithelium of CD11c$^-$ YFP$^+$ mice, the infiltration of CD11c$^-$ YFP$^+$ cells was significantly upregulated 48 h after polyI:C stimulation; it was significantly downregulated in the stromal layer of their central and peripheral cornea. On the other hand, in the corneal epithelium of TLR3 KO CD11c$^-$ YFP$^+$- and wild-type mice, the movement of CD11c$^-$ YFP$^+$ cells was different from CD11c$^-$ YFP$^+$ mice. This suggests that TLR3 knock-out (KO) interferes with their movement from the peripheral- to the central cornea or lymph nodes and that it may be similar in IPS-1 KO CD11c$^-$YFP$^+$ - and wild-type mice. Under normal conditions, the number of CD11c$^-$ YFP$^+$ cells in the central and peripheral corneal epithelium, but not in the stromal layer, is significantly greater in TLR3 KO CD11c- YFP$^+$- than CD11c$^-$ YFP$^+$ mice. In IPS-1 KO CD11c$^-$ YFP$^+$ mice, their number in the stromal layer, but not in the epithelium of the central and peripheral cornea, was significantly greater than in CD11c$^-$ YFP$^+$ mice. Our findings suggest that CD11c$^+$ dendritic cell (DC) migration in the corneal epithelium is regulated by TLR3, whereas CD11c$^+$ DC migration in the stromal layer of the cornea is regulated by IPS-1. These observations, together with our earlier findings, imply that TLR3 and IPS-1 contribute distinctly to the regulation of innate immune responses and tissue inflammation elicited by CD11c$^+$ DCs to maintain homeostasis in corneal tissues.

1. Introduction

Innate immunity, the first-line host defense against microbes, has primarily been studied in immune-competent cells such as macrophages (Mps) and natural killer cells. Their ability to recognize pathogen-associated molecular patterns (PAMPs) depends on the expression of a family of Toll-like receptors (TLRs), the retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), and the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [1]. Although TLRs detect PAMPs, e.g. endosomes or lysosomes on the cell surface or the lumen of intracellular vesicles, RLRs and NLRs are cytosolic detection-systems for intracellular PAMPs. RLRs belong to the RNA helicase family that specifically detects RNA species derived from viruses in the cytoplasm; they coordinate anti-viral programs via the induction of type I interferon (IFN) [1].

We previously reported that the human ocular surface epithelium (corneal and conjunctival) expresses TLR3, which recognizes double-stranded (ds) RNA mimicking polyinosine-polycytidylic acid (poly I:C), a synthetic dsRNA, and that poly I:C stimulation induces the secretion of inflammatory cytokines such as interleukin (IL)-6, IL-8, and type I IFN [2,3]. Our gene expression analysis of primary human conjunctival epithelial cells using microarrays revealed that CXCL11, RIG-I, IL28 A, CXCL10, CCL5, CCL4, MDA5, IL7R, TSLP, CCL20, and ICAM-1 were upregulated more than 10-fold by poly I:C stimulation [4], suggesting that it not only induces anti-viral innate immune responses, but that it could also regulate allergic reactions [4,5]. We also found that TLR3 positively regulated ocular surface inflammation in a murine allergic conjunctivitis model [6] and skin inflammation in contact- [7] and

* Corresponding author at: Department of Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajiicho, Hirokoji, Kawaramachi, Kamigyoku, Kyoto, 602-0841, Japan.
E-mail address: mueta@koto.kpu-m.ac.jp (M. Ueta).

https://doi.org/10.1016/j.imlet.2018.12.004
Received 19 October 2018; Received in revised form 11 December 2018; Accepted 14 December 2018
Available online 17 December 2018

atopic dermatitis models [8].

The cytoplasmic helicase proteins retinoic-acid-inducible protein I (RIG-I, also known as Ddx58) and melanoma-differentiation-associated gene 5 (MDA5, also known as Ifih1) have been implicated in the recognition of viral dsRNA. We reported that they were also expressed in the ocular surface epithelium [5,9].

