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Mini Review

Immune privilege as new therapeutic strategies for success of corneal transplantation

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Inflammation is self-regulated to preserve the functions in the eye, because the eye has immune privilege. At present, three major mechanisms prevail regarding the molecular mechanisms of immune privilege in the eye: there are (a) anatomical, cellular, and molecular barriers in the eye; (b) eye-derived immunological tolerance, the so-called anterior chamber-associated immune deviation; and (c) immune suppressive intraocular microenvironment. In this mini-review, the mechanisms of immune privilege that have been learned from ocular inflammation animal models, especially corneal transplantation, are described. The functions of new molecules on local immune regulation within the cornea are reviewed. Therapeutic strategies for restoring immune privilege are also introduced.

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Introduction

Immunological response is the necessary system to protect the biological body. But ocular tissue damage due to excessive inflammation can lead to loss of sight, because the eye is constructed from tissue with little or no capacity for regeneration, specifically, corneal endothelial cells and retinal cells cannot proliferate *in vivo*.

For that reason, ocular tissue damage due to excessive inflammation can lead to loss of sight. Accordingly, the eye, like the brain and reproductive organs have inherent immune privilege[1, 2], and inflammation is self-regulated to preserve the organ functions. In addition, corneal transplants are the least-rejected among all organ transplants, and that characteristic is also attributable to immune privilege[1, 2]. When corneal transplantation is performed on eyes that have been deprived of immune privilege, the rejection rate becomes as high as in the cases of heart and skin transplantation[1-3].

Early experiments by Medawar and by Barker and Billingham indicated that the cornea has the capacity to escape destruction by the allo-immune rejection process[4, 5].

Table1 Body sites and tissues that are immune privileged

Sites	Tissues
Eye: cornea, anterior chamber, vitreous cavity and subretinal space	Eye: cornea, anterior chamber, vitreous cavity and subretinal space
Brain: ventricles and striatum	Brain and spinal cord
Pregnant uterus	Placenta
Ovary	Ovary
Testis	Testis
Adrenal cortex	Liver
Hair follicles	
Certain tumors	Certain tumors
	Certain progenitorter cells

Streilein and colleagues has elucidated, that it is not just due to immunological ignorance, but also an active immune suppression mechanism[1, 2]. Progress has been made in analyzing the regulatory mechanisms in ocular inflammation by using animal models of corneal transplantation and autoimmune uveitis.

Immune-privileged sites are defined operationally as sites in the body where foreign tissue grafts can survive for extended or indefinite periods of time, whereas similar grafts placed at conventional body sites are acutely rejected[1]. Immune-privileged tissues are defined operationally as foreign organs or tissues that experience extended, often indefinite survival, when placed at conventional body sites, whereas non-privileged tissues are acutely rejected at conventional sites. A partial list of such tissues and sites is provided in Table 1[5-7].

Certain progenitor cells also have inherent immune privilege[7]. The immunogenic and antigenic properties of the central nervous system (CNS) progenitor cells was studied by grafting into a conventional (i.e., non-immune-privileged) site, namely, beneath the kidney capsule. Allogeneic CNS progenitor cells survived at least 4 weeks in a conventional site, during which time they neither sensitize their hosts nor express detectable levels of major histocompatibility complex (MHC) class I or II[7]. These *in vivo* data were in accord with flow cytometric results showing that CNS progenitor cells do not express MHC class I or class II, either at baseline or upon differentiation in 10% serum[7]. These results revealed CNS progenitor cells to possess inherent immune privilege.

Molecular mechanism of immune privilege in the eye

At present, three major lines of thought prevail regarding the molecular mechanisms of immune privilege in the eye: (1) there are anatomical, cellular, and molecular barriers in the eye; (2) eye-derived immunological tolerance, the so-called anterior chamber- associated immune deviation (ACAID); and (3) immune suppressive microenvironment in the eye.

