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Elevated galectin-10 expression of IL-22 producing T cells in atopic dermatitis patients

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To the editor

Atopic dermatitis (AD) is a highly pruritic, chronic, relapsing skin disease characterized by typically distributed skin lesions. Recently, subsets of T cells including T_H1 , T_H2 , T_H17 , T_H22 , and regulatory T (Treg) cells have been implicated as contributing factors to the immune dysregulation of AD. In this study, by using different quantitative proteomics, we identified differentially expressed proteins in $CD3^+$ T cells from AD patients and healthy controls (HCs), and found galectin-10 was elevated in IL-22 producing $CD4^+$ T cells in AD patients.

This study was approved by the institutional review board and written informed patient consent was obtained from each patient. A total of 45 AD patients and 20 HCs were enrolled in this study. Diagnosis of AD was made according to the criteria of Hanifin and Rajka (Hanifin and Rajka, 1980). Patients were classified based on their allergic history, skin prick test, CAP test and serum IgE (Table E1). Detailed methods were described in the supplementary materials.

In order to study the differential protein expression from $CD3^+$ T cells in HCs and AD patients, we performed LC/MS/MS analysis. Quantitative analysis based on the normalized spectral index (SI_N) of identified proteins (Griffin *et al.*, 2010) resulted in Table E2. Among total 636 proteins identified, 75 and 81 showed higher expressions of five times more in $CD3^+$ T cells in AD patients and in HCs, respectively. Extracted ion chromatograms of peptides from Galectin-10 and S100A9 increased in AD patients (Fig. 1a) were presented with their MS/MS spectra (Fig. E1), confirmed by western blot analysis to be highly expressed in AD patients (Fig. 1b). Confocal laser microscopy, also showed higher intensities of galectin-10 staining in $CD3^+$ T cells from AD patients compared with HCs (Fig. 1c).

As the upregulation of galectin-10 was mainly attributed to $CD4^+$ T cells (Fig. 2a), we performed flow cytometry using $CD3^+CD4^+$ T cells in the following analysis. Galectin-10 was significantly upregulated in IL-22 expressing $CD3^+CD4^+$ T cells from AD patients compared to HCs (Fig. 2b). There was no significant difference of galectin-10 expression in $CD3^+CD4^+$ T cells producing other cytokines

such as IL-17, IFN- γ , IL-4, or IL-10 (Fig. 2b and Fig. E2). In the immunohistochemical staining, galectin-10 was highly expressed in CD3⁺ T cells in AD skin, whereas its expression was nearly undetectable in the normal skin (Fig. 2c). Interestingly, significant acanthosis was also observed adjacent to the dermal area where galectin-10⁺CD3⁺ T cells were present, implying that epidermal hyperplasia in AD may result from galectin-10⁺ T cells producing IL-22.

Next, we performed correlation study between serum galectin-10 and various clinical and laboratory variables (Noh *et al.*, 2014). The serum galectin-10 were significantly higher in AD patients compared to HCs (Fig. 2d, and table E3) showing a positive correlation between galectin-10 levels and EASI score ($r=.409$, $P=.018$), and IGA ($r=.553$, $P=.001$). No significant correlation was observed between serum galectin-10 and cell counts, serum IgE, eosinophil cationic protein (ECP), vitamin D or eczema area. There was no significant difference of galectin-10 between extrinsic AD and intrinsic AD (data not shown).

In this study, we firstly performed large-scale analyses such as the normalized spectral index (SI_N) to identify differentially expressed proteins. As a local approach, we used extracted ion chromatography which determines abundance of specific target peptide converted from mass spectrometry-derived peptide data (Zybailov *et al.*, 2005) to minimize errors caused by large-scale proteomics. Among S100A9 and galectin-10, galectin-10 was chosen for further analyses, as galectin-10 was recently shown to be associated with allergic diseases such as asthma.

