Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Galectin-9 contributes to the survival of fully allogeneic skin grafts by modulating effector T cells and Tregs imbalance

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ARTICLE INFO

Original paper

Article history:
Received: July 10, 2023
Accepted: December 15, 2023
Published: December 31, 2023

Keywords:

CD4+CD25+Foxp3+Treg, effector T cells, Galectin-9, Th17

ABSTRACT

Allogeneic skin transplantation is an important clinical treatment for many diseases. Although Galectin-9 demonstrates multifaceted roles in modulating immune cell homeostasis and inflammation, its precise impact on balancing effector T cells and Tregs during allogeneic skin transplantation remains uncertain. This work was performed to evaluate the modulation of the survival time of allogeneic skin grafts by Gal-9 and to explore the critical mechanism involved in this process. Skin graft transplantation was conducted using C57BL/6 and BALB/c mice. Additionally, the levels of IL-2, IFN-γ, IL-4, and IL-17A were measured. Hematoxylin and eosin staining assay was performed to analyze the pathological conditions of skin grafts of experiment mice. The results revealed that Gal-9 noticeably prolonged the survival of the allogeneic skin graft and ameliorated the damage caused by acute immune rejection. Furthermore, Gal-9 resulted in selective reduction of effectors T cells such as Th1, and Th17. Simultaneously, Gal-9 didn't attenuate the protective function for allograft, which alleviated the immune rejection caused by abnormal immune imbalance. Gal-9 exhibited a therapeutic effect on the allogeneic skin graft by selectively reducing CD4+TIM-3+ T effector cells and inducing a shift from a Th1 to an anti-inflammatory Th2 response. Furthermore, Gal-9 did not attenuate the protective function. Our present study may represent a novel therapeutic candidate for modulating effector T cells and Tregs imbalance-based therapy of allograft transplantation.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.15.15

Introduction

Allogeneic skin transplantation is an important clinical treatment for many diseases such as large area burns. However, due to the immune rejection after allogeneic skin transplantation, the survival time is limited, which affects the growth and expansion of allogeneic skin grafts, resulting in limited therapeutic effect. Meanwhile, in order to avoid systemic infection, immunosuppressants are not applicable after skin transplantation. Therefore, if allogeneic skin transplantation can solve the immune rejection after transplantation, it will greatly promote the therapeutic effect of allogeneic skin transplantation (1).

Galectins are S-type lectins that bind to carbohydrates and play multiple roles in immune cell homeostasis and inflammation (2). Galectin-9 is characterized by two domains that are linked together by a peptide (3). There has been further confirmation that Galectin-9 binds to TIM-3 physiologically (3); and as a result of interaction between Galectin-9 and TIM-3, calcium is influxed and caspase-1 is activated, causing cell death (4).

Numerous early studies have demonstrated the crucial involvement of the TIM-3/Galectin-9 pathway in Th1-mediated auto- and alloimmune responses. It has been demonstrated that either TIM-3 antibodies inhibit the pathway and accelerate Th1-type reactions in experimental autoimmune encephalomyelitis (EAE) (5); while activation of this pathway by Gal-9 induces apoptosis of Th1 cells and ameliorates GVHD and CVB3 induced myocarditis in mice (6,7). The TIM-3/Galectin-9 pathway is a promising strategy for the induction of immunotolerance in the allograft. Therefore, to evaluate the modulation of the survival time of allogeneic skin grafts by Gal-9 and to explore the critical mechanism involved in this process, hG9NC (human stable form Gal-9, sGal-9) was administered daily to mice post-skin transplantation.

Materials and Methods

Animal sources

BALB/c mice (H-2d) and C57BL/6 mice (H-2b) were housed and maintained at the 309th hospital. Beijing-based Experiment Animal Technology Limited Company, Wei Tong Li Hua, approved all mice involved in this study.

Skin graft transplantation and Galectin-9 treatment

In accordance with our previous description, mice were transplanted with skin (8). It was administered intraperitoneally 50mg/kg of pentobarbital to anesthetize the recipient mice. The back skin from the C57BL/6 mice was excised and substituted with 1.5×1.5 cm back skin pieces from BALB/c mice, which were then put in cold saline for 24 hours. Grafts were in pressure dressing for 3 days, then the sterile gauze was removed. Rejection was defined according to the GB/T 14927122 2001 criterion for inbred strain mice skin transplant published by the Ministry of Science and Technology of China (9). Approximately 90% of viable epidermal graft tissue was lost during the study.