(1)Anatomical and cellular barriers in the cornea

Normal cornea lacks blood and lymphatic vessels[8], because VEGFR (vascular endothelial growth factor receptor)-3 and soluble VEGFR-1 expressing in the cornea respectively block VEGF (vascular endothelial growth factor)-C and VEGF-A to inhibit lymphangiogenesis[9-11]. Therefore, considerable time passes after corneal transplantation before antigen recognition occurs in the regional lymph nodes and effector cells reach the graft. In addition, the corneal epithelial cells, keratocytes, and endothelial cells, do not express MHC class II molecules and also express only low levels of MHC class I molecules[8, 12]. This means that the main targets of a rejection reaction are not MHC antigens, but the minor H antigens in the corneal allografts[13]. The central part of the cornea, which is used as donor tissue, contains only a small population of major histocompatibility complex (MHC) class II-expressing antigen-presenting cells (APCs)[14]. Although bone marrow-derived cells have been reported to be present within normal cornea, most such cells display an immature phenotype lacking MHC class II expression[15]. Therefore, the role of antigen presentation in the regional lymph nodes after corneal transplantation is carried out mainly by host- derived APCs rather than donor-derived APCs, and it is thought that recognition of donor antigens occurs in indirect fashion, by host $CD4^+$ T cells via donor antigen-bearing host MHC class II molecules on host APCs[13, 16].

Thus, it can be surmised that the post-transplantation immune-response is weaker in the case of corneal transplants compared with other organ grafts because of not only the anatomical characteristics of the corneal tissue but also its low antigenicity and the above-described mechanisms of antigen presentation and recognition.

(2)Anterior chamber-associated immune deviation

Anterior chamber-associated immune deviation refers to a phenomenon in which antigen-specific systemic immuno-

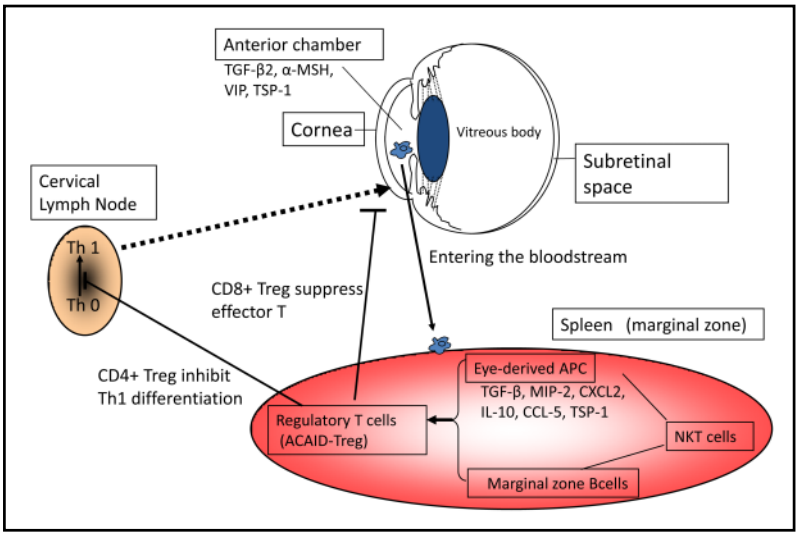

Fig.1 Induction of ACAID

In the anterior chamber, which contains TGF-β2 and TSP-1, the eye-derived antigen-presenting cells have captured antigens. The eye-derived APCs enter the bloodstream, reach the marginal zone of spleen, and produce TGF-β, MIP-2, and CXCL2. These cells attract and bind, via CD1d molecules, to NKT cells. The NKT cells produce TGF-β, IL-10, CCL5 and TSP-1; attract marginal zone B cells; and then form clusters comprised of these three cell types. T cells, which have presented the antigens in clusters, then differentiate into ACAID-Treg. $CD4^+$ ACAID-Treg inhibit the differentiation of Th1 cells in the lymph nodes, while $CD8^+$ ACAID-Treg inhibit the function of effector Th1 and Th2 cells in the local site.

logical tolerance is induced to an antigen that has been introduced to the anterior chamber in rodent eyes[1, 2]. ACAID is a phenomenon in which antibody responses are preserved while cellular responses such as delayed type hypersensitivity (DTH) and cytotoxic T cell (CTL) are suppressed. ACAID is induced in relation to various kinds of antigens, including allo-transplantation antigens, soluble protein antigens, viral antigens, and tumor antigens, and it has been demonstrated to be involved in various events such as acceptance of corneal transplants, autoimmune uveitis, acute retinal necrosis (ARN) in a fellow eye that experienced herpes virus infection in the anterior segment, or progression of intraocular malignant melanoma, in mouse models[1].