In CD11c$^-$ YFP$^+$ mice, CD11c$^+$ dendritic cells (DCs) are labeled by yellow fluorescent protein (YFP); YFP$^+$ CD11c$^+$ DCs could be detected under fluorescence- and confocal microscopes [10].

We investigated the function of TLR3 in the cornea of CD11c$^-$ YFP$^+$- and TLR3KO CD11c$^-$ YFP$^+$ mice and studied the function of IPS-1, an adaptor molecule common to RIG-I and/or MDA5, in the cornea of IPS-1 KO CD11c$^-$YFP$^+$ mice because not only TLR3, but also RIG-I and MDA5 detect viral dsRNA.

2. Materials and methods

2.1. Mice and reagents

Balb/c background CD11c$^-$ YFP$^+$ mice were a gift from Professor Ishii; C57BL/6 background IPS-1$^{-/-}$ mice [9] and Balb/c background TLR3$^{-/-}$ mice [6] were gifted by Professor Akira. Balb/c background IPS-1$^{-/-}$ mice were produced by back-crossing C57BL/6 background IPS-1$^{-/-}$ mice with Balb/c mice for more than 6 generations.

All mice were used at 8–12 weeks of age; they were maintained on a 12-hr light/dark cycle under specific pathogen-free conditions. All experimental procedures were approved by the Committee on Animal Research of Kyoto Prefectural University of Medicine and all studies were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

PolyI:C was purchased from Invivogen (San Diego, CA). To prepare the eyedrops, we made a 1000 μg/ml poly I:C solution in phosphate-buffered saline; eyedrops (10 μl) were administered only once.

2.2. Confocal microscopy

Before- and 24-, 48-, and 72 h after delivering the eyedrops, the cornea, including the limbus, was resected. Radial cuts were observed under a confocal microscope (hardware: FV1200, software: FV10-ASW4.2, Olympus, Japan). To examine the distribution of CD11c$^-$ YFP$^+$ in the cornea, we created tiled scans of the specimens by using motorized stages that move the sample; full-thickness Z stacks were used (Fig. 1A). To show the vertical distribution of CD11c$^-$ YFP$^+$ in the corneal layer, we also used full-thickness Y stacks (Fig. 1B).

2.3. Counting of CD11c$^-$ YFP$^+$ cells in the cornea under a fluorescence microscope

Because CD11c$^-$ YFP$^+$ cell infiltration into the corneal center was most remarkable 48 h after delivering the eyedrops, we manually counted their number at 0- and 48 h under a fluorescence microscope (DP72, Olympus, Japan). The cornea, including the limbus, was resected and radial cuts were made. Then the central cornea was punched out with a 2-mm trephine and CD11c$^-$ YFP$^+$ cells in the epithelium and the stromal layer of the central- and peripheral cornea (including the limbus) were counted manually (Supplementary Fig. 1). We also compared their number in the corneal epithelium and stroma of CD11c$^-$ YFP$^+$ mice against their number in the cornea of TLR3 KO CD11c$^-$ YFP$^+$- and IPS1KOCD11c$^-$ YFP$^+$ mice (CD11c$^-$ YFP$^+$ mice: 0 h, n = 12; 48 h, n = 14. TLR3KOCD11c$^-$ YFP$^+$ mice: 0 h, n = 10; 48 h, n = 8. IPS1KOCD11c$^-$ YFP$^+$ mice: 0 h, n = 7; 48 h, n = 10).

3. Results

Confocal microscopy showed that a single eyedrop (10 μl poly I:C, 1 mg/ml) elicited the infiltration of CD11c$^-$YFP$^+$ cells into the central cornea in a time-dependent manner until 48 h post-administration (Fig. 1A); it tended to decrease at 72 h (data not shown). These cells were observed in both the epithelium and stroma of the murine cornea (Fig. 1B).