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Human stable-form Galectin-9 was prepared as described previously (10). In this study, hG9NC (null) was donated by Kagawa University of Japan and Galpharma Limited Company. It was dissolved with ultra-pure water and asepticized by a 0.22um filter for use. In the study group, sGal-9 was administered 100ug daily by intra-peritoneal injection until 7th day post-transplant; while 0.30ml saline was served as control. Each group was tested on at least six mice. Mean survival times were indicated for each treatment.

Flow cytometric analysis

We used spleen and peripheral blood cell suspensions from BALB/c and C57BL/6 recipients after transplantation at seven and fourteen days after transplantation. Anti-CD4 (clone GK1.5) was staining with FITC-conjugated anti-mAb; anti-Tim-1 (RMT1-4); anti-TIM-3 (RMT3-23) was staining with PE-conjugated anti-mAb. For $CD4^+CD25^+$ $Foxp3^+$Treg analysis, using [w/APC Foxp3 (clone: FJK-16g and eBR2a), FITC CD4 (clone: RM4-5), PE CD25 (clone: PC 61.5), Treg Kit]; For Th17 analysis, using IL-17A (clone: eBio17B7). A FACS can flow cytometer (Becton Dickinson) was used for analysis of all samples.

ELISA assay

The animals were euthanized for a week following skin transplantation while under isoflurane anesthesia. Blood was harvested by extirpation of the eyes of mice. Mouse IL-2, IFN-γ, IL-4, and IL-17A kits were utilized in standard ELISA procedures to measure cytokine concentrations in blood plasma.

Histologic analysis

Following fixation using 10% paraformaldehyde and embedding in paraffin, sections with a thickness of 4mm were produced and underwent staining with hematoxylin and eosin.

Data analysis

Survival time was estimated using the Kaplan-Meier curve, while the efficacy was assessed using the student t-test. SPSS 22.0 was adopted to determine statistical significance. We considered differences statistically noticeable at $p<0.05$.

Results

Longer survival time of Gal-9 *in vivo* allogeneic skin grafts

The graft survival was observed daily post-transplantation either in the study group or in the control group. Pooled data for the Kaplan–Meier test are indicated in Figure 1. The findings indicated that the use of Galectin-9 had a notable effect on prolonging the survival of the skin graft, as demonstrated by an increase in the average duration of graft survival (MST) of 16 days to 12.5 days in the saline group.

Gal-9 ameliorates the lymphocyte infiltration in skin graft

on 7th day post graft transplantation, we harvested skins and made histologic studies as indicated in Figure 2. The skin from the saline group showed lesions caused by acute rejection and with a heavy lymphocytic infiltrate. Additionally, Gal-9 noticeably alleviated the damage caused by acute rejection and ameliorated lymphocyte infiltration in skin grafts. Collectively, Gal-9 ameliorates lymphocyte infiltration and immune rejection in skin grafts.

Gal-9 selectively reduces the $CD4^+TIM\text{-}3^+$T cells and promotes the $CD4^+TIM\text{-}1^+$T cells

When Th1 cells are incubated with Galectin-9, it leads to rapid cell death, whereas Th2 cells exhibit little to no cell death. The most distinctive sign of Th1 cell activation was found to be TIM-3 (5). TIM-3+ cells remarkably augmented after post-transplantation from 1.70±0.84% to 4.82±1.01% in spleen and 3.14±1.20% to 7.13±1.82% in PBL ($p<0.05$). Administration of 100μg sGal-9 noticeably lessened TIM-3 positive cells from 4.82±1.01% to 2.84±0.89% ($p<0.01$) in spleen and from 7.13%±1.82% to 4.94±1.56% ($p<0.05$) in PBL comparing with using saline (Figure 3B). Nevertheless, at 14th day post-transplantation

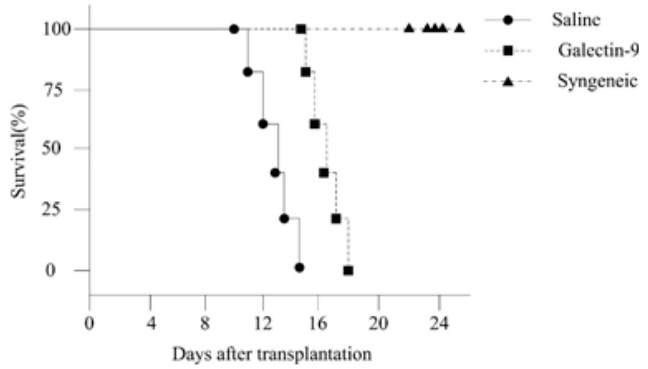

Figure 1. Galectin-9 slows down the rejection process of grafts when there is a mismatch in MHC. C57BL/6 ($H\text{-}2^d$) male mice were grafted with BALB/c ($H\text{-}2^b$) skin and treated with either saline (n=6, MST=12.5), or 100μg of Galectin-9 (n=6, MST=16). ($p<0.05$, vs saline).