In several areas, the findings from studies in the mouse model can be extrapolated to understanding the pathogenesis in human patients. For example, patients with ARN develop an ACAID-like response to viral antigens in the intraocular compartment that disappears as the disease resolves[17-19].

The eye and the spleen are involved in the induction of ACAID (Fig. 1).

Transforming growth factor (TGF)-β2, alpha-melanocyte-stimulating hormone (MSH), vasoactive intestinal peptide (VIP), and thrombospondin (TSP)-1 in the anterior chamber are involved in the induction of APC mediators of ACAID, and eye-derived APCs such as macrophages that express F4/80 molecule pass across the trabecular meshwork, enter the bloodstream, and reach the spleen[20-24]. The eye-derived APCs that reach the marginal zone in the spleen produce TGF-β2, macrophage inflammatory protein 2 (MIP-2), and CXC-chemokine ligand 2 (CXCL2) and attract natural killer T (NKT) cells[25]. Then CD1d molecules that are expressed on the surface of the APCs bind with receptors that are expressed on the surface of the NKT cells, thereby presenting the antigens[26]. NKT cells produce TGF-β, IL-10, RANTES, CC-chemokine ligand 5 (CCL5), and TSP-1[27, 28]. When marginal zone B cells are also present in this environment rich in immunomodulatory factors, clusters comprised of these three cell types form[29, 30]. When $CD4^+$ and/or $CD8^+$ T cells that are attracted to those clusters recognize the antigens being presented by the eye-derived APCs and the marginal zone B cells, they differentiate into ACAID- inducing regulatory T cells (ACAID-Treg). $CD4^+$ ACAID- Treg inhibit the differentiation for Th1 cells in secondary lymph tissues such as lymph nodes, while $CD8^+$ ACAID- Treg inhibit the function of effector T cells (Th1 and Th2) in the local site[31, 32]. It was recently reported that thymocytes and splenic $\gamma\delta$ T cells are also necessary for induction of ACAID, and it can be understood that immune privilege in the eye is sustained through the cooperation of various cells from organs other than the eye itself (Fig. 1)[1, 2, 33].

Following corneal transplantation, ACAID is induced by the mechanism described above after the transplantation antigens on the endothelial surface of the cornea are taken up by the eye-derived APCs in the anterior chamber and transported to the spleen[1, 2]. Induction of ACAID leads to inhibition of the allo-antigen specific DTH and results in long-term survival of the graft[16, 34, 35]. ACAID cannot be induced in the case of infiltration of lymph vessels into the cornea, suturing, inflammation, trauma, or neurotomy in the cornea. In

Table 2 Immunomodulatory factors expressed in the anterior segment of the eye

Soluble factors in the anterior chamber (target cells/factors to suppress)	Cell surface molecules of the cornea and iris-ciliary body
α-MSH (T cells, macrophages, neutrophils)	B7-H1 (PD-L1) (T cells)
VIP (T cells)	B7-H3 (?)
Somatostatin (T cells)	B7-2 (via CTLA4) (T cells)
CGRP (macrophages)	Fas L (CD95 L) (T cells, neutrophils)
TGF-β2 (T cells, macrophages, NK cells)	MHC class Ib (T cells, NK cells)
TSP-1 (macrophages)	CD46, CD55, CD59 (complement)
MIF (NK cells)	galectin (Gal)-9 (Tcell)
IL-1Ra (IL-1)	GITR lignad (Tcell)
sFas L (T cells, neutrophils)	
CD46, CD55, CD59, C3ib (complement)	

α-MSH α-Melanocyte stimulating hormone, VIP vasoactive intestinal peptide, CGRP calcitonin gene-related peptide, TGF-β2 transforming growth factor-β2, TSP-1 thrombospondin, MIF macrophage migrating inhibitory factor, IL-1Ra interleukin 1 receptor antagonist, sFas L soluble Fas ligand, CTLA4 cytotoxic T lymphocyte antigen 4, GITR glucocorticoid-induced tumor necrosis factor (TNF) receptor family-related protein

this situation, the eye is said to be at high risk of rejection, and in animal models of corneal transplantation, the rejection rate within 3 weeks after transplantation is 100%[3, 34]. Clinically, as well, rejection reactions readily occur in patients presenting this condition.