Next we compared the infiltration of CD11c$^-$ YFP$^+$ cells in CD11c$^-$YFP$^+$-, TLR3 KO CD11c$^-$ YFP$^+$-, and IPS-1 KO CD11c$^-$ YFP$^+$ mice that had, or had not been treated with eyedrops 48 h earlier (Table 1). Because the number of CD11c- YFP$^+$ cells that had infiltrated the central corneal epithelium and stroma of CD11c$^-$ YFP$^+$ mice was most pronounced at 48 h after eyedrop administration, we recorded infiltration at 48 h (Fig. 1).

In the central corneal epithelium of treated CD11c$^-$ YFP$^+$ mice, the infiltration of CD11c$^-$ YFP$^+$ cells was significantly upregulated; it was significantly downregulated in the stromal layer of their central and peripheral cornea. In the epithelium of their peripheral cornea, their number tended to be lower (Fig. 2A). On the other hand, in the central corneal epithelium of TLR3 KO CD11c$^-$ YFP$^+$ mice, the infiltration of CD11c$^-$ YFP$^+$ cells tended to be downregulated at 48 h; it tended to be upregulated in the peripheral corneal epithelium (Fig. 2B). Thus, the movement of CD11c$^-$ YFP$^+$ cells in the corneal epithelium appears to be different in wild-type- and TLR3 KO mice, suggesting that TLR3 KO impedes their movement from the peripheral to the central cornea or lymph nodes. In IPS-1 KO CD11c$^-$ YFP$^+$ mice, the infiltration by CD11c$^-$ YFP$^+$ cells of the stromal layer of the central and peripheral cornea and of the peripheral corneal epithelium was significantly downregulated (Fig. 2C), suggesting that the movement of CD11c$^-$ YFP$^+$ cells is similar in the wild- and the IPS-1 KO state.

We also compared the infiltration of CD11c$^-$ YFP$^+$ cells in CD11c$^-$ YFP$^+$-, TLR3 KO CD11c$^-$ YFP$^+$-, and IPS-1 KO CD11c$^-$ YFP$^+$ mice. Comparison of untreated wild-type- and TLR3 KO mice showed that the number of CD11c$^-$ YFP$^+$ cells was significantly greater in both the central and peripheral corneal epithelium of TLR3 KO CD11c$^-$ YFP- than of CD11c$^-$ YFP$^+$ mice. There was no significant difference between CD11c$^-$ YFP$^+$- and TLR3 KO CD11c$^-$ YFP$^+$ mice in the number of CD11c$^-$ YFP$^+$ cells observed in the stromal layer of the cornea. At 48 h after polyI:C stimulation, only in the peripheral corneal epithelium was the number of CD11c$^-$ YFP$^+$ cells significantly greater in TLR3KO CD11c$^-$YFP$^+$- than CD11c$^-$YFP$^+$ mice (Fig. 3A). These findings suggest that TLR3 regulates CD11c$^-$ YFP$^+$ cells in the corneal epithelium.

When we compared untreated wild-type- and IPS-1 KO mice we found that the number of CD11c$^-$ YFP$^+$ cells in the stromal layer of the central and peripheral cornea was significantly higher in IPS-1 KO CD11c$^-$ YFP$^-$- than CD11c$^-$YFP$^+$ mice. As shown in Fig. 3B, there was no significant difference in the number of these cells in the corneal epithelium of CD11c$^-$ YFP$^+$- and IPS-1 KO CD11c$^-$ YFP$^+$ mice; at 48 h after eyedrop administration, the number of CD11c$^-$YFP$^+$ cells in the stromal layer of only the peripheral cornea was significantly greater in IPS-1 KO CD11c$^-$YFP$^+$- than CD11c$^-$ YFP$^+$ mice. These observations suggest that IPS-1 regulates CD11c$^-$ YFP$^+$ cells in the stromal layer of the cornea.