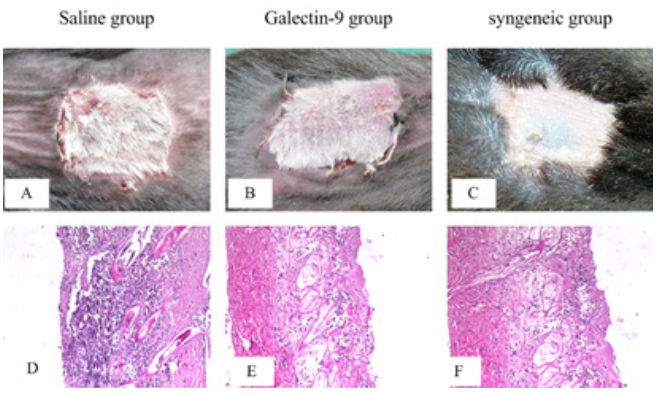

Figure 2. The images and histological analysis presented here demonstrate the effects of Galectin-9 treatment on skin grafts (n=6 in each group). Administration of saline resulted in partial skin necrosis and loss of viable epidermal graft tissue, whereas treatment with 100μg of Galectin-9 led to reduced skin necrosis and no loss of viable epidermal graft tissue. The syngeneic graft of C57BL/6 appeared normal at day 10 post-transplant. On the other hand, saline treatment at 7th day post-transplantation resulted in scarring and partial necrosis of deep dermal tissues, accompanied by a heavy lymphocytic infiltrate as revealed by HE staining. However, treatment with 100μg of Galectin-9 at 7th day post-transplantation showed normal dermis and subcutaneous tissue with a less lymphocytic infiltrate. (F) Histologic result of syngeneic graft at 7th day post-transplant.

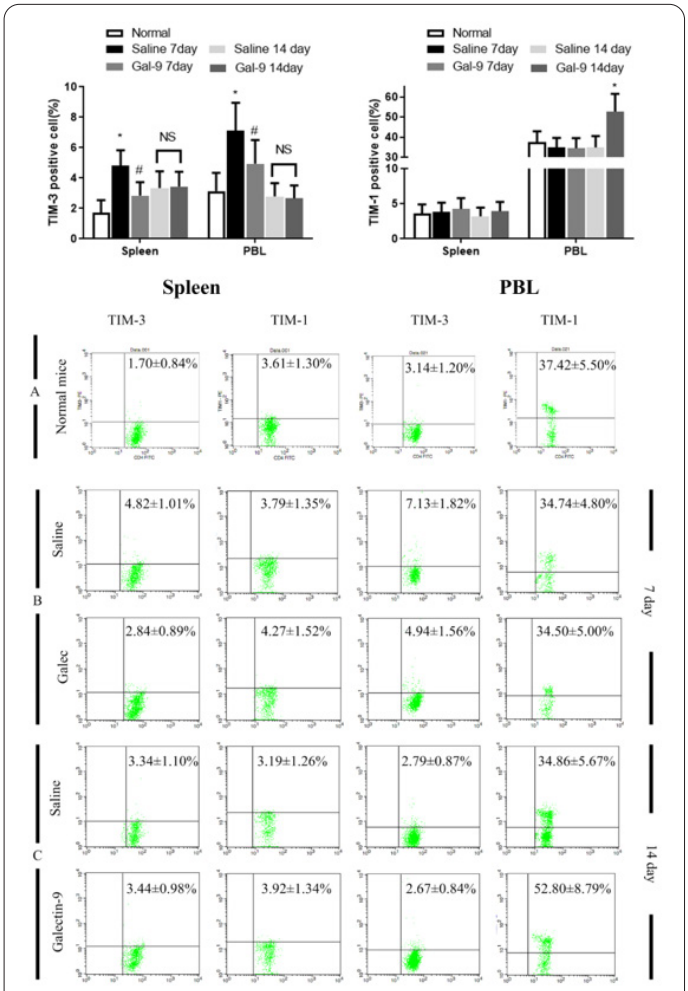

Figure 3. Flow cytometry was used to determine the levels of TIM-3 and TIM-1 positive cells in both spleen and PBL after skin transplantation. The baseline levels of TIM-3 and TIM-1 positive cells were determined in the spleen and PBL of normal mice. Subsequently, the recipients were administered either saline or 100μg Galectin-9 and the lymphocytes of spleen and PBL were harvested 7 and 14th days after transplantation to assay for TIM-3 and TIM-1 positive cells.