(3)Molecules maintaining an immune suppressive intraocular microenvironment

As the anatomical and cellular barriers, or ACAID, are not absolute, innate and adaptive immune cells and molecules can still access the eye. In response to threats to vision, the eye has soluble and cell surface immunomodulatory factors that act within the oculi to suppress cells and molecules that mediate innate and adaptive immune inflammation[1]. This intraocular milieu is called the immune suppressive microenvironment. The functions of the various cells and factors that manage natural immunity and acquired immunity are inhibited by the various factors that are expressed in the anterior segment and are shown in Table 2[1, 2, 20, 21, 36-47]. Among those factors, α-melanocyte-stimulating hormone, vasoactive intestinal peptide, calcitonin generelated peptide, TGF-β2, and TSP-1 regulate the functions of macrophages and dendritic cells. TGF-β2 and TSP-1 are essential factors for the induction of ACAID as described in the previous section. As shown in Table 2, various immunomodulatory factors are expressed in corneal endothelial cells and iris-ciliary body.

Our group have elucidated that the inhibitory costimulatory signaling molecules such as B7-H1[45], B7-H3[46], glucocorticoid-induced tumor necrosis factor receptor family-related protein ligand (GITR-L)[47], and galectin (Gal)-9[48], are involved in immune suppression in the cornea. These molecules are introduced below (Fig. 2).

i)T-cell apoptosis mediated by B7-H1 within the eye

B7-H1 (PD-L1) was identified as a new B7 family molecule that binds to programmed death (PD)-1 on the sur-

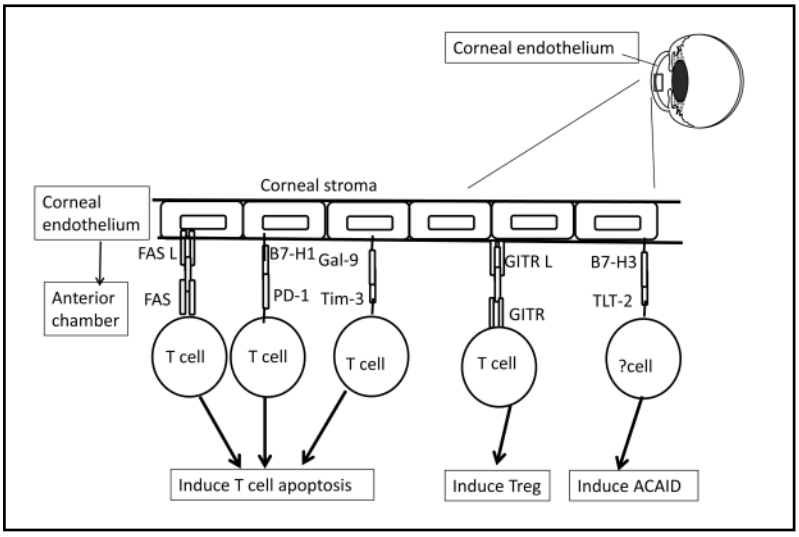

Fig.2 The mechanism of the molecules to inhibit T cell

B7-H1 induces apoptosis of PD-1+T cells and Fas L induces T-cell apoptosis via Fas. Gal-9 also induces apoptosis of T cells and protects corneal endothelium. GITRL has the functions to induce Foxp3$^+$CD25$^+$CD4$^+$Treg via GITR. B7-H3 is involved in induction of ACAID.

face of activated T cells and sends inhibitory signals to the T cells[49]. In ocular tissues, B7-H1 is constitutively expressed in endothelial cells of the cornea, some stromal cells, iris-ciliary body, and the neural retina. The rejection reaction after corneal transplantation is intensified by blockade of B7-H1 or PD-1 with antibodies[45]. B7-H1 expressed in the cornea induces apoptosis of PD-1-expressing T cells, and deletion of effector T cells in the cornea results in inhibition in the effector phase of the rejection reaction[45]. It is interesting that the T-cell apoptosis mediated by B7-H1 has been only observed in immune privileged tissues or sites such as tumors, liver, and cornea so far[50, 51]. Corneal endothelial cells also constitutively express Fas ligand, and apoptosis of effector T cells is induced via Fas[41-43]. It is unclear whether, in this state of the effector T cells having been eliminated from the eye, B7-H1 and Fas ligand interact on the surface of the corneal endothelial cells (Fig. 2).

