·Basic Research·

The expression and distribution of α-Gal gene in various species ocular surface tissue

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Abstract

• AIM: To examine the α -Gal gene expression and distribution in the different species/genus and developing phase animal ocular surface tissue.

• METHODS: α -Gal binding assay were carried out on various animal eye sections. Photograph, slit-lamp observation on various eye showed normal corneal transparence.

• RESULTS: A strong α -Gal expression in invertebrates and some vertebrates ocular tissue, but no α -Gal binding in birds, fish and mammal. α -Gal expression change in the development of mice ocular surface tissue (except sclera) and display genus dependency in the different murine ocular surface tissue.

• CONCLUSION: This study identified specific α -Gal epitopes binding area in the ocular surface of several species and may solve the problem that naive ocular surface may be used as natural α -Gal gene knockout model/high risk immunologic rejection model or ocular surface scaffold material.

• KEYWORDS: α -Gal; xenotransplantation; animal; ocular surface; tissue engineering
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INTRODUCTION

Xenotransplantation, when ultimately successful, will alleviate the current severe organ shortage for allotransplantation. However, multiple immune barriers preclude its clinical application. We have known that the major immunologic barrier in xenotransplantation is the interaction between anti-Gal and α-gal epitope [1-3]. α-Gal is the most abundant carbohydrate epitopes on cells of non-primate mammals, prosimians and New World monkeys [4]. At present, porcine-vascularized organs transplanted into humans are hyperacutely rejected due to the existence of a-Gal antibodies (Ab) in man. These Ab are mainly directed against the a-Gal epitope that is present on most cells or tissues.

Since immunological rejection in vascular tissue represents the most complicated factor preventing routine analysis of the relations between α-Gal and xerotransplantation, additional studies are necessary to simplex factor analysis of the correlation in the immune privileged model. The cornea is an avascular, transparent, and immune privileged tissue. Specific cornea is more difficult for transplantation than other low homology cornea and recovers more rapidly after xerotransplantation, suggesting that the expression of α-Gal gene may be different in various animal ocular surface tissue. Currently, little is known about α-gal epitope expression in the animal eyes. It is possible that such mechanisms are also involved in ocular surface xenotransplantation from different phylogenetic species.

To address these issues, we first provide an overview of suggested the GSIB4 binding cell/tissue in comparison with our findings on various animal eye specimens. Furthermore, we also assess the expression of α-Gal epitopes in the different developing phase of mice corneas and try to obtain