TIM-3 positive cells in the saline group recovered to a low level and had no difference with the Gal-9 group either in the spleen (3.34±1.10% vs 3.44±0.98%, p>0.05) or in PBL (2.79±0.87% vs 2.67±0.84%, p>0.05).

The Th2 cell line expresses TIM-1, but the Th1 cell line does not. Our results showed that there exhibited no difference in TIM-1 in the spleen on the 7th day after the operation (3.79±1.35% vs 4.27±1.52% vs 3.61±1.30%, p>0.05). The same results appear in the blood (34.74±4.80% vs 34.50±5.00% vs 37.42±5.50%) (Figure 3B). However, on the 14th day after the operation, the proportion of peripheral blood in the experimental group augmented from 34.86 ±5.67% to 52.80 ±8.79% (P<0.05), and there exhibited no noticeable change in spleen tissue (3.19±1.26% vs 3.92±1.34%, p>0.05) (Figure 3C).

Gal-9 noticeably depletes Th17 cell generation

Th17 is a pro-inflammatory cell, acting as an essential role in allograft rejection. Current studies have identified the expressed TIM-3 on Th17 cells and demonstrated that Galectin-9 suppresses the generation of Th17 in a dose-dependent relationship in vitro (11). Our results indicated that Th17 cells notably augmented at 7th day post-transplantation either in the spleen (5.54±1.38% vs 3.67±1.01%, p<0.05) or in PBL (12.42±3.67% vs 5.77±1.70%, p<0.05). Administration of 100μg Galectin-9 remarkably lessened Th17 cells frequency in PBL at 7th day post-transplantation (5.79±1.68% vs 12.42±3.67%, p<0.01); while a similar result occurred in spleen at 14th day (4.61±1.78% vs 7.91±2.34%, p<0.05) (Figure 4B, C).

Gal-9 administration has no visible effect on $CD4^+CD25^+$ $Foxp3^+$ Treg

We also carefully analyzed the change of $CD4^+CD25^+Foxp3^+$Treg *in vivo*. $CD4^+CD25^+Foxp3^+$Treg as a regulatory role is very important for immunological tolerance induction, to some degree the amount of Treg cells in the recipient decides the prognosis of the allogeneic grafts.

The results showed sGale-9 in vivo had no noticeable effect on CD4+CD25+Foxp3+Treg either in spleen (7th day 10.14±0.85% vs 10.57±2.56% vs 10.02±3.04%, p>0.05; 14th day 11.49±2.87% vs 12.93±3.12%, p>0.05) or in PBL (7th day 8.05±1.10% vs 8.00±1.78% vs 8.20±2.01%, p>0.05; 14th day 9.76±2.45% vs 10.90±2.67%, p>0.05) (Figure 4B, C). This result implied that the administration of Gal-9 didn't interrupt the protective function of Treg for allogeneic grafts.

