MINIREVIEW
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Galectin-9 Changes Its Function to Maintain Homeostasis

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Abstract
Galectin-9 (Gal-9)/Ecalectin was first identified as a T cell-derived eosinophil chemoattractant. We found that Gal-9 plays a role in not only the accumulation but also the activation of eosinophils in experimental allergic models and human allergic patients; Gal-9 was shown to induce eosinophil chemotaxis in vitro and in vivo and activated eosinophils in various aspects. Recent studies, however, showed that Gal-9 has other functions in the differentiation, maturation, aggregation, adhesion, and death of various cells. Presently, we and other groups are in the process of investigating the function of Gal-9 in a variety of physiological and pathological conditions. In this article, we will show the in vivo therapeutic effects of Gal-9 in various disease models (both hyper-immune and immune-compromised conditions), suggesting that Gal-9’s immune function changes according to the context. Indeed, the accumulated evidence suggests that Gal-9 orchestrates a variety of biological phenomena to maintain homeostasis.

A. Introduction
In 1998, we purified and identified the human homologue of the guinea-pig eosinophil chemoattractant that mediated tissue eosinophilia in 24-h-old allergic skin lesions. The human homologue, Ecalectin, was first identified as a variant of Galectin-9 (Gal-9), and as the first and only galectin purified as a biologically active molecule, opened the door to speculation that other galectins might have a secretion signal, like most galectins, it is supposed to be secreted by so called “a non-classical secretion pathway,” and hence Gal-9 does not have a carbohydrate modification.

By alternative splicing, three types of human Gal-9 are generated differing in the length of the linker peptides: L- (355 aa), M- (323 aa) and S-type (311 aa) Gal-9. The linker peptide is highly susceptible to proteolysis, resulting in the truncated Gal-9 at the linker and in the loss of the activity; nevertheless the fundamental activity of three variants is the same, at least in vitro. Since the degradation occurs by a trace amount of contaminating proteases, it is very difficult to supply Gal-9 sufficiently for reproducible experiments with recombinant wild-type Gal-9.

In order to solve the shortcoming of wild-type Gal-9, we developed a mutant protein lacking the entire linker peptide of Gal-9, called stable Gal-9. Stable Gal-9 is highly resistant against proteolysis, and retains all the activities of wild-type Gal-9 (2). We can store stable Gal-9 for years in refrigerator without any activity loss. Such easy-handling nature of stable Gal-9 has contributed a lot for the expansion of Gal-9 research. The yield is also improved compared to wild-type Gal-9, probably because of lower degradation during expression and purification. In this review, we used stable Gal-9 throughout the experiments as Gal-9. One may anxious that the improved stability might result in accumulation of stable Gal-9 when administered in vivo. However, our pharmacokinetics study using 125I-labeled stable Gal-9 demonstrated >90% excretion from the injected mice in 24 h. A new version of stable Gal-9 was...
recently prepared by 10-amino acid deletion and a single amino acid substitution in the C-terminal CRD of stable Gal-9, which resulted in about 400% increase in solubility and yield without an adverse effect on its activity in vitro (3). In vivo experiments using this version of stable Gal-9 is awaited.

Galectin family binds β-galactosides in general but each member has different preference in the structure of the glycan. Hirabayashi et al. utilized a frontal affinity chromatography and found that Gal-9 preferentially binds to both branched N-glycans and glycans with poly-N-acetyllactosamine sequences, and this was found for both the N- and C-terminal CRDs. By contrast, the N-terminal, but not the C-terminal CRD, showed significant binding with low μM in dissociation constants to Forssman glycolipid-derived pentasaccharides and to blood group A hexasaccharide (4). Gal-9 binds plural natural Gal-9 ligands and modulates immune response. Among these ligands, Tim-3 (unpublished data), IgE (5), 4-1BB (6) and DR3 (7) were examined for Gal-9 binding by surface plasmon resonance, which demonstrated high binding affinity of 0.038, 0.036, 0.011 and 0.18 µM in dissociation constants to Forssman glycolipid-derived pentasaccharides and to blood group A hexasaccharide (4).