The culture system of corneal tissue and T cells *in vitro* has been established and shown that B7-H1 expressed in the cornea shows local immunosuppressive activity[45]. This system permits complete elimination of any involvement of the secondary lymphatic organs and makes it possible to isolate and analyze only the effector phase of the rejection reaction when the corneal endothelial cells has been damaged by effector T cells. The results showed that B7-H1 expressed in the corneal cells not only inhibited corneal endothelial damage by allo-reactive T cells but also inhibited bystander damage caused by activated T cells that are specific to third party antigen. In addition, PD-1 on the surface of the T cells was up-regulated as a result of contact with the corneal cells, thus accelerating apoptosis.

As described above, the PD-1/B7-H1 pathway is more involved in interactions between the effector T cells and the corneal cells within the eye than in the immune responses in the secondary lymphatic organs[45]. Thus, these molecules contribute to maintenance of the local immune suppressive microenvironment in the eye.

ii)GITR Ligand—Mediated Local Expansion of Regulatory T Cells within the eye

The pathway between glucocorticoid-induced tumor necrosis factor (TNF) receptor family-related protein (GITR) and GITR ligand (GITRL) have been shown to control the function of regulatory T cells. GITR is a type I transmembrane protein of the TNF receptor superfamily[52, 53]. GITRL was expressed constitutively in the cornea and iris-ciliary body[47] (Fig. 2). If GITRL was blocked by peritoneal injection of antagonistic mAbs in recipients of corneal allografts, the allografts became more vulnerable to rejection[47]. This is caused by that GITRL inducing the expansion of $Foxp3^+$ $GITR^+CD25^+CD4^+$ Treg within the cornea after corneal transplantation. And it was evaluated that corneal endothelial cells were destructed by CD4 T cells in vitro, Destruction of corneal endothelial cells by T cells was significantly enhanced in GITRL-blocked cornea compared with control cornea. $Foxp3^+CD25^+CD4^+$Tcells were increased after incubation with GITRL-expressing cornea, but not with GITRL- blocked cornea. GITRL-dependent expansion of Treg within the cornea is one mechanism underlying immune privilege in corneal allografts[47].

iii)T-cell apoptosis mediated by galectin-9/Tim-3

T-cell immunoglobulin and mucin domain (Tim)-3 is a regulatory molecule for T-cell function, and galectin (Gal)-9 is a Tim-3 ligand[48]. Gal-9 is constitutively expressed on the corneal epithelium, endothelium and iris-ciliary body in normal mouse eyes (Fig. 2). Allograft survival in recipients treated with anti-Tim-3 monoclonal antibody (mAb) or anti-Gal-9 mAb was significantly shorter than that in control recipients[48]. *In vitro*, destruction of corneal endothelial cells by allo-reactive T cells was enhanced when the cornea was pretreated with anti-Gal-9 mAb[48]. And when the co-culture of allo-reactive T cells and corneal endothelial cells were treated with anti-Gal-9 mAb, apoptosis of $CD4^+$ T cells was significantly suppressed compared to control[48].

It was proposed that constitutive expression of Gal-9 plays an immunosuppressive role in corneal allografts. Gal-9 expressed on corneal endothelial cells protects them from destruction by allo-reactive T cells within the cornea.

iv)ACAID induction mediated by B7-H3

B7-H3 was recently identified as a new B7 family molecule[46], and in the eye, B7-H3 is constitutively expressed in corneal endothelial cells and the iris-ciliary body, and that a rejection is induced after corneal transplantation in experimental hosts to which anti-B7-H3 blocking antibody had been intraperitoneally administered (Fig. 2)[54]. It is of interest that ACAID is not induced in such animals administered with anti-B7-H3 antibody. That is, B7-H3 is involved in induction of ACAID-Treg that is dependent on the spleen and mediated by eye-derived antigen-presenting cells, and it thus plays a different role from that of B7-H1[54]. Also, the possibility that

B7-H3, like the previously described B7-H1, plays a role in eliminating or inhibiting effector immune cells within the cornea remains to be established.