Effects of sGal-9 on cytokines of effectors T cells in vivo

The ELISA was used to check the recipients' blood

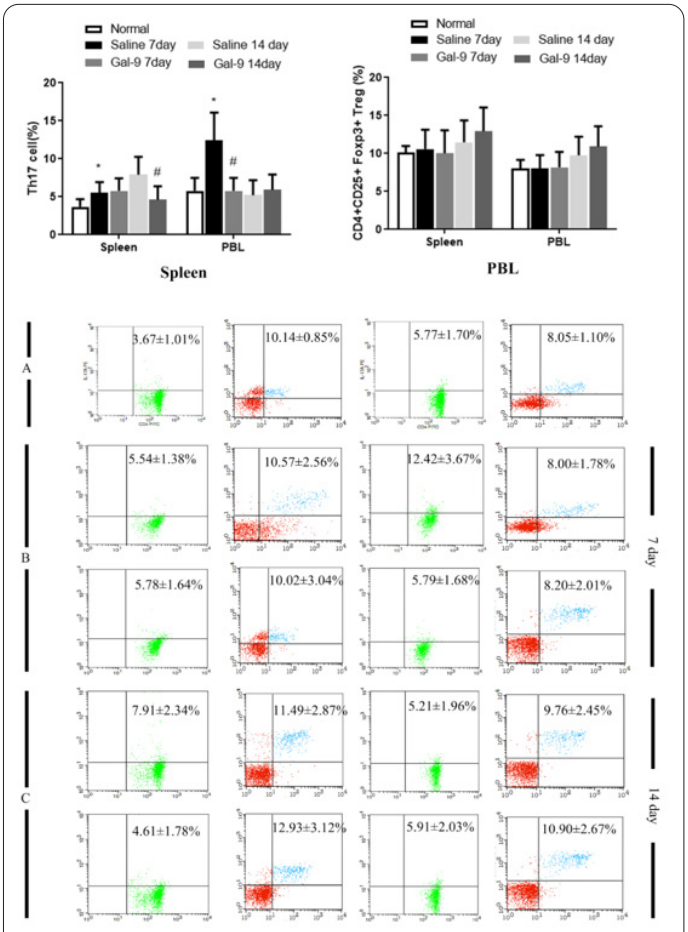

Figure 4. The flow cytometer was used to assess Th17 and CD4+CD25+Foxp3+ Treg cells in both spleen and peripheral blood lymphocytes (PBL) after skin transplantation. Firstly, the baseline level of these two types of cells in the spleen and PBL of normal mice was evaluated. Then, the recipients were administered either saline or 100μg Galectin-9 and their lymphocytes were harvested on 7th day and 14th day post-transplantation. The Th17 and CD4+CD25+Foxp3+ Treg cells were then analyzed using the flow cytometer.

serum samples for the presence of cytokine levels. Based on the results shown in Figure 5. Galectin-9 administration *in vivo* reduced the IFN-γ production (51.33±8.16pg/ml vs 98.41±15.01pg/ml, $p<0.01$) and IL-17A (48.50±8.48 vs 15.17±5.46, $p<0.01$) almost 50%. Galectin-9 did not have a noticeable effect on IL-2 (23.17±4.22pg/ml vs 26.50±3.78pg/ml, $p>0.05$) and IL-4 (86.83±13.33 vs 99.67±11.64, $p>0.05$) levels following in vivo transplantation.

Discussion

Gal-9 noticeably prolonged the survival of the allogeneic skin graft and mitigated the damage caused by acute immune rejection. In terms of mechanism, the curative effect of sGal-9 was ascribed to the selective reduction of effectors T cells such as Th1, and Th17. Simultaneously, Gal-9 didn't attenuate the protective functions of $CD4^+CD25^+Foxp3^+$Treg for allograft, which alleviated immune rejection caused by abnormal immune imbalance.

Precise immunoregulation post-transplantation is the key point to decide the prognosis of the grafts. In this complex and dynamic process, T effectors cells are critically involved including Th1, CTL and Th17 (11). In order to induce immunological tolerance, immune intervention to selectively suppress T effector cells and inflammatory cytokines is the most appropriate method. With its carbohydrate recognition domain, Galectin-9 can bind to the TIM-3 IgV domain and recognizes the oligosaccharide chains (11). By activating calcium-calpain-caspase-1, Galectin-9 induces Ca2+ influx and apoptosis in T cells (4). TIM-3 is composed of five sections (12). It was initially discovered that IFN-γ-producing Th1 and Tc1 cells are specifically expressed in mice (13). However, subsequent studies have shown that TIM-3 is not only expressed on IFN-γ producing Th1 and Tc1 cells but also in proinflammatory Th17 cells (14,15). TIM-3 on T effector cells is crucial in allogeneic reactivation, and the effect of TIM-3-Galectin-9 interaction on target cells strongly supports the importance of targeting the TIM-3-Galectin-9 pathway in treating autoimmune diseases.

The current study showed that the administration of Gal-9 noticeably prolonged the survival of allogeneic skin grafts, leading to an increase in the MST from 12.5 days to 16.0 days. This effect was directly attributable to the amelioration of lymphocyte infiltration in cutaneous lesions. Deep insight into the mechanism revealed that Gal-9 selectively reduced the $CD4^+TIM\text{-}3^+$T cells at 7th day post-transplantation either in PBL or in the spleen. Meanwhile, Gal-9 noticeably upregulated $CD4^+TIM\text{-}1^+$T cells on the 14th day in PBL from 34.86±5.67% to 52.80±8.79%, which was consistent In previous reports (16,17). However, the question is by what way sGal-9 elevates $CD4^+TIM\text{-}1^+$T frequency since it is a ligand for TIM-3 but not for TIM-1. We thought there were two reasons attributed to this phenomenon: 1) The descent of $CD4^+TIM\text{-}3^+$T frequency caused an indirectly rising ratio of $CD4^+TIM\text{-}1^+$T cells in total. 2) The differentiation of Th1 and Th17 cells can inhibit the differentiation of Th2 cells (18). Therefore, Gal-9-induced inhibition of differentiation of Th1 and Th17 terminated the suppressive functions. Thus, Gal-9 is prone to induce immune deviation from Th1 to Th2 type reaction *in vivo*, although it doesn't interact with $CD4^+$ TIM-1^+T directly.