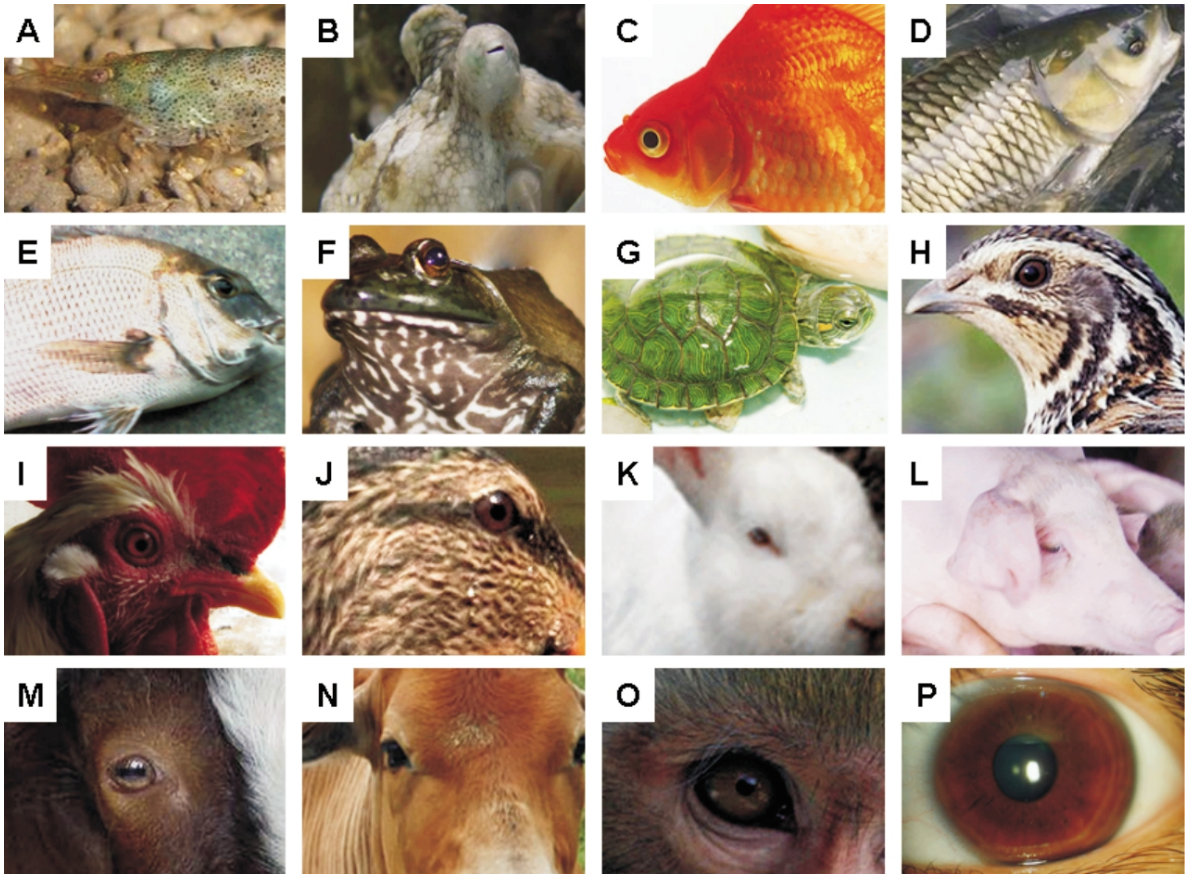

Figure 1 Eye photograph in various adult animals is seen using camera and slit lamp **(A–P)** Optical ocular examinations were conducted to make sure that there were no apparent signs of ocular disease in adult shrimp (A), octopus (B), goldfish (C), chinese idle (D), porgy fish (E), pond frog (F) , tortoise (G) ,quail (H), chick (I), duck (J), New zealand rabbit (K), porcine (L), goat (M), bovine (N), Macaca rhesus (O) and human (P).

the evidence about the change of α-Gal epitopes in BALB/c and C57BL6 mice corneas. Finally, we investigate expression of α-Gal epitopes and study the interspecies variation in the ocular surface tissue in various genus of murine.

MATERIALS AND METHODS

Materials

Human eye tissues All studies using human tissues were in accordance with the tenets of the Declaration of Helsinki and in accordance with the policies of the institutional review board for human subjects, and were approved by the Ethics Committee of Xiamen Eye Center. Normal (transplant quality) human eye tissue were obtained from the Cornea and Ocular Surface Clinic of Xiamen Eye Center.

Animal eye tissue All procedures with experimental animals were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and in accordance with the policies of the institutional animal care and use committee. Brief physical and optical ocular examinations were conducted to make sure that there were no apparent signs of systemic or ocular disease. Enucleations were processed on the aeroembolism after the general anaesthesia.Adult Macaca rhesus eyes were obtained from Xishan Zhongke Laboratory Animals Co., Ltd (Suzhou, China). Adult New Zealand white rabbit, Wistar/SD rat, guinea pig, murine and the development phase of BALB/c and C57BL6 mouse were obtained from Shanghai Laboratory Animals Co., Ltd (Shanghai, China). Adult shrimp, lobster, octopus, gold fish, porgy fish, chinese idle, lizard, tortoise, terrapin, pond frog, rana catesbiana, quail, chick, duck, goose, domestic rabbit, porcine, sheep, goat, bovine and cow eyes were obtained from local abattoirs (Figure 1). Normal porcine aortic vessel was acquired from local abattoirs served as controls.

Methods

Frozen sections Eye tissues (n=5，respectively) were snap frozen in liquid nitrogen and then embedded in optimal cutting temperature (OCT) compound (Tissue Tek, IN). 6μm thick cross sections of eye tissue were cut with a cryotome (model 3050; Leica, IL). The sections (n=5) were mounted on glass slides, air dried, and stored at -20℃ until used for GSIB4 staining.

