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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Serum galectin-1, galectin-3, and galectin-9 are bystander molecules for chronic spontaneous urticaria

To the Editor,

The most frequent cause of chronic spontaneous urticaria (CSU) is autoimmunity, that is, supported by the autologous serum skin test (ASST).¹ A positive test indicates the presence of functional circulating IgE autoantibodies to autoallergens and/or IgG autoantibodies to IgE or IgE receptor. However, only one half of CSU patients score ASST-positive, and one third show circulating autoantibodies against IgE or IgE receptor.¹ Therefore, there must be other inflammatory mediators to be involved in CSU development.

Galectins are carbohydrate-binding proteins that are involved in many immunological processes by affecting cell proliferation, differentiation, apoptosis, and function. Of the 15 galectins in mammals, galectin-1, -3, and -9, particularly galectin-10 are expressed by eosinophils and participate the development of allergic diseases by regulating the recruitment and function of eosinophils.² In addition, galectin-1 was shown a therapeutic potency for intestinal allergy by inhibiting mast cell activation and facilitating Treg development.³ Moreover, the four galectins are all recognized as IgE-binding proteins, however little has been known about their roles in CSU, one of the commonest allergic diseases mediated by mast cells, eosinophils and IgE.² The object of the present study was to evaluate the involvement of the four galectins in CSU development.

Chronic spontaneous urticaria was defined as the spontaneous appearance of wheals, angioedema, or both for ≥ 6 weeks in the absence of known triggers as reported.¹ Patients with physical urticaria, other allergic and autoimmune diseases, infectious diseases,

and cancer were excluded in this study. Healthy controls were individuals without obvious abnormalities in immunologic indexes, routine blood and urine examinations, hepatic and renal function test. The study was approved by the research ethics board of Sun Yat-sen Memorial Hospital, and the informed consent was obtained from all participants. Fifty-five patients (35 female and 20 male) were enrolled in this study with the average age of 34.33 ± 11.82 years and the disease duration of 14.87 ± 22.17 months (mean $\pm$ SD). Serum was collected at the time when patients were experiencing wheals and itch. Twenty-seven individuals were selected for healthy controls with age (mean $\pm$ SD 30.74 ± 10.28 years) and gender (F:M = 19:8) matched to the patients. We first examined the serum level of each galectin using enzyme-linked immunoassay (ELISA) according to the manufacturer's instruction. The mean levels (ng/mL) of galectin-1, -3, and -9 were 32.15 ± 1.38 , 8.59 ± 0.69 , and 10.99 ± 0.43 in patients, respectively, higher than in healthy controls (26.94 ± 1.165 , 5.77 ± 0.50 , and 9.475 ± 0.42 , respectively) ($P = 0.035$, $P = 0.015$, and $P = 0.046$, respectively). However, the mean level of galectin-10 was comparable between the two groups (10.06 ± 2.62 vs 12.37 ± 4.15 , $P = 0.63$) (Figure 1). Therefore, we only examined the association of galectin-1, -3, and -9 with the disease parameters of CSU in the following analysis. Galectin-1, -3, and -9 was comparable between male and female patients and did not vary with age. Galectin-1 and -3, but not -9 was positively associated with disease duration. None of the three galectins was correlated with the Chronic Urticaria

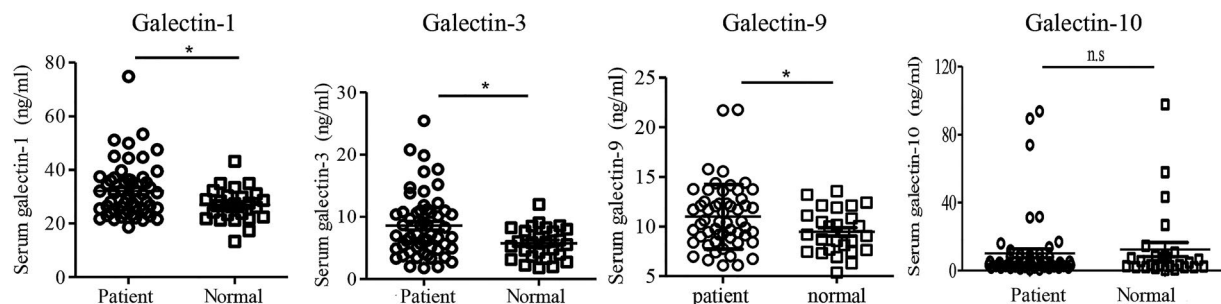


FIGURE 1 Serum levels of galectin-1, galectin-3, galectin-9, and galectin-10 in patients with chronic spontaneous urticaria. Serum levels of galectin-1, -3, -9, and -10 were determined by the enzyme-linked immunosorbent assay (ELISA). Data represent two or more independent experiments. Mean $\pm$ SD, * $P < 0.05$. n.s. = no significance