Therapeutic strategies for restoring corneal immune privilege

As it is described above, the eye modifies and regulates immune responses in order to prevent inflammation-mediated tissue destruction. This is accomplished by using a variety of molecules. Understanding how immune privilege can be modified in the cornea will lead to the development of new therapeutic approaches to tissue transplantation and autoimmune disease.

(1)Modifying immunogenic potential of the tissue:

The different layers of the cornea display either immunogenicity or immune privilege[43].The properties of one layer can influence the properties and fate of another layer. The primary immunogenicity of the cornea as an allograft resides within the epithelium[43]. On the other hand, the corneal endothelium not only lacks inherent immunogenicity, but it also prevents allosensitization by the corneal stroma[43]. Thus, the immune privilege of the cornea resides solely with the endothelium. Further, constitutive expression of FasL and B7H1(PD-L1) is critical to the corneal immune privileged status[42, 45]. Simply covering an epithelium-deprived allogeneic corneal graft (stroma plus endothelium) with an epithelium that was genetically identical to the graft recipient virtually eliminate rejection when transplanted into low-risk and even into high-risk recipients beds. Recipients of these composite corneal grafts show no evidence of donor-specific sensitization, implying that graft acceptance might result from immunological ignorance[3, 55].

(2) Restoring the anatomical and molecular barriers:

Inhibition or suppression of corneal haemangiogenesis and lymphangiogenesis is one approach to restore the anatomical barriers in the cornea. Systemic or topical application of antagonistic antibodies to vascular endothelial growth factor (VEGF)-A and VEGFR-3 has been reported to promote corneal allograft survival[9, 10]. Administration of soluble VEGFR-2 that inhibits developmental and reparative lymphangiogenesis by blocking VEGF-C function, suppresses lymphangiogenesis but not hemagiogenesis induced by corneal suture injury or transplantation, enhanced corneal allograft survival[11].

Gene therapies to transfer the following immune modulating factors are also potential strategies for restoring immune suppressive microenvironment in the eye. Overexpression of soluble TNF receptor, soluble CTLA(cytotoxic T lymphocyte-associated antigen)-4, IL(interleukin)-10, IL-12p40 subunit, indoleamine 2, 3-dioxygenase, nerve growth factor have been shown to modify immune privilege and lead to prolonged corneal graft survival in animal models[12, 56-60]. Topical application of doxycycline, which is one of the chemically modified tetracyclines, has been reported to up-regulate the expression of Fas L and prolong the allograft survivial[61].

Induction of anti-inflammatory and immunosuppressive molecules in the aqueous humor is also one of the effective strategy for corneal allograft survival. Treatment with local alpha-MSH suppressed allo-specific DTH and T cell infiltration into the allograft, and resulted in acceptance of corneal allograft[62].

(3) Inducing T regulatory cells (Treg):

Inducing Treg is an effective approach to modify and enhance immune privilege. Keino et al. has recently shown that an active metabolite of vitamin A, all-trans retinoic acid (ATRA), synergized with TGF-β to induce Foxp3(+) T regulatory cells and reciprocally inhibited development of Il-17-producing T helper cells (Th17) induced by TGF-β and IL-6. ATRA treatment reduced inflammation in experimental autoimmune uveoretinitis (EAU)[63].

Induction of ACAID-inducing Treg is also one of the effective strategy for corneal allograft survival. Several laboratories have showed that induction of ACAID, through the intracameral injection of alloantigens before keratoplasty, results in a marked reduction in corneal graft rejection[35, 64-66].

Conclusion

The eye, which is endowed with immune privilege, is a rare organ that permits analysis of the self-regulatory mechanisms for inflammation in organs. The normal eye possesses an anatomical barrier mechanism, ACAID, and a molecular mechanism of an immune suppressive intraocular microenvironment. This information not only helps discern potential mechanisms underlying many ocular disease conditions but also elaborates on mechanisms underlying the induction and maintenance of immunologic tolerance, an integral component of immune privilege. Therefore these studies provide the background for the development of new therapeutic strategies applicable to a broad range of tissue transplants and inflammatory diseases relevant to the eye and other sites.

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Conflicts of interest

Both authors have no conflict of interests.

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