4. Discussion

Because the cornea, one of the typical mucosal surfaces, is constantly exposed to diverse environmental stimuli, it is the portal of entry for pathogens and allergens. Corneal tissue homeostasis is maintained by networks of resident DCs and Mps, i.e. by the innate immune responses [11,12]. As only few earlier investigations examined the distinct roles in tissues played by TLR3 and MDA5/RIG-1, whose ligand is the same, we first identified the distinctly regulated localization of DCs by examining the roles of TLR3 and IPS1 on the murine ocular surface.

DCs are diverse and specialized hematopoietic cells; they serve as an essential bridge between innate and adaptive immune systems [13,14]. In the cornea, Mps occupy only the posterior stroma; DCs, on the other hand, are present in both the stroma and the epithelium and they

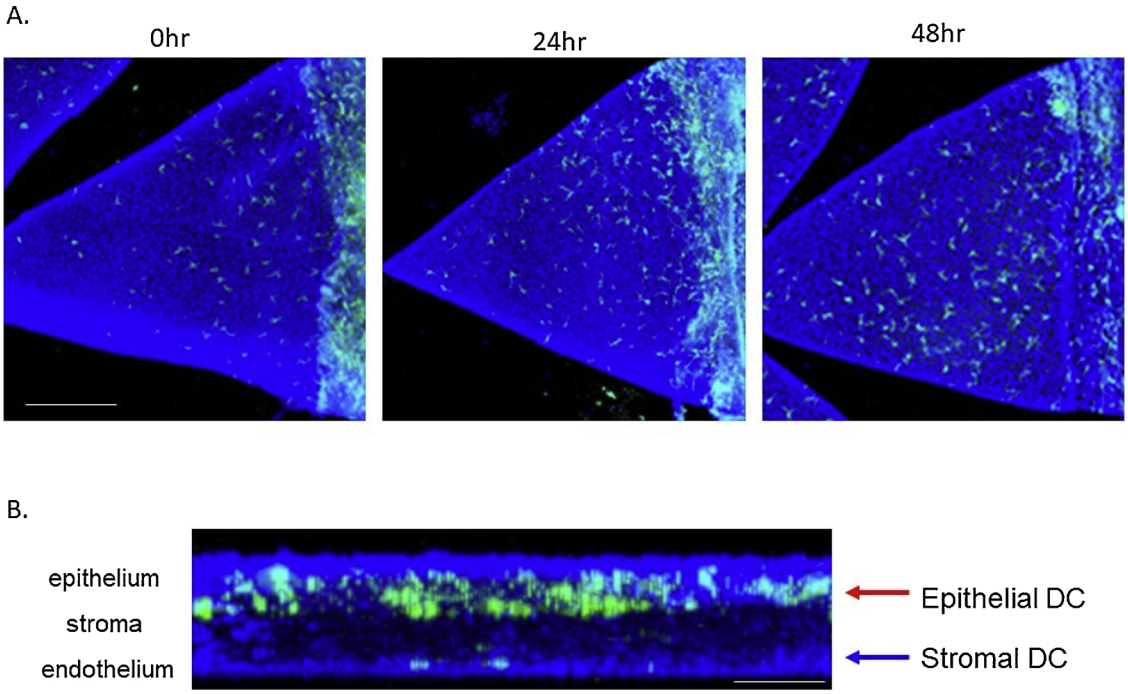

Fig. 1. Distribution of CD11c- YFP+ cells in the cornea. (A) Time-dependent increase of infiltrated CD11c$^-$ YFP$^+$ cells in the central cornea. Confocal microscope. The number of infiltrated CD11c$^-$ YFP$^+$ cells in the central cornea increased time-dependently. It was highest 48 h after eyedrop administration. Green: CD11c$^-$ YFP$^+$ cells, blue: DAPI (nuclear staining). Tiled scans of the specimens (about 1/8 cornea) and full-thickness Z stacks were used. Bar: 500 μm. (B) Vertical distribution of CD11c$^-$ YFP$^+$ cells in the corneal layer CD11c$^-$ YFP$^+$ cells are observed in both the epithelium and the basal stromal layer of the cornea. Full-thickness Y stacks were used. Green: CD11c$^-$ YFP$^+$ cells, blue: DAPI (nuclear staining) under a confocal microscope Bar: 50 μm (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Average number of CD 11c YFP positive cells.