Galectins, a family of animal lectins that bind N-acetyllactosamine-containing glycans deciphering the biological information of glycosylation signature, are known to have multiple roles in innate and adaptive immunity. For example, deletion of galectin-1 enhanced antigen-specific T_H1 and T_H17 responses and autoimmune inflammation (Blois *et al.*, 2007; Toscano *et al.*, 2007) whereas deletion of galectin-3 decreased pathogenic T_H1 and T_H17 responses reducing neuroinflammation (Jiang *et al.*, 2009).

Among galectin family members, galectin-10 was initially described exclusively in eosinophils and

basophils (Ackerman *et al.*, 1993; Ackerman *et al.*, 1982; Archer and Blackwood, 1965). However, CRTH2⁺CD4⁺ T_H2 memory cells was reported to undergo further T_H2 polarization and upregulated galectin-10, when activated by thymic stromal lymphopoietin (TSLP)-activated dendritic cells (Wang *et al.*, 2006). Recently, mRNA of galectin-10 was reported to be overexpressed in the peripheral blood of patients with aspirin-induced asthma compared with aspirin-tolerant asthma (Devouassoux *et al.*, 2008). Galectin-10 was reported to be expressed by human CD25⁺ Treg cells and essential for their suppressive function (Kubach *et al.*, 2007). In our study, galectin-10 was significantly increased in IL-22 producing T cells from peripheral blood and lesional skin of AD patients compared with HCs. As we performed intracellular staining for flow cytometry without any activation, AD patients present circulating activated T cells producing IL-22. As IL-22 causes epidermal hyperplasia, galectin-10 can be closely associated with epidermal acanthosis which is a hallmark of chronic AD lesion (Fig. 2c). CD3⁺Galectin-10⁺ T cells from HCs showed expression pattern of central memory T (T_{CM}, CD45RO⁺CCR7⁺CD62L⁺), not the patterns of naive or effector memory T (T_{EM}) cells (Fig. E3). As T_{CM} have skin homing properties, further experiments are required to study galectin-10⁺CD3⁺ T cells in lesional skin (Clark *et al.*, 2012).

The observations that serum level of galectin-10 was increased in AD patients than HCs and that these levels correlate positively with AD severity offer the possibility of galectin-10 as a disease biomarker. The origin of serum galectin-10 was not determined in this study. The observation that there is no significant correlation between eosinophil, ECP, basophil, and lymphocyte count and serum galectin-10 level indicates that serum galectin-10 might be directly correlated with a certain subtype of T cells like T_H22 cells.

In summary, using proteomic analysis, we identified galectin-10 to be overexpressed in circulating CD3⁺ T cells from AD patients and in chronic AD skin compared with HCs. Increased expression of galectin-10 was also observed in IL-22 producing CD4⁺ T cells from AD patients. Serum levels of galectin-10 were higher in AD patients relative to HCs, and this level correlated positively with AD severity. Further study of galectin-10⁺ T cells in chronic AD is warranted to elucidate the clear role of

galectin-10 in the pathogenesis of AD, and large-scale studies could reveal the possibility of galectin-10 as a disease biomarker for AD.

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Conflict of interest: none declared

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Table E1. Clinical characteristics of study subjects

	Mean $\pm$ SD			<i>P</i> value
	AD (Extrinsic)	AD (Intrinsic)	Healthy controls	
Age, y	25.0 $\pm$ 5.0	29.3 $\pm$ 14.8	27.4 $\pm$ 2.7	.176
Gender; total (N)	39	6	20	
Male	21	3	10	
Female	18	3	10	
Eczema area and severity index	20.0 $\pm$ 9.0	17.2 $\pm$ 9.5	0	<.001
Serum IgE level (kU/L)	2146 $\pm$ 1724	33.9 $\pm$ 24.4	38.9 $\pm$ 24.4	<.001
Allergic rhinitis	77% (30/39)	0%	0%	
Allergic asthma	26% (10/39)	0%	0%	
Positive CAP or skin prick test	100% (39/39)	0%	0%	

Table E2. 636 proteins in AD vs. HC identified by normalized spectral index (SI_N) based label-free quantification. Among total 636 proteins identified, 75 (red) and 81 (blue) showed higher expressions of five times more in $CD3^+$ T cells in AD patients and in HCs, respectively. Galectin-10 and S100A9 were highlighted with yellow color.