Xenoantigen α −Gal epitopes binding assay GSIB4 staining was performed to detect α-Gal epitopes as previously described [5]. In brief, corneas, grafts and eyeballs slices after fixation were treated with 0.3% hydrogen peroxide for 30 minures at room temperature to inhibit

Table 1 Expression of α-Gal in various animal ocular tissue

Species	Cornea/limbus			Conjunctiva		Sclera	Others
	epithelium	stroma	endothelium	epithelium	stroma		
Invertebrates							"+"
Shrimp	+/+	−/−	−/−	+	−	−	Adenocarcinoma (R)[7]
Lobster	+/+	−/−	−/−	+	−	+	[1]Aorta endothelium (C, D, H, P)
Octopus	++/+	+/+	−/−	+	−	+	[1]Aorta smooth muscle cells (C, Do)
Vertebrates							
Fish							
Goldfish	−/−	−/−	−/−	−	−	−	[1]B lymphocyte cell line (NWM)
Porgy	−/−	−/−	−/−	−	−	−	Cornea stroma (P)[8]
Chinese idle	++/++	−/−	−/−	+	+	−	Ehrlich ascites cells (M)[9]
Amphibians							
Pond frog	++/++	−/−	−/−	+	−	−	Fibrosarcoma cells (R)[10]
Rana catesbiana	++/++	−/−	−/−	+	−	−	Kidney fibroblasts (B, Ra, C, Dol, S, P)[11]
Repitiles							
Lizard	++/++	−/−	+/+	+	−	++	[1]L cells (M)
Tortoise	++/++	−/−	+/+	+	−	++	[1]Lens epithelium (C, M, Ra, P)
Terrapin	++/++	−/−	+/+	+	−	++	[1]Lung fibroblasts (B, M)
Birds							
Quail	−/−	−/−	−/−	−	−	−	Lymphoma cells (M)[12]
Chick	−/−	−/−	−/−	−	−	−	Red cells (B, D, Ha, Ra)[13-15]
Duck	−/−	−/−	−/−	−	−	−	[1]Thymocytes (C)
Goose	−/−	−/−	−/−	−	−	−	[1]Thyroid cells (P, Ra, B, S)[16,17]
Mammal							
Mouse	++/++	−/−	−/−	+	−	(+)	[1]Transformed fibroblasts (M, Ra)
BALB/C	++/++	−/−	−/−	+	−	(+)	[1]Skin fibroblasts (Ca, H, NWM, Pr)
C57Bl6	++/++	−/−	−/−	+	−	(+)	[1]SP/2 (M)
Nude	−/−	−/−	−/−	−	−	−	
KM	+/+	−/−	−/−	+	−	(+)	"−"
Rat							
Wistar	++/++	+/+	−/−	+	+	+	Brain (Ra, S, P)[17]
SD	+/+	+/+	−/−	+	+	+	[1]Be-13 (H)
Guinea pig	++/++	+/+	−/−	+	+	+	[1]EB3 (H)
Rabbit							
Domestic	−/−	−/−	−/−	+	−	++	[1]Fibroblasts (Ar, Ch, D, F, G,I, Q)
New Zealand	−/−	−/−	−/−	+	−	++	[1]HeLa (H)
Porcine	−/−	+/+	−/−	−	−	++	[1]HL60 (H)
Goat	−/−	(+)/(+)	−/−	+	+	+	[1] Kindey fibroblasts (A, H, OWM)
Sheep	−/−	(+)/(+)	−/−	+	+	+	[1]Myeloma cell line (A)
Cow	−/−	+/+	−/−	+	+	+	Skin fibroblasts (A, H, OWM)
Bovine	−/−	+/+	−/−	+	+	+	[1]SV-40 Transformed fibroblasts(H)
Macaca rhesus	−/−	−/−	−/−	−	−	−	
Human	−/−	−/−	−/−	−	−	−	

−: undetectable; (+): weak positivity; +: moderate positivity; ++: strong positivity. [1] see review 5. A: apes; Ar: Armadillo; B: bovine; Ba: bat; Be-13: T-125 memia cell line; C: cow; Ca: cat; Ch: chick; D: Duck; Do: dog; Dol: dolpjin; EB_3: Burkitt lymphoma cell line; F: Frog; G: goldfish; H: human; Ha: haster mouse; HeLa: Carcinoma cell line; HL60: Myeloid cell line; Ho: horse; I: iguana; M: mouse; Mi: mink; NWM: New World moneys; OWM: Old World moneys; P: pig; Pr: prosimians; Q: Armadillo; R: rat; Ra: rabbit; S: sheep; SP/2: Myeloma cell line.