	Central Cornea								Peripheral Cornea							
	Epithelial				Stromal				Epithelial				Stromal			
	0hr	48hr	p-value	up or down	0hr	48hr	p-value	up or down	0hr	48hr	p-value	up or down	0hr	48hr	p-value	up or down
wild	46	85	0.009	up**	29	15	0.042	down*	526	479	0.347	downX	224	120	0.003	down***
TLR3KO	87	71	0.425	downX	40	43	0.831	no change	645	772	0.068	upX	292	245	0.456	downX
IPS1KO	67	69	0.905	no change	92	16	p < 0.001	down****	569	393	0.008	down**	520	173	p < 0.001	down****

X no significant.
* p < 0.05.
** p < 0.01.
*** p < 0.005.
**** p < 0.001.

exhibit phenotypically distinct subtypes [15–18].

The number of CD11$^+$ cells in the corneal epithelium is higher in TLR3 KO-than wild-type mice. This implies that under physiological conditions, TLR3 on the ocular surface regulates the mobilization of DCs in the corneal epithelium and that the regulation of DC dynamics by TLR3 signaling is more polarized in the corneal epithelium.

After poly I:C stimulation of TLR3 KO mice, the movement of CD11c$^+$ DCs in the cornea, especially its epithelium, was different from their movement observed in wild-type mice. In TLR3 KO mice, the infiltration of the central corneal epithelium by CD11c$^-$YFP$^+$ cells tended to be downregulated while infiltration into the peripheral corneal epithelium tended to be upregulated. In wild-type mice, on the other hand, their infiltration into the central corneal epithelium was significantly upregulated while it tended to be downregulated in the peripheral corneal epithelium. These findings might suggest that in the corneal epithelium of TLR3 KO mice, the migration of CD11c$^-$ YFP$^+$ cells from the peripheral- to the central cornea or lymph nodes is disturbed. In *A. fumigatus*-infected corneas, DCs gradually migrated not only from the basement membrane to the corneal epithelium, but also from the corneal limbus to the central cornea [19]. More DCs are present in the basal layer of the peripheral- than the central corneal epithelium and some DCs in the central cornea insert processes between epithelial cells; these processes are similar to those observed in vertically-oriented sensory nerve endings [20,21].

The number of CD11$^+$ cells in the stromal layer of both the central and peripheral cornea was higher in IPS-1 KO- than wild-type mice. This implies that the regulation of DC dynamics by IPS-1 signaling is mainly directed at the stromal layer while TLR3 regulates these dynamics in the epithelium. As in wild-type mice, poly I:C-triggering reduced the number of CD11c$^+$ cells in the stromal layer of both the central and peripheral cornea of IPS-1 KO mice. Consequently, IPS-1 may not be involved in the mobilization of DCs in the stromal layer of corneal tissues. Our findings suggest that TLR3 might regulate epithelial CD11c$^+$ cells and IPS-1 might regulate stromal CD11c$^+$ cells.