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C. Gal-9 in Acute Inflammation

Since lipopolysaccharide (LPS) is widely used for induction of acute inflammation, we used LPS-induced inflammation to clarify whether Gal-9 exhibits therapeutic effects for acute inflammation. Peritoneal administration of Gal-9 induced infiltration of Gr-1+ cells (neutrophil and/or macrophage (Mφ)) into the peritoneal cavity, and suppressed TNF-α, IFN-γ and IL-12 production. The fact that Gal-9 failed to suppress LPS-induced TNF-α, IFN-γ and IL-12 production in Gr-1+ cell-depleted mice induced by anti-Gr-1 antibody (Ab), and to protect those mice from the Schwartzman reaction, suggests the importance of Gr-1+ cells in Gal-9’s protective role. Interestingly, Gr-1+ cells attracted by Gal-9 produce prostaglandin E2 (PGE2), which can suppress TNF-α production by peritoneal Mφs, while Gr-1+ cells attracted by casein produce less PGE2 and fail to suppress TNF-α production, suggesting that Gal-9 regulates LPS-induced inflammation and protects mice from the Schwartzman reaction by selectively attracting PGE2-producing Gr-1+ cells, and that Gr-1+ cells with different functions are attracted according to the type of chemoattractant (22).

We also found that Gal-9 suppresses pathological changes of LPS-induced acute lung injury (ALI). Gal-9 reduced the levels of proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6 and keratinocyte-derived cytokine, but Gal-9 increased IL-10 and the accumulation of Gr-1+ CD11b+ F4/80+ Mφs in the bronchoalveolar lavage fluid (BALF) of ALI mice. In Gal-9-deficient mice, pathological changes of ALI were exaggerated, and the number of neutrophils and the TNF-α level were increased. The above Gr-1+ CD11b+ Mφs increased in the spleen of both Gal-9-treated and PBS-treated ALI mice, but only Mφs of Gal-9-treated ALI mice increased the ability of CCR2-expressing Mφs to migrate toward MCP-1. Transfer of Gr-1+ CD11b+ Mφs obtained from Gal-9-treated mice ameliorated ALI. Gr-1+ CD11b+ Mφs obtained from Gal-9-treated but not PBS-treated mice suppressed...
TNF-α and keratinocyte-derived cytokine production from LPS-stimulated Mφs, and down-regulated TLR-4 and TLR-2 expression on thioglycolate-elicited Mφs. FACS analysis revealed that CD14 is negligible on PDCA1⁺ Ly-6C⁺ CD11b⁺ Gr-1⁺ F4/80⁺ plasmacytoid dendritic cell-like Mφs (pDC-Mφs) obtained from Gal-9-treated mice, although Mφs obtained from PBS-treated mice expressed both PDCA-1 and CD14. Moreover, Gal-9 down-regulated the expression of CD14 on pDC-Mφs from PBS-treated mice in a manner independent of Gal-9/Tim-3 interaction, which results in the acquisition of suppressive function. It was thus suggested that the down-regulation of CD14 expression by Gal-9 is critical for the suppressive activity of pDC-Mφs (23).

**D. Gal-9 in Allergic Inflammation**

As mentioned above, we first purified Gal-9 from 24-h-old guinea-pig allergic skin lesions with eosinophilia but without rubor and/or edema (24). On this basis, we developed the hypothesis that Gal-9 (and eosinophils at 24-h-old skin lesions) plays a role in the suppression of allergic inflammation. Interestingly, we found that almost all eosinophils infiltrating 24-h-old lesions are Gal-9-positive, whereas many of those in 6-h-old lesions are Gal-9-negative (unpublished data), indicating the heterogeneity of eosinophils. Although several investigators have suggested that Gal-9 is involved in the pathogenesis of allergic disease, we found that in vivo Gal-9 administration in animal allergy models results instead in the suppression of several allergic inflammations. These results suggest that Gal-9 is a therapeutic candidate for Type 1 allergic inflammation.