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Table E3. Correlation analysis between serum galectin-10 and clinical variables

Variables	Correlation coefficient	P value
Severity of AD		
Eczema Area and Severity Index (EASI)*	.409	.018
Investigator's Global Assessment (IGA)**	.553	.001
Eczema Area		
Head & Neck	-.028	.882
Trunk	.177	.348
Upper limb	-.124	.514
Lower limb	-.072	.707
Total area	.000	.999
Cell counts (cells/mm ³)		
White Blood Cells	.005	.978
Eosinophil	.048	.805
Basophil	-.033	.865
Lymphocyte	.076	.697
Neutrophils	-.156	.386
Serum IgE (kU/L)	-.254	.176
Eosinophil cationic protein (ug/L)	-.078	.730
Serum Vitamin D (ng/mL)	.446	.096

* $P < 0.05$; ** $P < 0.01$, Spearman's Rho was used for analysis of IGA and eczema area while other variables were analyzed using Pearson correlation

FIGURE LEGENDS

Figure 1. Proteomic analysis and western blot analysis of CD3⁺ T cells from AD patients and HCs. **(a)** Extracted ion chromatograms representing that peptides from galectin-10 and S100A9 were increased in T cells of AD patients vs. HCs. **(b)** Western blot analysis of identified proteins in CD3⁺ T cells from AD patients and HCs. **(c)** Confocal laser microscopy of purified CD3⁺ T cells stained with anti-galectin-10 (green) and DAPI (blue). Data shown are representative of 3 independent experiments. Scale bar=10 μ m.

****** $P<0.01$

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Figure 2. Increased expression of galectin-10 in IL-22 producing T cells, lesional skin, and sera of AD patients. **(a)** Galectin-10 expression in CD3⁺CD4⁺ T cells and CD3⁺CD8⁺ T cells, the increase of galectin-10 in AD was mainly attributed to CD3⁺CD4⁺ T cells. **(b)** The expression of galectin-10 was significantly increased in IL-22 producing CD3⁺CD4⁺ T cells from AD patients compared with HCs while its expression was not significant in IL-17 producing CD3⁺CD4⁺ T cells. Data shown are representative of 3 independent experiments. **(c)** Galectin-10 expression in the skin of HCs and AD patients. The superficial perivascular infiltrated CD3⁺ T cells in AD were strongly stained with galectin-10 marked by arrow with straight line. Arrows with dashed line indicate epidermal acanthosis adjacent to infiltrated CD3⁺ galectin-10⁺ T cells. Scale bars denotes for 100 μ m (200X) and 50 μ m (400X), respectively. **(d)** Serum levels of galectin-10 were increased in the moderate and severe AD patients. * P <0.05; ** P <0.01.

Figure E1. MS/MS spectra of peptide, YQVMVNGQSSYTFDHR from Galactin-10 and NIETIINTFHQYSVK from S100-A9

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Figure E2. IFN- γ , IL-4, and IL-10 expression in the galectin-10 expressing CD3⁺CD4⁺ T cells. There were no significant differences of cytokine expression as IFN- γ , IL-4, or IL-10 expression in galectin-10⁺CD3⁺CD4⁺ T cells from AD patients vs. HCs. Data shown are representative of 3 independent experiments.

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Figure E3. Flow cytometry analysis of CD3⁺galectin-10⁺ T cells. CD3⁺Galectin-10⁺ T cells from HCs showed expression pattern of central memory T (T_{CM}, CD45RO⁺CCR7⁺CD62L⁺), not the patterns of naive or effector memory T (T_{EM}, CD45RO⁺CCR7⁻CD62L⁻) cells.