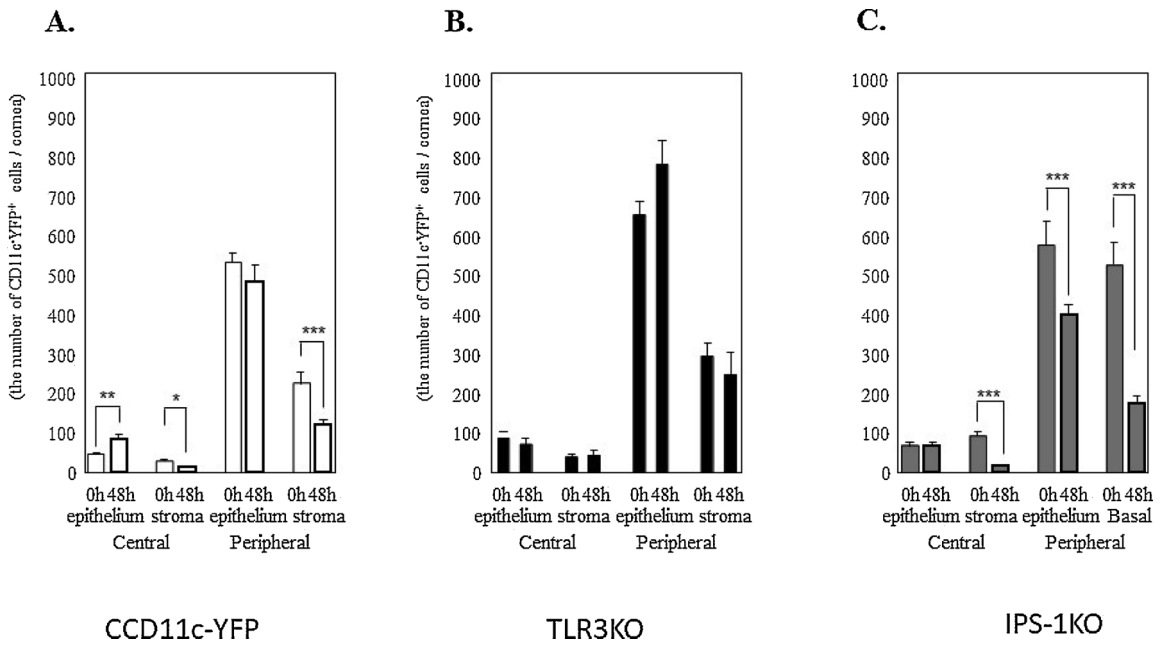

Fig. 2. Comparison of the time-dependent change in the number of infiltrated CD11c- YFP+ cells. Comparison of infiltrated $CD11c^-$ YFP^+ cells in $CD11c^-$ YFP^+- (A), TLR3 KO $CD11c^-YFP^+$- (B), and IPS-1 KO $CD11c^-$ YFP^+ mice (C) not treated, or treated 48 h earlier with a polyI:C eyedrop. 'Central'= a 2-mm diameter part of the central cornea 'Peripheral' = part of the cornea outside the 2-mm punched-out portion of the cornea. The limbus is included. $CD11c^-$ YFP mice: 0 h, n = 12; 48 h, n = 14. TLR3KO$CD11c^-$ YFP mice: 0 h, n = 10; 48 h, n = 8. IPS1KO$CD11c^-$ YFP mice: 0 h, n = 7; 48 h, n = 10.