Various modes of action by which Gal-9 may suppress allergic response have been discovered. First, Gal-9 directly binds to IgE and inhibits IgE-Ag complex formation, resulting in the suppression of mast cell degranulation (5). Second, Gal-9 induces apoptosis of proinflammatory eosinophils, which are probably Gal-9-negative (25). Third, Gal-9-induced inhibition of CD44-hyaluronan interaction results in the suppression of infiltration of Th2 cells in the allergic lesions (7). Fourth, Gal-9 activates and expands Th1 cells (26) and Treg cells (11). Fifth, Gal-9 suppresses production of TNF-α and RANTES from Mφs and T cells (27). Sixth, Gal-9 augments differentiation of pDC-Mφs with suppressive function (23).

de Kivit et al. found that Gal-9 expression by intestinal epithelial cells and serum Gal-9 levels were increased in mice and humans by dietary intervention with prebiotic galacto- and fructooligosaccharides in combination with Bifidobacterium breve M-16V (GF/Bb), and were correlated with reduced acute allergic skin reaction and mast cell degranulation. GF/Bb enhanced Th1 and Treg cell differentiation in mesenteric lymph node cells and peripheral blood mononuclear cells when they are exposed to Gal-9 (28).

In a mouse summer-type hypersensitivity pneumonitis model induced by *Trichosporon asahii*, Gal-9 reduces the levels of IL-1, IL-6, IFN-α, and IL-17 in BALF, resulting in the reduction of lung inflammation. Gal-9 expanded pDC-Mφs in BALF, and the expanded pDC-Mφs were responsible for the various suppressions, indicating that Gal-9 expands immunosuppressive pDC-Mφs to ameliorate Th1/Th17 cell-mediated hypersensitivity pneumonitis by suppressing Th1/Th17 production (29). It thus becomes quite intriguing to clarify whether such pDC-Mφs also involve in the amelioration of other allergic animal models, such as bronchial asthma and food allergy. Moreover, we also found that Gal-9 ameliorates the clinical and pathological symptoms of mouse contact dermatitis by decreasing IFN-α and IL-17-producing T cells (10).

**E. Gal-9 in Autoimmune Diseases**

The effects of Gal-9 on a mouse collagen-induced arthritis (CIA) model were assessed to clarify whether Gal-9 suppresses CIA by regulating T cell immune responses. Gal-9 administration strongly suppressed CIA in a dose-dependent manner, and such suppression was observed even when treatment was started 7 days after the booster, indicating that Gal-9 has preventive and therapeutic effects. Gal-9 induced the decreased levels of the proinflammatory cytokines IL-12, IL-17 and IFN-α in the joint. Gal-9 also induced a decrease in the number of CD4⁺ TIM-3⁺ T cells in peripheral blood. We further found that Gal-9 induces differentiation of naive T cells to Treg cells, and suppresses differentiation to Th17 cells *in vitro*. The results suggested that Gal-9 ameliorates CIA by suppressing the generation of Th17 (10), and by promoting the induction of Treg cells (11).