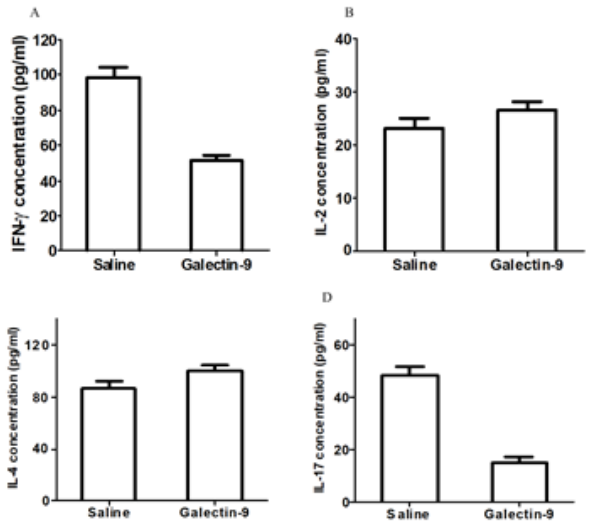

Figure 5. The impact of Galectin-9 on cytokines in vivo was evaluated. A) Recipients were treated with NS or 100μg of Galectin-9, and the serum concentration of IFN-γ was determined by ELISA on 7th day. Results are shown as means±SD of triplicates from one experiment. B) The serum level of IL-2 was measured. C) The serum level of IL-4 was assessed. D) The serum level of IL-17A was analyzed.

Th17 cells are characterized by their ability to secrete IL-17. As well as Th1 cells, it has been demonstrated to attach importance to autoimmune diseases (19-21). TIM-3 has also been found expressed on Th17 in recent studies (22). Further research *in vitro* by Seki gave evidence that Gal-9 could repress the generation of Th17 from native T cells triggered by IL-2, TGF-β and IL-6 in a dose-effect manner (23). In this study, we demonstrated sGal-9 could also exert the suppressive function on Th17 differentiation in vivo. The frequency of Th17 cells was noticeably lessened from 12.42±3.67% to 5.79±1.68% in PBL on the 7th day and from 7.91±2.34% to 4.61±1.78% in the spleen on the 14th day post allogeneic skin transplantation. IL-17A expression levels detected by ELISA have also confirmed the above results. Thus, the down-regulation of TIM-3 ligands on Th17 cells appears to be crucial in inducing immune tolerance after organ transplantation, making it a promising proof-of-concept. Other than our findings, attenuation of Th17 reaction by Gal-9 was also found in other studies. Haruna reported Gal-9 lessened IL-17-producing T cells in contact hyper-sensitivity and psoriatic mouse models (24). Kazuki's study demonstrated that sGal-9 can alleviate acute graft-versus-host disease (GVHD) by inducing T-cell apoptosis, including Th17 cells (7).

To comprehensively understand the immunological regulation by Gal-9, we also carefully analyzed the frequency changes of Treg cells for the first time. That being said, $CD4^+CD25^+$ $Foxp3^+$Treg cell is well known as a regulatory role on the other side of the "immunoregulation seesaw", and the strategies for augmenting Treg cells in recipients or allografts display promising outcomes in some initial research (25). The most deterministic thing is that the latest studies found Gal-9 was not only critically involved in the differentiation of naive T cells to Tregs, but also participated in the function exertion of Treg cells by interaction with TIM-3 (26). So, what happens on $CD4^+CD25^+Foxp3^+$Tregs by sGal-9 administration is not yet conclusive**.** The results showed sGal-9 had no noticeable impact on its frequency either in the spleen

or PBL when measured at the 7th and 14th days post-skin transplantation (Figure 4). Our findings lead to controversial conclusions with the other studies. Haruna found the frequency of $CD4^+CD25^+Foxp3^+$Treg cells was augmented by sGal-9 administration in psoriatic mouse models (24). Furthermore, Kun reported that administration of Galectin-9 improved CVB3-induced myocarditis by promoting regulatory T cell proliferation and alternatively activating Th2 cells (6).