endogenous peroxidase, and washed with PBS for 15 minutes. Samples were then reacted with Alexa fluor 568 conjugated GSIB4 (5g/mL in PBS) for 10 minutes at room temperature. After 3 washes with PBS for 15 minutes, the nuclei were counterstained with DAPI (Vector Laboratories, USA). The samples were investigated and photographed with laser confocal microscopy. The human corneal tissue was used as a negative control and the porcine aortic vessel as a positive control[6].

RESULTS

Expression of A-Gal in Various Animal Ocular Tissue

In order to contribute to clarification of existing controversies, we provided an overview of suggested the GSIB4-positive cell and tissue in comparison with our findings on some animal cornea, conjunctiva and sclera specimens (Table 1)[7-17].

Cornea and Limbus Tissue Considerably high levels of α-Gal expression were found in the adult shrimp, lobster, chinese idle, octopus, pond frog, rana catesbiana, guinea pig, lizard, tortoise, terrapin，rat cornea/limbus tissues from epithelium. α-Gal epitope was detected in all of the following anterior corneal stroma include the porcine, goat, sheep, cow and bovine. Absence of α-Gal expression was observed in the cornea of goldfish, porgy fish, quail, chick, duck, goose, rabbit, Macaca rhesus and human.

Conjunctiva and Sclera Tissue α-Gal epitopes was

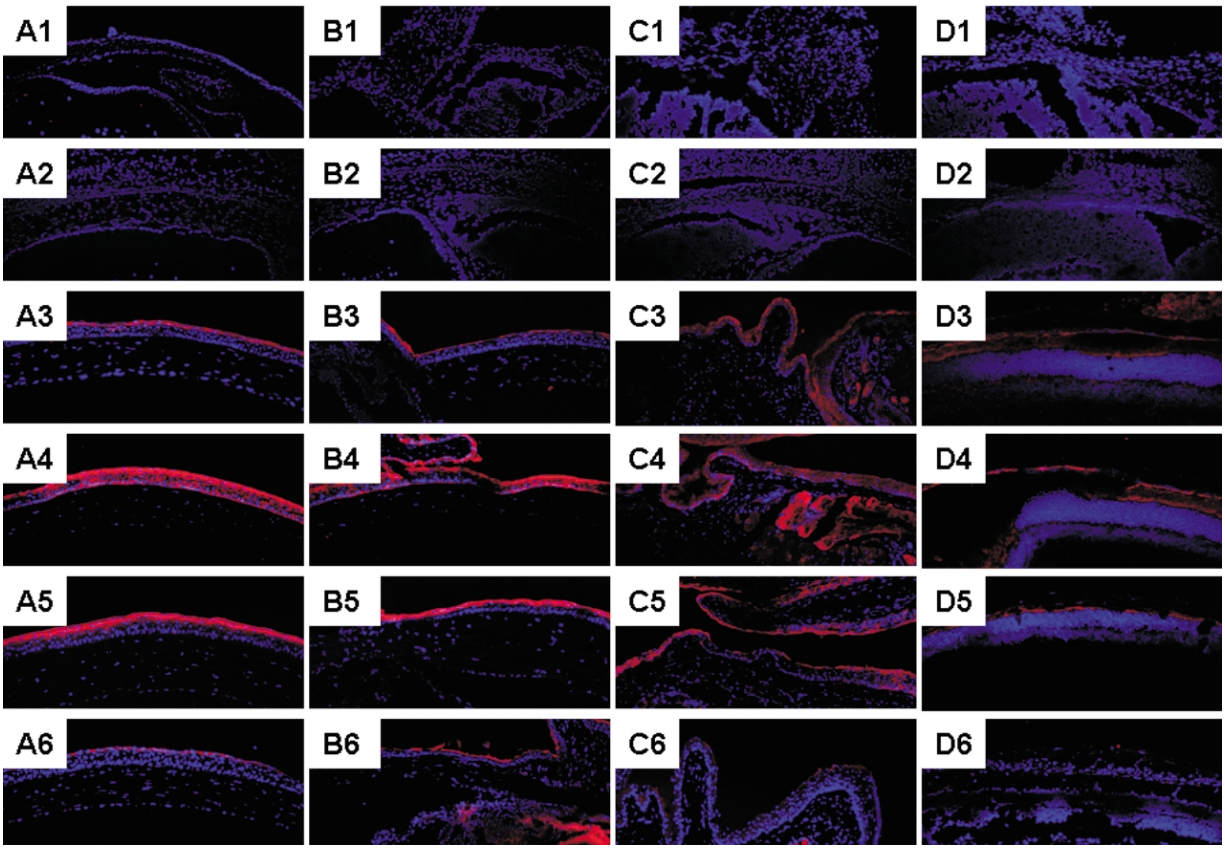

Figure 2 α−Gal gene expression in the different developing phase of BALB/c mice ocular tissue (A1−D6) (A1−A6: centre cornea, B1−B6: limbus, C1−C6: conjunctiva, D1−D6: sclera; GSIB4: red, DAPI: blue; magnification:×200) As early as mouse embryonic day 12 (A1-D1) and newborn day 1 (A2-D2), there was no GSIB4 expression in the BALB/c mice. α-Gal gene expression in the supine surface cornea/limbus, conjunctiva epithelium and sclera tissue in the 2 weeks (A3-D3) and 12 months (A6-D6). On 6 weeks, α-Gal epitopes was present on the full- thickness cornea/limbus,conjunctiva epithelium and sclera tissue (A4-D4). Cells that displayed α-Gal at 6 month often tended to be located at the supine surface cornea/limbus and conjunctiva epithelium (A5-D5).