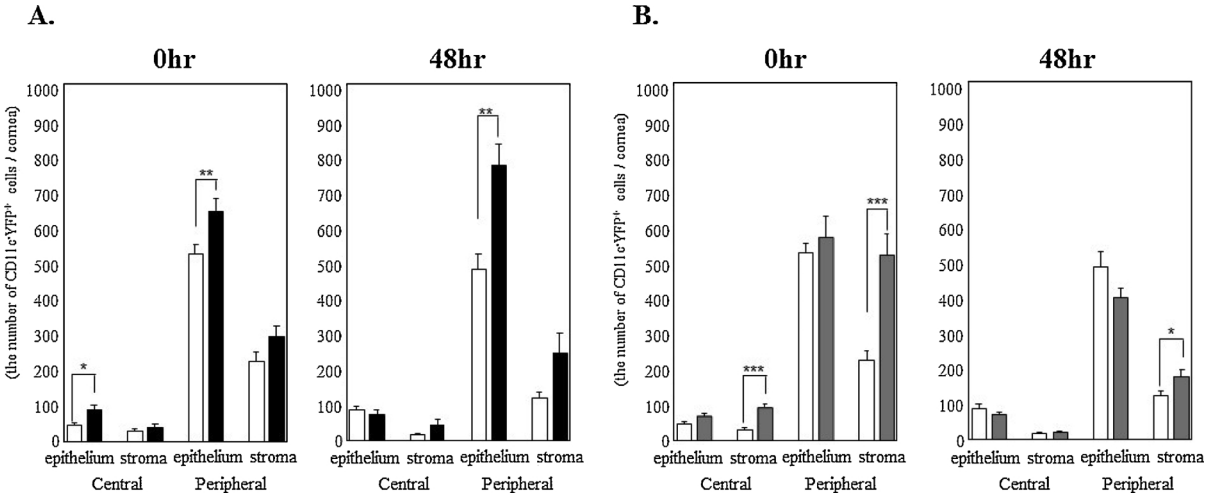

Fig. 3. Comparison of infiltrated CD11c- YFP+ cells at 0 h and 48 after eyedrop administration. (A) $CD11c^-$ YFP^+- vs TLR3 KO $CD11c^-$ YFP^+ mice. (B) $CD11c^-$ YFP^+- vs IPS-1 KO $CD11c^-$ YFP^+ mice. The data are derived from Fig. 2 and presented in a different way.

According to Kumar et al. [22], poly I:C incorporated by DCs is accessible to both TLR3 and MDA5 and results in the activation of specific signaling pathways for the robust induction of cytokines. Our preliminary study on the expression of CD11b in the cornea indicated that some corneal stromal CD11c-YFP^+ cells also expressed CD11b protein (Supplementary Fig. 2), suggesting that epithelial- and stromal $CD11c^+$ cells might not be of quite the same phenotype. To identify the causal variants related to the regulation of alternative splicing, further investigations and analyses are needed.

Elsewhere [9] we reported that some genes were dominantly and solely regulated by TLR3, that other genes were regulated by TLR3 and IPS-1 (RIG-I or/and MDA5), and that yet others were significantly down-regulated in the conjunctival epithelium of TLR3 KO-, but not of wild-type mice. Together, the findings we reported here and elsewhere support the suggestion that **??in our mice,??** TLR3 and IPS-1contributed distinctly to innate immune responses and to tissue inflammation elicited by $CD11c^+$ DCs.

Declaration of all sources of funding

This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, the JSPS Core-to-Core Program, A. Advanced Research Networks, and partly by grants-in-aid for scientific research from the Japanese Ministry of Health, Labor and Welfare.

Author contributions

M.U. designed the project. M.U. and S.O. conducted the experiments. M.U. analyzed the results. M.U. and J.H. wrote the main manuscript. M.U. and S.K. critically revised the manuscript for important intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Financial disclosure

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imlet.2018.12.004.