Our further experiments on a rat CIA model revealed that Gal-9 exhibits evident therapeutic effects. As shown in Fig. 1, in this rat arthritis model, paw volume was evidently increased on day 14, and destruction of bone and cartilage, inflammatory cell infiltration, and synovial cell proliferation were also detected in the joint on day 14. We thus started subcutaneous Gal-9 administration (0.06 mg/kg, 3 times /week) on day 14. Even at such a low concentration, Gal-9 exhibited evident therapeutic effects, and the volume became close to normal on day 32 with little or no inflammatory signs in the joints. Meanwhile, on day 32, joints of the untreated CIA model were almost completely destroyed (unpublished results). These findings suggest that Gal-9 has not only anti-inflammatory effects but also beneficial effects on synovial cells, chondrocytes, and osteocytes. Moriyama *et al.* also observed marked suppression of bone destruction when Gal-9 was injected into rats with adjuvant-induced arthritis. They suggested that inflammatory bone destruction is ameliorated through the Tim-3/Gal-9 system(30).
In an in vitro experiment, Gal-9 preferentially induced apoptosis and suppressed the proliferation of synovial cells of patients with rheumatic arthritis (RA), but not synovial cells from patients with osteoarthropathy. Gal-9 administration also induced apoptosis of synovial cells of RA patients implanted into SCID mice in vivo. In a mouse model of CIA, apoptotic cells were detected in the joints of Gal-9-treated mice but not PBS-treated mice, and the treatment reduced pannus formation with inflammatory cell infiltration and bone/cartilage destruction, thus appearing to suppress CIA. In contrast to Gal-9, other galectins, such as Gal-1, Gal-3, and Gal-8, did not induce apoptosis or suppress the proliferation of human synovial cells derived from RA patients (31).

It is well known that immune complex (IC) is also central to the onset of autoimmune diseases including RA. Thus, we assessed the effects of Gal-9 administration on an IC-induced mouse arthritis model. Gal-9 treatment reduced the severity of IC-induced arthritis, as well as reducing pro-inflammatory cytokine levels in inflamed joints and C5a levels in serum. FcγRIIb (inhibitory receptor) expression on Mφs from Gal-9-treated mice was enhanced, whereas FcγRIII (stimulatory receptor) expression was suppressed. Mφs from Gal-9-treated mice produced less TNF-α and IL-1β but more IL-10 than PBS-treated Mφs (32). We further found that Gal-9 exhibits more therapeutic effects on IC-induced arthritis than biologics acting on TNF-family receptors (Fig. 2, unpublished observation).

Antibodies against 4-1BB (CD137) are currently in clinical trials and have been shown to both augment immunity in cancer and promote Treg cells that inhibit autoimmune disease (6, 33). We found that the action of agonistic anti-4-1BB in the suppression of autoimmune and allergic inflammation is completely dependent on Gal-9. Gal-9 binds directly to 4-1BB, as a natural ligand, and Gal-9 facilitates 4-1BB aggregation, signaling, and functional activity in T cells, DCs, and NK cells. Conservation of the Gal-9 interaction in humans has important implications for effective clinical targeting of 4-1BB and possibly other TNFR superfamily molecules (7).

We further showed that Gal-9 administration ameliorates a variety of clinical symptoms, such as proteinuria, arthritis, and hematocrit in MRL/lpr lupus-prone mice (a mouse model of systemic lupus erythematosus) (34). Gal-9 of course reduced the frequency of Th1, Th17, and activated CD8 T cells in MRL/lpr lupus-prone mice. Anti-dsDNA antibody was increased in MRL/lpr lupus-prone mice. Anti-dsDNA antibody production was increased in MRL/lpr lupus-prone mice, and Gal-9 suppressed anti-dsDNA antibody production, at least partly, by decreasing the number of plasma cells via induction of apoptosis. About 20% of CD19−/low CD138+ plasma cells expressed Tim-3 in MRL/lpr lupus-prone mice. But it was suggested that Tim-3 was not directly involved in the Gal-9-induced apopto-

Fig. 1. Therapeutic effect of Gal-9 in a rat CIA model. Rats were sensitized and boosted with type II collagen on day 0 and 7, and Gal-9 was given 3 times/week from day 14 subcutaneously. On day 14 or day 32, rats were sacrificed, and radiological and pathological analyses were assessed using inflamed lesions.
sis, because an anti-Tim-3 antibody did not block Gal-9-induced apoptosis. Collectively, it is likely that Gal-9 attenuates the clinical severity of MRL lupus-prone mice by regulating T cell function and inducing plasma cell apoptosis. Indeed, our preliminary experiments further revealed that Gal-9 administration controls the differentiation and maturation of germinal center B cells by inducing apoptosis of certain B cell lineage cells (probably centroblasts) (unpublished data), suggesting the involvement of Gal-9 in antibody production.