Considering the above disagreement, we seriously speculated the following hypothesis for explanation: 1) Pathogenic T cells can paradoxically provide a protective effect in autoimmune diseases (27). Since sGal-9 induces apoptosis of T effector cells, apoptosis of T effector cells also terminates Treg differentiation. 2) Gal-9 administration *in vivo* it maybe also inhibit the excessive regulation of Treg by inducing the cell death of Tregs, because TIM-3 is also expressed in Treg cells. Considering the above reasons, no matter what mechanism involved we at least confirmed that Gal-9 *in vivo* did not attenuate the protective function for allograft.

In conclusion, Gal-9 is effective in the allogeneic skin graft via selectively lessened $CD4^+TIM\text{-}3^+$ T effector cells and induced deviation from Th1 to anti-inflammatory Th2 reaction. Gal-9 cannot attenuate the protective function of $CD4^+CD25^+Foxp3^+$Treg. As a result of this strategy, allograft transplantation might represent a new therapeutic option.

Author contributions

Yu Luo was a major contributor to writing the manuscript. Bing-Yi Shi collected the patient data. Ye-Yong Qian performed both surgeries and followed up with the patients. Hong-Wei Bai and Feng Liu realized the scarcity of the two cases, did literature searches, and revised the manuscript. All authors read and approved the final manuscript.

References

1. Sun BK, Siprashvili Z, Khavari PA. Advances in skin grafting and treatment of cutaneous wounds. Science 2014; 346(6212): 941-945. https://doi.org/10.1126/science.1253836
2. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol 2009; 9(5): 338-352. https://doi.org/10.1038/nri2536
3. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol 2005; 6(12): 1245-1252. https://doi.org/10.1038/ni1271
4. Kashio Y, Nakamura K, Abedin MJ, Seki M, Nishi N, Yoshida N, Nakamura T, Hirashima M. Galectin-9 induces apoptosis through the calcium-calpain-caspase-1 pathway. J Immunol 2003; 170(7): 3631-3636. https://doi.org/10.4049/jimmunol.170.7.3631
5. Sánchez-Fueyo A, Tian J, Picarella D, Domenig C, Zheng XX, Sabatos CA, Manlongat N, Bender O, Kamradt T, Kuchroo VK, Gutiérrez-Ramos JC, Coyle AJ, Strom TB. Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. Nat Immunol 2003; 4(11): 1093-1101. https://doi.org/10.1038/ni987
6. Lv K, Xu W, Wang C, Niki T, Hirashima M, Xiong S. Galectin-9 administration ameliorates CVB3 induced myocarditis by promoting the proliferation of regulatory T cells and alternatively activated Th2 cells. Clin Immunol 2011; 140(1): 92-101. https://doi.org/10.1016/j.clim.2011.03.017
7. Sakai K, Kawata E, Ashihara E, Nakagawa Y, Yamauchi A, Yao H, Nagao R, Tanaka R, Yokota A, Takeuchi M, Hirai H, Kimura S, Hirashima M, Yoshimura N, Maekawa T. Galectin-9 ameliorates acute GVH disease through the induction of T-cell apoptosis. Eur J Immunol 2011; 41(1): 67-75. https://doi.org/10.1002/eji.200939931
8. Yamanokuchi S, Ikai I, Nishitai R, Matsushita T, Sugimoto S, Shiotani T, Yamaoka Y. Asialo GM1 positive CD8+ T cells induce skin allograft rejection in the absence of the secondary lymphoid organs. J Surg Res 2005; 129(1): 57-63. https://doi.org/10.1016/j.jss.2005.07.009
9. Wang Y, Lei T, Wei L, Du S, Girani L, Deng S. Xenotransplantation in China: Present status. Xenotransplantation 2019; 26(1): e12490. https://doi.org/10.1111/xen.12490
10. Nishi N, Itoh A, Fujiyama A, Yoshida N, Araya S, Hirashima M, Shoji H, Nakamura T. Development of highly stable galectins: truncation of the linker peptide confers protease-resistance on tandem-repeat type galectins. FEBS Lett 2005; 579(10): 2058-2064. https://doi.org/10.1016/j.febslet.2005.02.054
11. Hirahara K, Nakayama T. CD4+ T-cell subsets in inflammatory diseases: beyond the Th1/Th2 paradigm. Int Immunol 2016; 28(4): 163-171. https://doi.org/10.1093/intimm/dxw006
12. Sabatos CA, Chakravarti S, Cha E, Schubart A, Sánchez-Fueyo A, Zheng XX, Coyle AJ, Strom TB, Freeman GJ, Kuchroo VK. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. Nat Immunol 2003; 4(11): 1102-1110. https://doi.org/10.1038/ni988
13. Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA, Freeman GJ, Kuchroo VK. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 2002; 415(6871): 536-541. https://doi.org/10.1038/415536a
14. Anderson AC, Anderson DE, Bregoli L, Hastings WD, Kassam N, Lei C, Chandwaskar R, Karman J, Su EW, Hirashima M, Bruce JN, Kane LP, Kuchroo VK, Hafler DA. Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. Science 2007; 318(5853): 1141-1143. https://doi.org/10.1126/science.1148536
15. Nakae S, Iikura M, Suto H, Akiba H, Umetsu DT, Dekruyff RH, Saito H, Galli SJ. TIM-1 and TIM-3 enhancement of Th2 cytokine production by mast cells. Blood 2007; 110(7): 2565-2568. https://doi.org/10.1182/blood-2006-11-058800
16. Wang F, He W, Yuan J, Wu K, Zhou H, Zhang W, Chen ZK. Activation of Tim-3-Galectin-9 pathway improves survival of fully allogeneic skin grafts. Transpl Immunol 2008; 19(1): 12-19. https://doi.org/10.1016/j.trim.2008.01.008
17. He W, Fang Z, Wang F, Wu K, Xu Y, Zhou H, Du D, Gao Y, Zhang WN, Niki T, Hirashima M, Yuan J, Chen ZK. Galectin-9 significantly prolongs the survival of fully mismatched cardiac allografts in mice. Transplantation 2009; 88(6): 782-790. https://doi.org/10.1097/TP.0b013e3181b47f25
18. Sifnaios E, Mastorakos G, Psarra K, Panagopoulos ND, Panoulis K, Vitoratos N, Rizos D, Creatsas G. Gestational Diabetes and T-cell (Th1/Th2/Th17/Treg) Immune Profile. In Vivo 2019; 33(1): 31-40. https://doi.org/10.21873/invivo.11435
19. Chen Z, Laurence A, Kanno Y, Pacher-Zavisin M, Zhu BM, Tato C, Yoshimura A, Hennighausen L, O'Shea JJ. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci U S A 2006; 103(21): 8137-8142. https://doi.org/10.1073/pnas.0600666103
20. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. J Leukoc Biol 2002; 71(1): 1-8.
21. Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17

