

CONCISE COMMUNICATION

Elevated serum galectin-9 levels in patients with atopic dermatitis

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ABSTRACT

Galectin-9 is a member of the galectin family that has a wide spectrum of biological functions. Among them, galectin-9 has been known mainly as a potent chemoattractant for eosinophils. In addition, galectin-9 alters the T-cell balance by negatively regulating T-helper (Th)1 and Th17 cells, resulting in Th2 polarization. Atopic dermatitis (AD) is a skin allergic disease characterized by peripheral eosinophilia, mast cell activation and predominance of Th2 cells. To investigate possible roles of galectin-9 in AD, we measured serum galectin-9 levels in AD patients and investigated galectin-9 expression in lesional skin by immunohistochemistry. Serum galectin-9 levels in patients with AD were significantly higher than those in healthy controls and correlated with the Eczema Area and Severity Index. Serum galectin-9 levels were decreased after treatment, accompanied by improvement of skin lesions. Immunohistochemical study revealed that galectin-9 was expressed on epidermal keratinocytes and mast cells in lesional skin of AD. Our results suggest that elevated galectin-9 expression is associated with progression of AD and that galectin-9 could be a therapeutic target in AD.

Key words: atopic dermatitis, Eczema Area and Severity Index, epidermal keratinocytes, galectin-9, mast cells.

INTRODUCTION

Galectin-9 is a member of the tandem-repeat galectin family, which is widely distributed in human tissues including epithelial tissues and expressed in several cell types.¹ Galectin-9 binds to various partners expressed on the cell surface, such as T-cell-immunoglobulin- and mucin-domain-containing molecule-3 (TIM-3), CD44 and immunoglobulin (Ig)E.^{2–4} Due to the presence of multiple binding partners, galectin-9 has a wide spectrum of potential target cells and biological functions. First, galectin-9 has been well characterized as an eosinophilic chemoattractant.⁵ On the other hand, galectin-9 is known to play a variety of cellular roles, including modulation of cell differentiation, adhesion, aggregation and cell death.⁶ Through these functions, this galectin can regulate multiple physiological and pathological processes including allergy.

Atopic dermatitis (AD) is a chronic or relapsing inflammatory skin disease characterized by eczematous skin lesions and intense pruritus. Multiple heterogeneous factors including skin barrier dysfunction, scratch and allergy are associated with the pathogenesis of AD. Patients with AD usually exhibit elevated serum IgE levels and the presence of specific IgE for environmental antigens, such as food and allergens. In addition, they often have other allergic diseases, including food allergy, asthma and allergic rhinitis. In this study, we examined serum

galectin-9 levels and galectin-9 expression in lesional skin in patients with AD to determine galectin-9 involvement in AD.

METHODS

Patients

Thirty-six patients with AD (mean $\pm$ standard error of the mean [SEM] age, 30.2 ± 2.0 years; 25 men and 11 women) and 29 healthy controls subjects (mean $\pm$ SEM age, 29.2 ± 1.6 years; 10 men and 19 women) were enrolled in this study. The disease severity was determined by Physician Global Assessment and the Eczema Area and Severity Index (EASI). The medical ethics committee of the University of Tokyo approved all described studies. Written informed consent was obtained to use blood samples. The 29 healthy controls had no history of allergic diseases.

Enzyme-linked immunosorbent assay

Immunoreactive galectin-9 in sera was quantified using a Human Galectin-9 Quantikine ELISA Kit (R&D systems, Minneapolis, MN, USA). Optical densities were measured at 450 nm using a Bio-Rad Model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The concentrations were calculated from the standard curve generated by a curve-fitting program.

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Immunohistochemistry

We performed immunohistochemical staining for galectin-9 with AD skin ($n = 6$) and normal skin ($n = 6$). Briefly, 5- μ m thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were dewaxed and rehydrated. These sections were stained with antihuman galectin-9 polyclonal antibody (Abcam, Cambridge, UK) or rabbit IgG followed by ABC staining (Vector Lab, Burlingame, CA, USA). Diaminobenzidine was used for visualizing the staining, and counterstaining with Mayer's hematoxylin was performed. In some experiments, toluidine blue staining was performed to identify mast cells.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test for comparison of two groups. Correlation coefficients were determined by using Spearman's rank correlation coefficient. Paired Student's *t*-test was used to investigate the difference between before and after treatment. $P < 0.05$ was considered statistically significant.

RESULTS

Serum galectin-9 levels

We first measured serum galectin-9 levels in patients with AD and healthy controls (Fig. 1a). Serum galectin-9 levels in AD patients were 3.19 ± 1.57 ng/mL, which was significantly higher than those in healthy individuals (2.10 ± 0.66 ng/mL; $P < 0.01$). We also analyzed serum galectin-9 levels according to the severity of AD. In patients with mild, moderate and severe AD, serum galectin-9 levels were 2.17 ± 0.54 , 2.50 ± 0.84 and 3.84 ± 1.76 ng/mL, respectively (Fig. 1b). The levels in severe AD patients were significantly higher than those in mild and moderate AD patients ($P < 0.01$ and 0.01 , respectively). Thus, serum galectin-9 levels were elevated in patients with AD, especially with severe AD.