Gal-9 also exhibited therapeutic effects in other autoimmune animal models: two models of multiple sclerosis, MOG35-55-induced (35) and PLP-induced (unpublished data) experimental autoimmune encephalomyelitis, and a model of Type 1 diabetes mellitus (DM) (36). Even in Type 2 DM (not autoimmune disease), Gal-9 suppressed hyperproliferation of the glomeruli by regulating the cell cycle (37). Serum Gal-9 levels in patients with Type 2 DM were closely linked to the glomerular filtration rate, and were related to alterations of the immune response and inflammation of patients with Type 2 DM and chronic kidney disease.

F. Gal-9 in Infectious Diseases

Regarding infectious disease, many investigators have shown elevated levels of Gal-9 in the serum of patients with infection, and many have speculated that Gal-9 is the cause of the severity by terminating the immune response through Gal-9/Tim-3 interaction. However, such speculation is too early because it is still not known whether Gal-9 administration in infected animals attenuates or aggravates disease.

Gal-9 levels vary during virus infection; Gal-9 levels are high early in the infection while decreases as a cure is achieved. Up-regulation of Gal-9 was shown to induce cell migration of human DCs towards infected lesions with dengue virus (DV) (38). Therefore, it is possible that Gal-9 is a therapeutic target for preventing immunopathogenesis induced by DV infection. Gal-9 also ameliorated respiratory syncytial virus (RSV)-induced pulmonary immunopathology through regulating the balance between Th17 and Treg cells, suggesting that regulation of the Gal-9/Tim-3 pathway is an effective and safe approach to treating RSV infection in the lungs (39).

Plasma Gal-9 concentrations were higher in patients with influenza infection than in patients with pneumococcal pneumonia and healthy subjects. Patients with influenza were easily differentiated from those with pneumococcal pneumonia or healthy subjects based on plasma levels of Gal-9. Furthermore, using a human airway epithelial cell line, we showed that the presence of PolyIC but not LPS increases the Gal-9 concentration in culture medium, suggesting that PolyIC enhances Gal-9 production. These findings support our proposal that Gal-9 production is induced by influenza virus infection in humans. It was thus suggested that plasma Gal-9 could be a new biomarker for patients with influenza infection (40). Sharma et al. found that Gal-9 regulates influenza A virus-specific humoral and CD8 T cell responses using Gal-9-knock out mice (41). We also assessed the effects of Gal-9 on the infection by influenza virus in vitro and on survival after influenza virus infection in vivo. Gal-9 inhibited infection by H1N1, H3N2 and H5N1 influenza A viruses in vitro. Half of the Gal-9-transgenic mice survived after H1N1 infection while all the wild-type mice died. Furthermore, Gal-9 administration suppressed influenza virus replication in the lungs and decreased the levels of proinflammatory cytokines. Combined administration of Gal-9 and oseltamivir was dramatically more effective than the use of Gal-9 or oseltamivir alone in the mouse model (42).

Shim et al. reported that herpes simplex virus-induced inflammation was ameliorated by Gal-9 through decrease of Th1 cells and increase of Treg cells (43). Simultaneously, Reddy et al. reported that combination therapy with Gal-9 and monoclonal antibody (mAb) against TNF receptor superfamily member 25 (TNFRSF25) results in the highly effective therapeutic effects on HSV infection-induced stromal keratitis. The beneficial outcome of the combination therapy was attributed to the expansion of the Treg cells.
cell population that additionally expressed activation markers such as CD103 needed to access inflammatory sites. Additionally, there was a marked reduction of IFN-γ-producing CD4+ T cells responsible for the tissue damage (44).