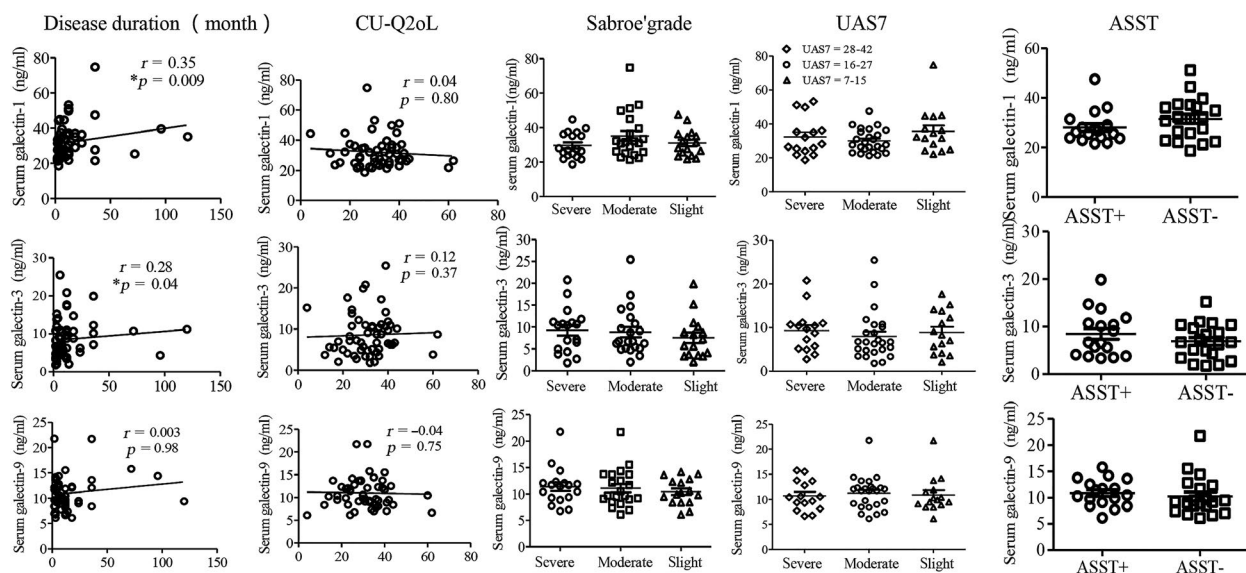


FIGURE 2 Association of serum galectin-1, galectin-3, and galectin-9 with disease features of chronic spontaneous urticaria. The quality of life was measured using the Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL). Disease activity was evaluated and graded into three categories (severe, moderate, and slight) according to Sabroe's grade and Urticaria Activity Score Over 7 Days (UAS7), respectively, at the time of sampling blood for autologous serum skin tests (ASST) 3 d after discontinuing anti-histamines. Data are given as means $\pm$ SEM and representative of two or more independent experiments. * $P < 0.05$

Quality of Life Questionnaire (CU-Q2oL, 31.36 ± 10.32 , mean $\pm$ SD). The disease activity of CSU was measured by the Urticaria Activity Score Over 7 Days (UAS7) and Sabroe's grade,^{4,5} respectively. Eighteen of 55 patients were graded with severe, 21 with moderate, and 16 with slight CSU according to Sabroe's grade, whereas 36, 10, and 9 patients were categorized with severe, moderate, and slight CSU disease according to UAS7. None of the three galectins varied with the disease activity regardless of the grading methods (Figure 2). Seventeen of 37 (45.9%) patients were tested ASST-positive. Serum galectin-1, -3, and -9 were comparable between ASST-positive and -negative patients (28.02 ± 1.58 vs 31.55 ± 1.87 ; 8.44 ± 1.15 vs 6.88 ± 0.82 ; 10.86 ± 0.62 vs 10.21 ± 0.84 ng/mL, respectively) ($P = 0.17$, $P = 0.26$, $P = 0.23$, respectively) (Figure 2). In addition, ASST had no association with gender, age, disease duration, CU-Q2oL, and CSU activity (data not shown). Together, these results suggested that galectin-1, -3, and -9, but not galectin-10 were

bystander molecules for CSU. However, the role of each galectin in CSU development requires further researches.

Elevated serum levels of galectin-1, -3, and -9 were not only found in allergic disorders but also in cancer and autoimmune diseases. For instance, our previous researches and other investigators reported that serum galectin-3 and -9 were significantly higher in autoimmune diseases including systemic lupus erythematosus, dermatomyositis, and systemic sclerosis and in immune-related diseases such as psoriasis and atopic dermatitis.⁶⁻⁸ However, the association of each galectin with disease characteristics not only varied with disease itself but also with studies. Of interest, serum galectin-3 was observed elevated and associated with IgE anti-galectin-3 antibodies in IgG4-related diseases; however, the antibodies were unlikely involved in the pathogenesis of this disease.⁹ Since allergen-specific IgE is critical for CSU, it would be of interest to characterize IgE anti-galectin-3 antibodies in this disease. Alternatively, galectin-1 and -3 plays an

anti-inflammatory and a pro-inflammatory role, respectively, whereas galectin-9 exerts both sides mainly by interacting with eosinophils in the Th2-mediated eosinophilic allergic disorders.² Eosinophils are the most abundant cells in skin biopsy, however remain nearly unknown in the pathogenesis of CSU. Our findings open an avenue to evaluate the importance of the balance of the galectins and the contribution of eosinophils to CSU development regarding the galectins.

The limitation of this study is the small size of patients group. Rather than an elevation in all CSU patients, there were small elevations in a small subgroup of CSU compared to healthy controls, most CSU patients were comparable to healthy controls. Apart from the limitations, our current findings suggest that serum galectin-1, -3, and -9 are bystander molecules for CSU.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

Keywords

chronic spontaneous urticaria, galectin-1, galectin-3, galectin-9

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Endothelial B2-receptor overexpression as an alternative animal model for hereditary angioedema

To the Editor

Hereditary angioedema (HAE) is a rare genetic disease mediated by bradykinin (BK) in most of the cases.¹ The existing HAE mouse models present only increased vascular permeability, but no spontaneous swelling episodes comparable to patients' characteristics.^{2,3} Therefore, we aimed to develop and characterize a transgenic

rat overexpressing the BK B2-receptor (B2R) in endothelial cells (B2Rover) as an alternative model for HAE and BK-mediated angioedema studies.

Endothelium-specific overexpression of B2R was achieved using the vascular endothelial cadherin promoter (VECDH—based on the