detected in conjunctiva of shrimp, lobster, octopus, pond frog, rana catesbiana, guinea pig, lizard, tortoise, terrapin, rabbit, goat, sheep, cow and bovine. Interestingly, α-Gal epitopes was undetectable in the goldfish, porgy fish, quail, chick, duck, goose, rabbit, Macaca rhesus and human. Conjunctiva epithelium of shrimp, lobster, octopus, pond frog, rana catesbiana, guinea pig, lizard, tortoise, terrapin, mouse, rat, rabbit, goat, sheep, cow and bovine showed staining for GSIB4, but conjunctiva stroma were negative. α-Gal epitopes were detected in sclera tissue from octopus, lizard, tortoise, terrapin, mouse, rat, guinea pig, rabbit, goat, sheep, cow and bovine sclera. Similar to the keratocyte, sclera fibroblast from same species did not react with GSIB4.

α−Gal Expression in the Development of Mice Ocular Surface Tissue In the developing and mature mouse ocular tissue, α-Gal was detected in the epithelial cell. The α-Gal expression was changed during the integral development phase in BALB/c mice ocular tissue (Figure 2). As early as mouse embryonic day 12 and newborn day 1, there was no GSIB4 expression in the BALB/c mouse. α-Gal gene was expressed in the supine surface cornea/limbus, conjunctiva epithelium and sclera tissue in the 2 weeks. On 6 weeks, α-Gal epitopes was present on the full-thickness cornea/limbus, conjunctiva epithelium and sclera tissue. Cells that displayed α-Gal at 6 months often tended to be located at the supine surface cornea/limbus and conjunctiva epithelium. By 12 months, α-Gal was mostly restricted to the supine surface cornea/limbus, conjunctiva epithelium. No significant change of α-Gal expression was found in the developing BALB/c sclera. A similar distribution in ocular tissue was observed in the developing of C57BL6 mice(Figure 3).

α −Gal Expression in the Different Murine Ocular Surface Tissue To determine the GSIB4 expression in the different murine, we measured the distribution of α-Gal epitopes in adult KM, nude mouse, Wistar/SD rat and guinea pig eye tissue using GSIB4 and DAPI staining (Figure 4). Similarly to what we have observed in the adult C57BL6 and BALB/c mouse, GSIB4 staining in KM and nude mouse confirmed that supine surface cornea/limbus epithelium, conjunctiva epithelium and sclera express α-Gal. In the Wistar/SD rats and guinea pig, α-Gal epitope was detected in any of the above ocular tissues except in the endothelium cell, where the staining was absent.