We also observed a rapid decrease of plasma Gal-9 levels in patients with acute HIV infection after therapy despite the Gal-9 level being high before treatment (45). Furthermore, we showed that Gal-9 binding to Tim-3 renders activated human CD4+ T cells less susceptible to HIV-1 infection (46). Since Gal-9 can inhibit infection by influenza virus, it is suggested that Gal-9 is also a useful therapy for HIV infection.

Sepsis is the leading cause of death in critically ill patients, and the incidence of sepsis is increasing. The mortality rate of severe sepsis is very high, up to 70%. Two types of animal sepsis models have been established: the LPS-induced inflammation described above, and the cecal ligation and puncture (CLP) model of microbial sepsis. LPS stimulates Mϕs to release large amounts of TNF-α and IL-1β that can precipitate tissue injury and lethal shock. Antagonists of TNF-α and IL-1β have shown limited efficacy in clinical trials, most likely because these cytokines are early mediators in the sepsis pathogenesis. Gal-9 probably exhibits crucial roles in bacteria-induced inflammation because Gal-9 can directly suppress LPS-induced production of proinflammatory cytokines (TNF-α and IL-1β) from Mϕs (unpublished data) though it stimulates DCs to release small amounts of TNF-α (47).

In CLP mice, Gal-9 administration suppressed the production of TNF-α, IL-6, IL-12, HMGB1 and IL-10, but enhanced the production of IL-15 and IL-17 from spleen cells prepared 24 h after a single Gal-9 administration immediately after the CLP procedure (48). We further showed that Gal-9 prolongs the survival of polymicrobial septic mice by expanding Tim3-expressing NK cells and pDC-Mϕs. Similarly to anti-PD-1 antibody (49). Gal-9 exhibited therapeutic activity even 1 day after CLP, suggesting the usefulness of Gal-9 as a therapeutic candidate for sepsis. Although the similarity between Gal-9 and PD-1 / PD-L1 is intriguing, further studies are required to elucidate their relationship.

G. Gal-9 in Malignant Tumors

As described above, Abs against 4-1BB (CD137) can augment immunity in cancer (33). Since Gal-9 is essentially required for anti-4-1BB to exhibit its therapeutic effects on autoimmune disease and allergic inflammation by promoting Treg cells, the possibility cannot be excluded that Gal-9 also exhibits therapeutic effects on cancer-bearing animals. Indeed, many papers have demonstrated that Gal-9 expression in tumor cells is associated with better prognoses in tumor-bearing patients. For instance, patients with high Gal-9-positive tumor cells have better prognoses and no or few metastases (50, 51). Indeed, we found that Gal-9 inhibits tumor cell metastasis by binding to adhesive molecules to block adhesion of tumor cells to the endothelium and extracellular matrices (16).

Furthermore, Gal-9 induces tumor cell death by inducing apoptosis and/or autophagy of tumor cells in vitro and/or in vivo (52, 53). It thus becomes important to clarify whether Gal-9 treatment contributes to a better prognosis in tumor-bearing animals. Nobumoto et al. reported that Gal-9 administration significantly prolonged the survival of B16F10 melanoma-bearing mice. Gal-9 increased the numbers of NK cells, CD8 T cells and Mϕs in tumor-bearing mice (54). However, the Gal-9-mediated anti-tumor activity was not induced in NK cell-, Mϕ- or CD8 T cell-depleted mice. NK cells from Gal-9-treated mice, compared to PBS-treated mice, exhibited significantly higher cytolytic activity. Co-culture of naïve NK cells with Mϕs from Gal-9-treated mice resulted in enhanced NK activity, although Gal-9 by itself did not enhance the NK activity, suggesting that Gal-9 activates Mϕs to enhance NK cell activity. We further found that the Mϕs were responsible for the enhanced NK activity. These results provide evidence that Gal-9 promotes NK cell-mediated anti-tumor activity by expanding pDC-Mϕs.