family and IL-17 receptors. Cytokine Growth Factor Rev 2003; 14(2): 155-174. https://doi.org/10.1016/s1359-6101(03)00002-9

22. Wang J, Li C, Fu J, Wang X, Feng X, Pan X. Tim-3 regulates inflammatory cytokine expression and Th17 cell response induced by monocytes from patients with chronic hepatitis B. Scand J Immunol 2019; 89(5): e12755. https://doi.org/10.1111/sji.12755
23. Seki M, Oomizu S, Sakata KM, Sakata A, Arikawa T, Watanabe K, Ito K, Takeshita K, Niki T, Saita N, Nishi N, Yamauchi A, Katoh S, Matsukawa A, Kuchroo V, Hirashima M. Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. Clin Immunol 2008; 127(1): 78-88. https://doi.org/10.1016/j.clim.2008.01.006
24. Niwa H, Satoh T, Matsushima Y, Hosoya K, Saeki K, Niki T, Hirashima M, Yokozeki H. Stable form of galectin-9, a Tim-3 ligand, inhibits contact hypersensitivity and psoriatic reactions: a potent therapeutic tool for Th1- and/or Th17-mediated skin inflammation. Clin Immunol 2009; 132(2): 184-194. https://doi.org/10.1016/j.clim.2009.04.012
25. Karczewski M, Karczewski J, Kostrzewa A, Wiktorowicz K, Glyda M. The role of Foxp3+ regulatory T cells in kidney transplantation. Transplant Proc 2009; 41(5): 1527-1529. https://doi.org/10.1016/j.transproceed.2009.03.065
26. Wang F, Wan L, Zhang C, Zheng X, Li J, Chen ZK. Tim-3-Galectin-9 pathway involves the suppression induced by CD4+CD25+ regulatory T cells. Immunobiology 2009; 214(5): 342-349. https://doi.org/10.1016/j.imbio.2008.10.007
27. Grinberg-Bleyer Y, Saadoun D, Baeyens A, Billiard F, Goldstein JD, Grégoire S, Martin GH, Elhage R, Derian N, Carpentier W, Marodon G, Klatzmann D, Piaggio E, Salomon BL. Pathogenic T cells have a paradoxical protective effect in murine autoimmune diabetes by boosting Tregs. J Clin Invest 2010; 120(12): 4558-4568. https://doi.org/10.1172/JCI42945