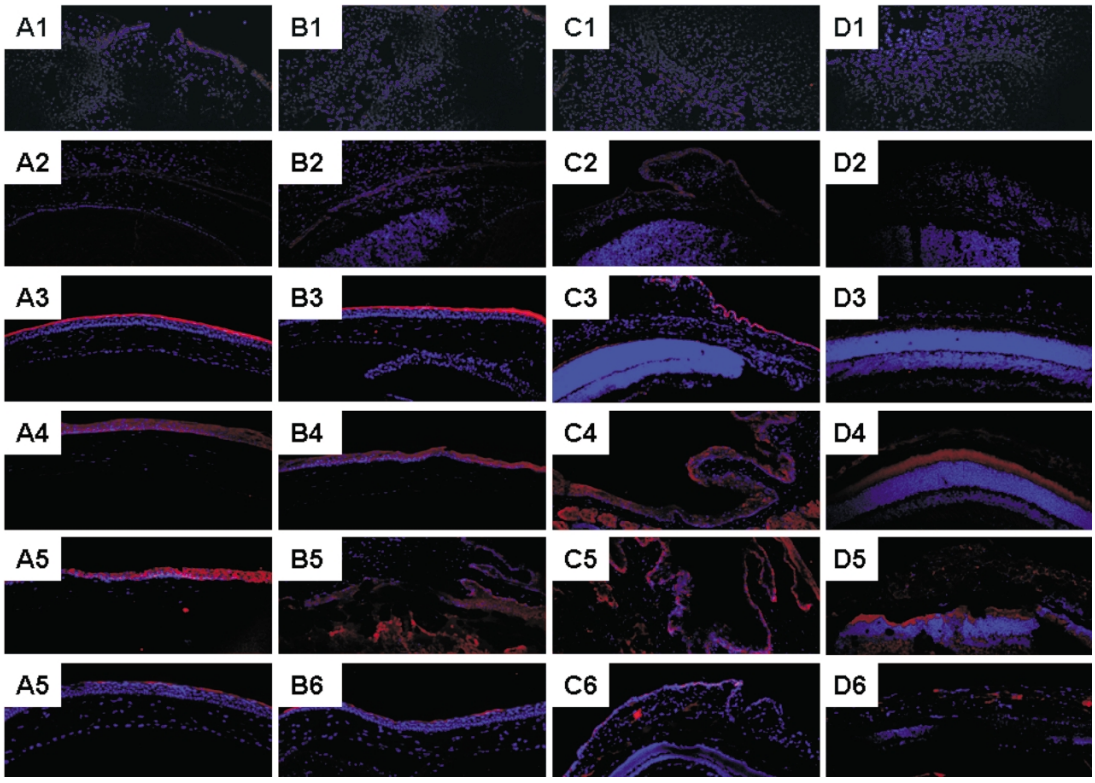

Figure 3 α-Gal gene expression in the different developing phase of C57BL6 mice ocular tissue (A1-D6) (A1-A6: centre cornea, B1-B6: limbus, C1-C6: conjunctiva, D1-D6: sclera; GSIB4: red, DAPI: blue; magnification:×2 000) There was no GSIB4 binding in embryonic day 12 (A1-D1) and newborn day 1 (A2-D2), whereas α-Gal was mostly restricted to the the supine surface cornea/limbus, conjunctiva epithelium in the 2 weeks (A3-D3) and 12 months (A6-D6). On 6 weeks, α-Gal epitopes was present on the full-thickness cornea/limbus,conjunctiva epithelium and sclera tissue (A4-D4). α-Gal often tended to be located at the supine surface cornea/limbus and conjunctiva epithelium at 6 month (A5-D5).