Gal-9 also expanded pDC-Mϕs in lung cancer-bearing mice and prolonged their survival (55). Gal-9 increased the frequency of CD11chigh cells in M-CSF- but not GM-CSF-induced Mϕs in vitro in a Tim-3-independent manner. CD11chigh cells differentiated with M-CSF+Gal-9 expressed pDC-Mϕ markers, such as PDCA-1 and F4/80 (42). These cells expressed high TLR7, TLR8 and TLR9, but low IFN-α mRNA levels. LPS or LLC stimulation further elevated pDC-Mϕ markers, indicating that M-CSF+Gal-9-induced Mϕs were pDC-Mϕ precursors. Moreover, LPS stimulation resulted in increased IRF7 and E2-2 levels, suggesting that the pDC-Mϕ precursors matured into pDC-Mϕs. These matured pDC-Mϕs augmented NK cell-mediated cytotoxicity though they did not produce IFN-α upon TLR7 or TLR9 stimulation. The results suggest that Gal-9 induces Mϕs to differentiate into pDC-Mϕs, and that this switch in differentiation favors the activation of NK cells that are able to prolong the survival of tumor-bearing mice.

Fujita et al. recently showed that Gal-9 inhibited the growth of human hepatocellular carcinoma cells by apoptosis but not by cell cycle arrest in vitro and in vivo. MicroRNA (miRNA)-1246 was shown to mediate signals of Gal-9, possibly through the miR-1246–DYRK1A–caspase-9 axis (56). Tadokoro et al. reported that Gal-9 suppresses tumor growth by implanted human gall-bladder carcinoma cells in a xenograft model (57). Gal-9 induced phosphorylation of the Ephrin type-B receptors, and the miRNA expression profile was markedly altered by Gal-9. Based on these results,
various miRNAs might contribute to the suppression of tumor growth by Gal-9. Furthermore, Wiersma et al. showed very interesting data that Gal-9 induces fatal frustrated autophagy in KRAS-mutant colon carcinoma, depending on elevated basal autophagic flux (38).

H. Gal-9 in Transplantation

Gal-9 administration has been mainly used to promote TIM-3 signaling in experimental models. Regarding the role of Gal-9 in transplantation, Gal-9 treatment is associated with prolonged allograft survival of both skin and cardiac allografts (59, 60). These studies used models of both acute and chronic allograft rejection, and all consistently demonstrated that Gal-9/TIM-3 interaction leads to Th1 and Th17 suppression while promoting Treg cell differentiation. Recently, it was further found that Gal-9 improves the survival of allografts, and that Gal-9 treatment in combination with rapamycin or EX-527 (a Sirtin-1 inhibitor) exhibited stronger beneficial activity (61) We should note, however, that Gal-9 binds to not only Tim-3 but also to many other molecules, such as 4-1BB, adhesive molecules and many cell surface proteins with β-galactoside; as of yet, there is no evidence that Gal-9 interaction with molecules other than TIM-3 is associated with its immune regulatory functions. Furthermore, it will be very important to clarify whether pDC-Mφs are involved in the above Gal-9-mediated immune regulatory function.

I. Conclusion

When the level of certain bioactive molecules varies directly with the severity of disease, many investigators jump to the conclusion that the substance is pathogenic. However, we should consider two possibilities: one, that the substance is indeed pathogenic and the other, that the substance has increased to the degree needed to suppress the disease. In order to clarify which is the right interpretation, we should not forget the principles that 1). Inflammation-mediating substances are present at the site, and the amount of the substances is proportional to the severity of inflammation, 2). Production of inhibitory substances follows the production of the mediating substances, and the amount of inhibitor is proportional to the amount of mediating substances. Therefore, one cannot determine that a certain substance is proinflammatory or anti-inflammatory unless the results of in vivo administration in disease model animals. We should keep in mind the maxim that one piece of direct evidence is more persuasive than numerous pieces of indirect evidence.