Decrease in serum galectin-9 levels after treatment

In five patients with moderate or severe AD, we measured serum galectin-9 levels before and after treatment. The treatment included topical corticosteroids, topical tacrolimus and oral antihistamine drugs. All cases showed improvement of

skin lesions. After treatment, serum galectin-9 levels significantly decreased from 6.42 ± 0.98 to 3.16 ± 2.07 ng/mL ($P < 0.01$; Fig. 2a).

Correlation between serum galectin-9 levels and EASI in patients with AD

We compared serum galectin-9 levels with other clinical and laboratory data. We found that serum galectin-9 levels were significantly correlated with EASI ($r = 0.44$, $P < 0.05$; Fig. 2b) but not frequencies or numbers of eosinophils in peripheral blood. These results suggest that serum galectin-9 levels can be a useful disease marker of AD reflecting skin conditions.

Galectin-9 expression in lesional skin of AD

To investigate the source of galectin-9 in lesional skin, we immunolabeled the lesional skin biopsy samples of AD and normal skin. In normal skin, galectin-9 was weakly expressed on epidermal keratinocytes, and there were no positive cells in the dermis (Fig. 3a). In AD skin, expression of galectin-9 on epidermal keratinocytes was stronger than that observed in normal skin (Fig. 3b). In addition, some infiltrating cells in the upper dermis expressed galectin-9 (Fig. 3b). Because most galectin-9-positive dermal infiltrating cells had abundant cytoplasm with oval nucleus, the characteristic appearance of mast cells, we performed serial section staining with galectin-9 and toluidine blue (Fig. 3c,d). The distributions of galectin-9-positive cells and mast cells were similar, indicating that mast cells were one of the sources of galectin-9 in AD skin. Collectively, galectin-9 was expressed on epidermal keratinocytes and mast cells in AD skin.

DISCUSSION

In this report, we first found that serum galectin-9 levels in AD patients were obviously higher than those in healthy controls. Galectin-9 has been known mainly for its chemotactic activity towards eosinophils.⁵ Galectin-9 also induces apoptosis in Th1 cells and Th17 cells, resulting in Th2 polarization.⁷ Indeed, galectin-9 expression was elevated in lung tissue in animal models of asthma and associated with Th2 cytokine expression and local eosinophilia.^{8,9} Among human allergic diseases,

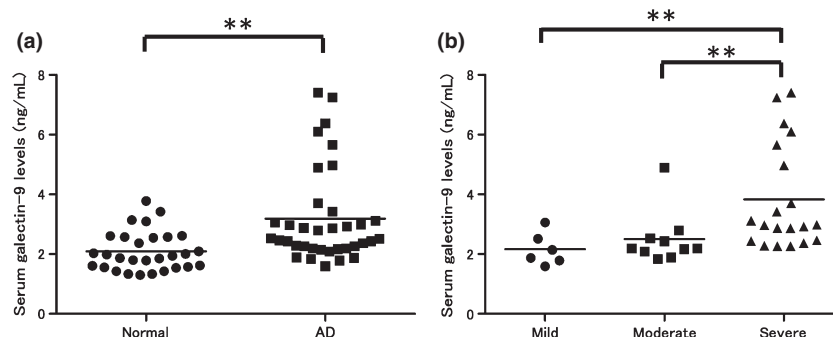


Figure 1. Serum galectin-9 levels in patients with (a) atopic dermatitis (AD) or (b) AD classified with disease severity and healthy controls. The measured values from individual patients are plotted by dots. Bars represent average. ** $P < 0.01$.