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Figure 2

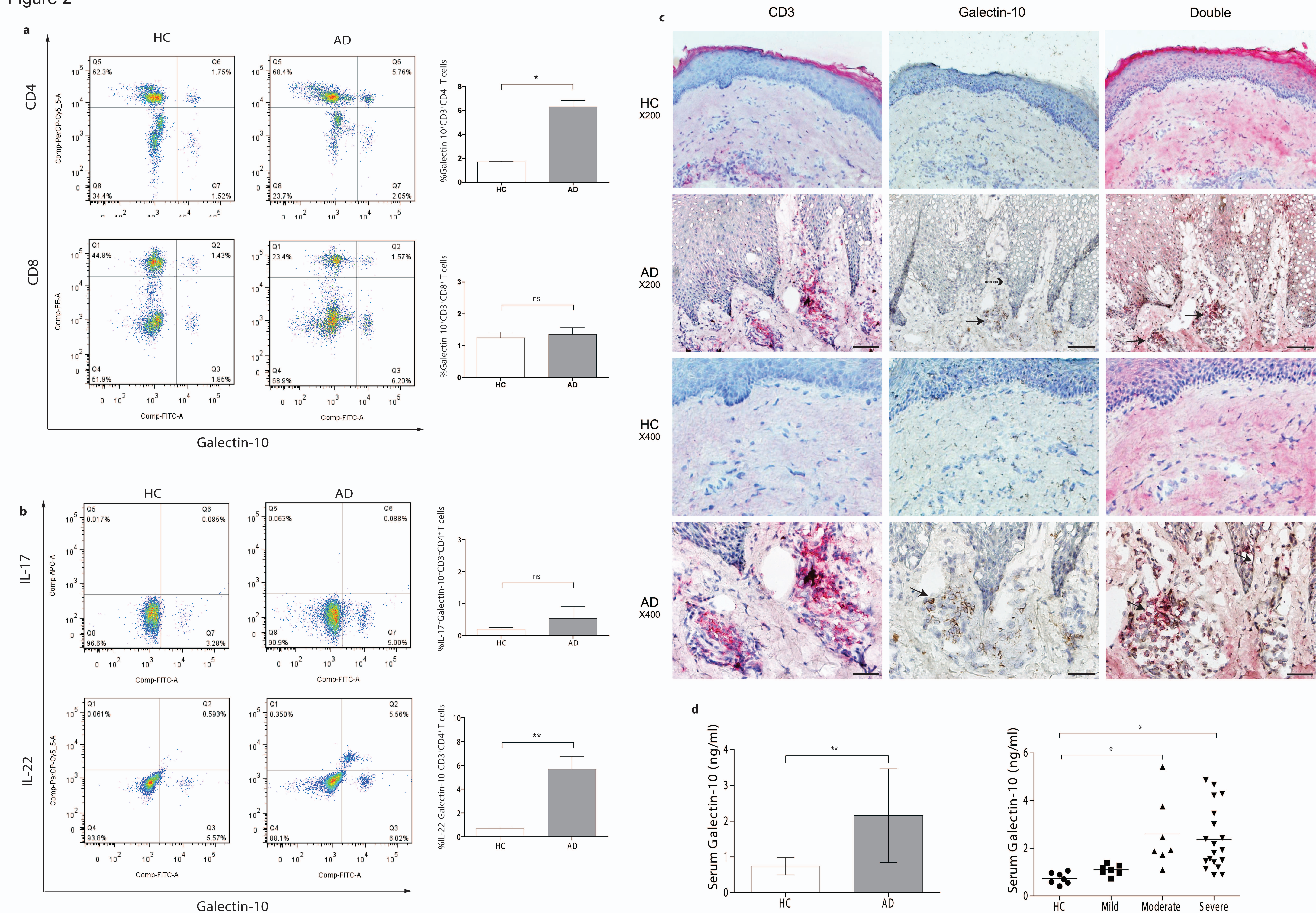


Figure 1