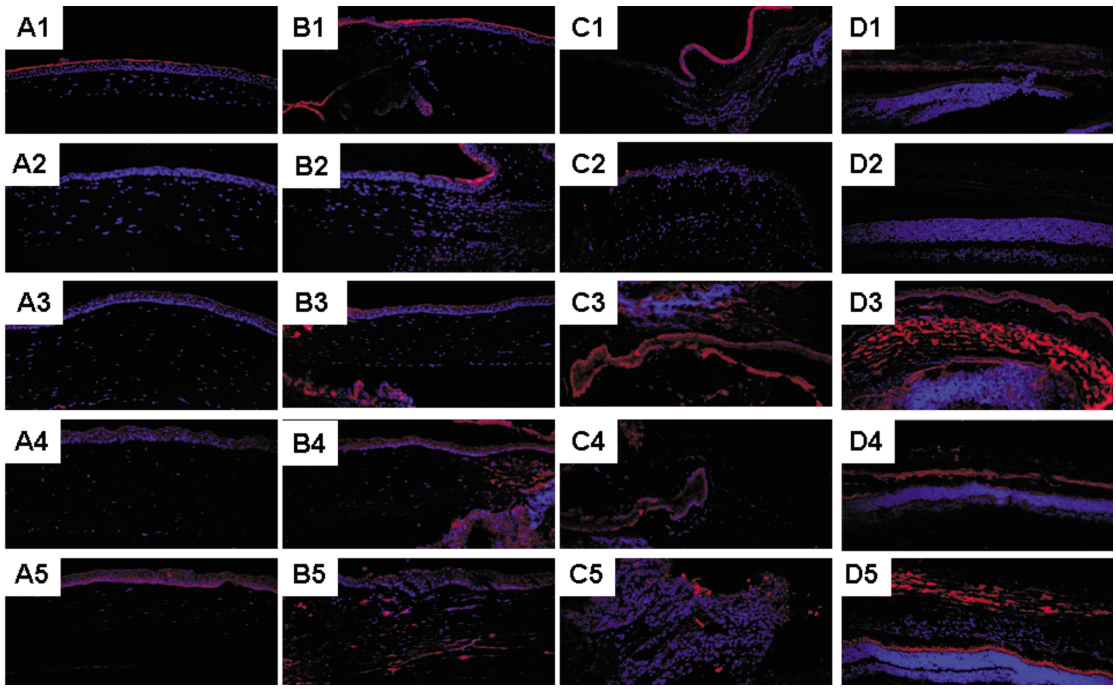

Figure 4 α-Gal gene expression in the different murine strains (A1-D5)]. (A1-A5: centre cornea, B1-B5: limbus, C1-C5: conjunctiva, D1-D5:sclera; GSIB4: red, DAPI: blue; magnification:×200) The expression and distribution of a-Gal gene were present only in the supine surface ocular surface in adult KM mouse (A1-D1) and nude mouse (A2-D2) and present in the epithelium and stroma area in the wistar rats (A3-D3), SD rats (A4-D4) and guinea pig (A5-D5).

DISCUSSION

This study identified specific α-Gal epitopes binding area in the ocular surface of several species and may solve the problem that naive cornea having no α-Gal expression, may

be used as natural α-Gal gene knockout model in xenotransplantation model. In contrast, strong α-Gal expression was detected in the invertebrates (shrimp, lobster and octopus), fish (chinese idle), amphibians (pond frog, rana catesbiana), reptiles (lizard, tortoise, terrapin), mammal (mouse, rat, guinea pig, porcine, goat, sheep, cow and bovine) in ocular surface and it can, therefore, be served as a high risk immunologic rejection model or ocular surface scaffold material. This study is the first to examine the developmental change or variation throughout ocular surface development. The results offer support that α-Gal are conserved gene across species. However, genus dependent expression of α-Gal significantly different in the various murine ocular tissue and the different development stage, this result may support the solid evidence about different response to immunity.

Since α-Gal gene play an essential role in control of functions of recipient-donor transplanted tissues in animal species, examining the expression of α-Gal in ocular surface may provide insight into the immunity mechanism of rejection in xeno tissues. Detailed expression of α-Gal gene in each development process and different ocular surface tissue may be modified or mutated in further study when more animal information becomes available. Further experiments are needed to characterize the α-Gal expression in various retinal, if there is any, and to test the effect of the retinal xerotransplantation. Since α-gal epitope is expressed in several cells in the ocular surface [18], another study was to use human serum, which contains anti-Gal[19], to decellularize the those α-gal positive cells. The findings of this study are useful in advancing our knowledge and research capability relating to correlation between the α-Gal gene and immunity rejection on ocular surface tissue.

In summary, the results showed that ocular surface without α-Gal expression may be used as natural α-Gal gene knockout model, while ocular surface with strong α-Gal expression may be served as a high risk immunologic rejection model or ocular surface scaffold material.

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