References

[1] T. Kawai, S. Akira, The roles of TLRs, RLRs and NLRs in pathogen recognition, Int. Immunol. 21 (2009) 317–337.
[2] M. Ueta, J. Hamuro, H. Kiyono, S. Kinoshita, Triggering of TLR3 by polyI:C in human corneal epithelial cells to induce inflammatory cytokines, Biochem. Biophys. Res. Commun. 331 (2005) 285–294.
[3] M. Ueta, S. Kinoshita, Innate immunity of the ocular surface, Brain Res. Bull. 81 (2010) 219–228.
[4] M. Ueta, K. Mizushima, N. Yokoi, Y. Naito, S. Kinoshita, Gene-expression analysis of polyI:C-stimulated primary human conjunctival epithelial cells, Br. J. Ophthalmol. 94 (2010) 1528–1532.
[5] M. Ueta, S. Kinoshita, Ocular surface inflammation is regulated by innate immunity, Prog. Retin. Eye Res. 31 (2012) 551–575.
[6] M. Ueta, S. Uematsu, S. Akira, S. Kinoshita, Toll-like receptor 3 enhances late-phase reaction of experimental allergic conjunctivitis, J. Allergy Clin. Immunol. 123 (2009) 1187–1189.
[7] N. Nakamura, R. Tamagawa-Mineoka, M. Ueta, S. Kinoshita, N. Katoh, Toll-like receptor 3 increases allergic and irritant contact dermatitis, J. Invest. Dermatol. 135 (2015) 411–417.
[8] R. Yasuike, R. Tamagawa-Mineoka, M. Ueta, N. Nakamura, S. Kinoshita, N. Katoh, The role of toll-like receptor 3 in chronic contact hypersensitivity induced by repeated elicitation, J. Dermatol. Sci. 88 (2017) 184–191.
[9] M. Ueta, T. Kawai, N. Yokoi, S. Akira, S. Kinoshita, Contribution of IPS-1 to polyI:C-induced cytokine production in conjunctival epithelial cells, Biochem. Biophys. Res. Commun. 404 (2011) 419–423.
[10] R.L. Lindquist, G. Shakhar, D. Dudziak, H. Wardemann, T. Eisenreich, M.L. Dustin, M.C. Nussenzweig, Visualizing dendritic cell networks in vivo, Nat. Immunol. 5 (2004) 1243–1250.
[11] K.A. Buela, R.L. Hendricks, Cornea-infiltrating and lymph node dendritic cells contribute to $CD4^+$ T cell expansion after herpes simplex virus-1 ocular infection, J. Immunol. 194 (2015) 379–387.
[12] V. Soumelis, P.A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, K. Smith, D. Gorman, S. Zurawski, J. Abrams, S. Menon, T. McClanahan, R. de Waal-Malefyt Rd, F. Bazan, R.A. Kastelein, Y.J. Liu, Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP, Nat. Immunol. 3 (2002) 673–680.
[13] A. Iwasaki, Mucosal dendritic cells, Annu. Rev. Immunol. 25 (2007) 381–418.
[14] A.C. Soloff, S.M. Barratt-Boyes, Enemy at the gates: dendritic cells and immunity to mucosal pathogens, Cell Res. 20 (2010) 872–885.
[15] P. Hamrah, Y. Liu, Q. Zhang, M.R. Dana, The corneal stroma is endowed with a significant number of resident dendritic cells, Invest. Ophthalmol. Vis. Sci. 44 (2003) 581–589.
[16] P. Hamrah, Q. Zhang, Y. Liu, M.R. Dana, Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells, Invest. Ophthalmol. Vis. Sci. 43 (2002) 639–646.
[17] T. Hattori, S.K. Chauhan, H. Lee, H. Ueno, R. Dana, D.H. Kaplan, D.R. Saban, Characterization of Langerin-expressing dendritic cell subsets in the normal cornea, Invest. Ophthalmol. Vis. Sci. 52 (2011) 4598–4604.
[18] K. Segawa, Electron microscopic studies on the human corneal epithelium: dendritic cells, Arch. Ophthalmol. 72 (1964) 650–659.
[19] L. Sun, C. Chen, J. Wu, C. Dai, X. Wu, TSLP-activated dendritic cells induce T helper type 2 inflammation in *Aspergillus fumigatus* keratitis, Exp. Eye Res. 171 (2018) 120–130.
[20] N. Gao, P. Lee, F.S. Yu, Intraepithelial dendritic cells and sensory nerves are structurally associated and functional**??ly??** interdependent in the cornea, Sci. Rep. 6 (2016) 36414.
[21] E.J. Lee, J.T. Rosenbaum, S.R. Planck, Epifluorescence intravital microscopy of murine corneal dendritic cells, Invest. Ophthalmol. Vis. Sci. 51 (2010) 2101–2108.
[22] H. Kumar, S. Koyama, K.J. Ishii, T. Kawai, S. Akira, Cutting edge: cooperation of IPS-1- and TRIF-dependent pathways in poly IC-enhanced antibody production and cytotoxic T cell responses, J. Immunol. 180 (2008) 683–687.