Gal-9 augments pDC-Mφ (PDCA-1<sup>+</sup> CD11b<sup>+</sup> CD11c<sup>high</sup> Ly-6C<sup>+</sup> F4/80<sup>+</sup>) differentiation, and pDC-Mφs enhance NK cell

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**Fig. 3. Presence of human pDC-Mφs in peripheral blood mononuclear cells.** Human peripheral mononuclear cells were stained with F4/80, PDCA-1, CD123 and CD303, and FACS analysis was performed.
activity in cancer-bearing animals. In contrast, Gal-9 expands similar pDC-Mϕs that release proinflammatory cytokines from Mϕs through down-regulation of TLR-4 and TLR-2 expression in hyperimmune conditions, suggesting the importance of pDC-Mϕs in the “orchestrating” role of Gal-9. We have detected PDCA-1<sup>high</sup>+ F4/80<sup>low</sup> cells in human PBMC, and those cells express CD123 and CD303, markers of pDC, suggesting the existence of human pDC-Mϕs similar to those in mice (Fig. 3). Of course, further study is required to ascertain the role of human pDC-Mϕs. We also found that cell surface Gal-9-expressing Th cells (ThGal-9 cells) reduce Th17 development but augment Foxp3<sup>+</sup> Treg development (62). Thus, it will be important to precisely clarify the relation between pDC-Mϕs and surface Gal-9-expressing Th cells.

Lastly, Gal-9 is not just a suppressor of immune reaction. Gal-9 acts as a suppressor in hyper-immune conditions, but it exhibits immune-enhancing activity in immune-compromised conditions. Moreover, many investigators have been confused by the results that Gal-9 binds numerous cell surface molecules as described above. However, from the results of in vivo experiments, it can be raised a hypothesis that Gal-9 has been designed as the substance that maintain homeostasis of not only of immune responses but also other biological phenomena, appearing to orchestrate a wide range of biological responses by binding many cell surface molecules.

References


Mitsuomi Hirashima earned his M.D. degree from Kumamoto University Medical School in 1972, and Ph.D degree from Kumamoto University, Graduate School of Medicine in 1976 and was appointed as an Assistant Professor at the Pathological Department, Kumamoto University Medical School Hospital in this year. He worked at Department of Immunology, the Good Samaritan Hospital, School of Medicine, Johns Hopkins University between 1979 and 1981 as a Postdoctoral Fellow under mentorship of Dr. Kimishige Ishizaka. He was appointed as an Assistant Professor at Department of Pathology, Kumamoto University Medical School in 1981, a University Lecturer in 1983, and an Associate Professor in 1984. In 1994, he moved to Kagawa Medical School as Professor of Department of Immunology and Immunopathology. In 2012, he retired and got a title of Emeritus Professor, and he is now working as a Research Collaborator at Department of Gastroenterology and Neurology, Kagawa University Faculty of Medicine. His research interest is to clarify the role of galectin-9 in health and various diseases. In 2000, he established a university-launched bio-venture company (Galpharma Co., Ltd.) as the representative executive to utilize galectin-9 clinically as a therapeutic agent and diagnostic pharmaceuticals for various diseases.

Toshiro Niki graduated from the Faculty of Integrated Arts and Sciences, Hiroshima University in 1989. In 1994 he received a Ph.D. from the same school for cell biology/biochemical research. Work for Bayer Yakuhin and its overseas affiliated companies to deepen knowledge of practical drug discovery research and development. In 2004 participated in Kagawa University-launched venture company GalPharma and contributed to the expansion of Galectin-9 research in the world. Since 2009 he is the company's representative director.

Tsutomu Masaki earned his M.D. degree from Kagawa University School of Medicine in 1990, and D. Med. Sci. degree from Kagawa University in 1994. He was appointed as an Assistant Professor in 2001, as a Lecturer in 2005 at Department of Internal Medicine, Faculty of Medicine, Kagawa University. He was further appointed as a Professor at Department of Gastroenterology and Neurology, Kagawa University Faculty of Medicine in 2008. His research interest is Hepatology, especially Diagnosis and Therapy of Liver Cancer and its Pathogenesis.