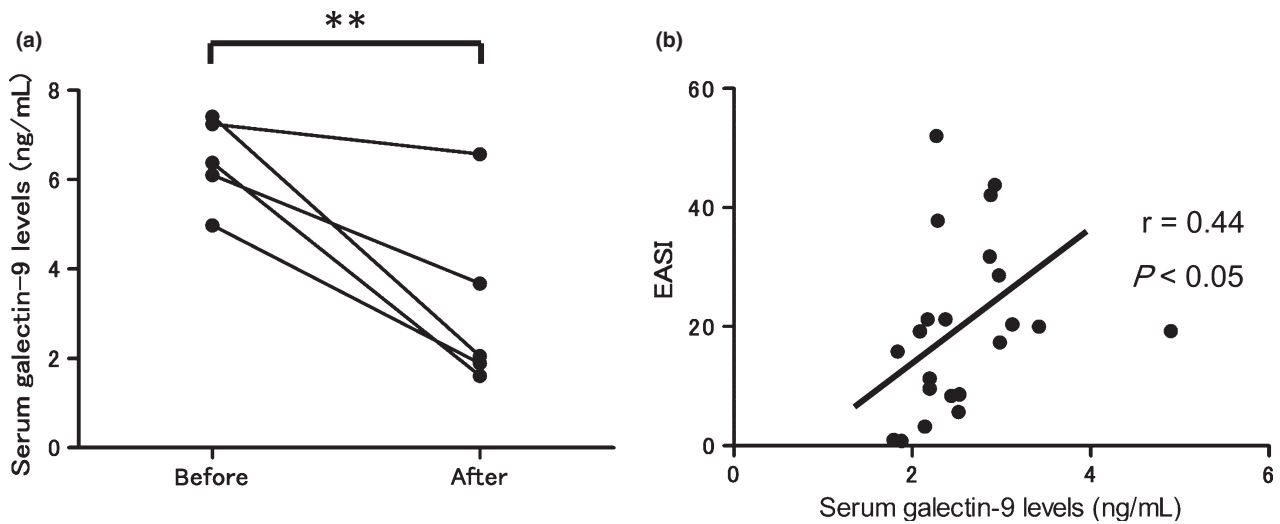


Figure 2. (a) Serum galectin-9 levels before and after treatment. The measured values from individual patients are plotted by dots. ** $P < 0.01$. (b) Correlation between serum galectin-9 levels and the Eczema Area and Severity Index (EASI) scores.

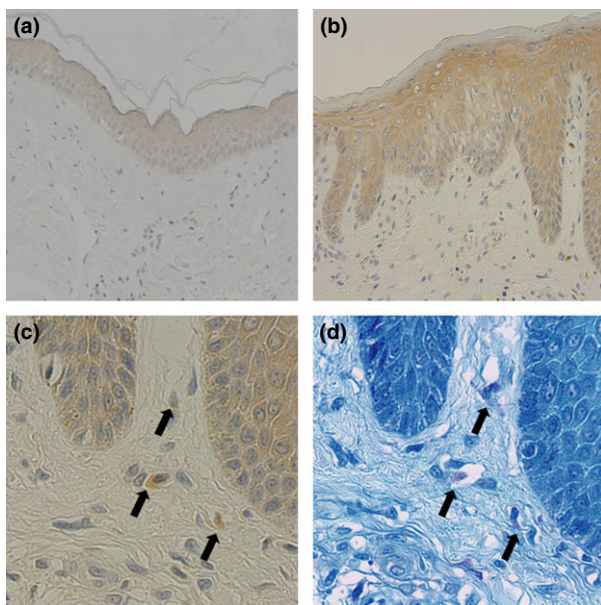


Figure 3. (a) Immunohistochemical staining for galectin-9 in lesional skin of atopic dermatitis (AD) and (b) normal skin (original magnification $\times 200$). (c) Serial section staining with galectin-9 and (d) toluidine blue in lesional skin of AD ($\times 400$). Arrows indicate galectin-9-positive cells or mast cells detected with toluidine blue staining.

serum galectin-9 levels in food allergy patients and galectin-9 levels in bronchoalveolar lavage fluid in patients with eosinophilic pneumonia were elevated.^{10,11} Similar to asthma and food allergy, AD is generally recognized as an allergic Th2-mediated disease. Therefore, our findings suggest that elevated galectin-9 levels play a role in eosinophilia and Th2 polarization in AD. We also revealed that serum galectin-9

levels were correlated with EASI. Serum galectin-9 levels decreased after treatment accompanied by the improvement of skin lesions, strongly indicating that serum galectin-9 levels correlate with disease activity. The blockade of the ligand of galectin-9 (TIM-3) resulted in ameliorated ovalbumin-induced asthma.¹² In a mouse model of food allergy, blocking galectin-9 inhibited the allergic hypersensitivity status and the antigen-specific Th2 response in intestine.¹⁰ Collectively, our results suggest that elevated galectin-9 expression in AD patients is associated with development of allergic status in AD, and that inhibition of endogenous galectin-9 function may improve the disease course of AD.

We also revealed that one of the main sources of galectin-9 in lesional skin of AD was epidermal keratinocytes. Consistent with our findings, galectin-9 expression on epidermal keratinocytes in lesional skin of bullous pemphigoid, which are dominantly mediated by Th2 responses, and galectin-9 expression on intestinal epithelial cells in patients with Th2-mediated food allergy were augmented.^{10,13} In intestinal epithelial cells, galectin-9 expression was augmented by co-culture with mast cells.¹⁰ Besides, mast cells accumulation is induced by Th2 cytokines and infiltration of mast cells in dermis is also observed in AD. Thus, it is possible that galectin-9 expression on epidermal keratinocytes is upregulated by mast cells. We also found that mast cells are the sources of galectin-9 in lesional skin of AD, consistent with previous reports.^{14,15} Taken together, our results suggest that the Th2-polarized microenvironment in lesional skin of AD induces galectin-9 upregulation through accumulation of mast cells, following possible interaction between mast cells and epidermal keratinocytes.

In summary, galectin-9 expression was elevated in sera and lesional skin of AD. Epidermal keratinocytes and mast cells were the main sources of galectin-9 in lesional skin. Our results suggest that a Th2-polarized microenvironment in AD induces elevated galectin-9 expression, resulting in exacerbation of Th2

polarization in AD and that galectin-9 could be a therapeutic target in AD.

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CONFLICT OF INTEREST: The authors state no conflict of interest.

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