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LETTER TO THE EDITOR

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Increased expression of IL-24 in chronic spontaneous urticaria

To the Editor,

In chronic spontaneous urticaria (CSU), mast cell degranulation has a central role in the occurrence of wheals; however, the pathogenesis of CSU also involves other complex immune mechanisms such as autoimmunity, inflammation, and coagulation. There are several arguments to classify CSU as an autoimmune disease, at least in some patients. Indeed, patients with CSU were found to more frequently have another autoimmune disease than the general population.[1] Moreover, subsequent work demonstrated that some autoantibodies such as IgG autoantibodies directed against the α subunit of the mast cell high-affinity IgE receptor (FcεRIα)[2] are more frequently detected in the CSU population than in healthy subjects and cause mast cell activation/degranulation. The presence of IgE autoantibodies directed against anti-thyroid peroxidase[3] in some CSU patients moreover suggests an "autoallergic" basis of disease in this subset. "Autoallergy," classified as type 1 autoimmunity, is thought to involve the local production of self-allergens against which specific IgE are directed, thus inducing mast activation/degranulation. More recently, Schmetzer et al detected IgE against IL-24 in the sera of CSU patients directly correlated with CSU activity.[4] They also showed that, in vitro, exogenous IL-24 induced histamine release from human mast cells sensitized with IgE against IL-24 from CSU patients, but not with IgE from healthy controls.[4] To our knowledge, expression of IL-24 in CSU has never been reported.

IL-24 is generally regarded as a cytokine mainly involved in skin inflammation, rather than as an auto-antigen. It has been implicated in inflammatory skin diseases such as psoriasis and atopic dermatitis.[5] Indeed, IL-24 is highly expressed in skin psoriatic lesions.[5] In a model of reconstituted human epidermis, IL-24 treatment led to epidermis hyperplasia and increased expression of inflammatory mediators of psoriasis.[6] IL-24 is also implicated in autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis.[7]

In light of the identification of IL24-directed IgE in CSU, we wondered whether local IL-24 is produced during the urticarial reaction. We evaluated the expression of *IL24* mRNA in both skin biopsies and cultured-PBMCs of CSU patients by quantitative reverse transcriptase-polymerase chain reaction. We also measured IL-24 in sera and supernatants of cultured-PBMCs by enzyme-linked immunosorbent assay (See Supporting information Material and methods section). In addition, we evaluated for possible correlations with response of autologous serum skin test (ASST), autoimmune status of CSU patients (based on history of autoimmune disease and/or presence of autoimmune antibodies), disease severity, and some inflammatory biomarkers. Sixty-nine CSU patients and 23 healthy controls (HCs) were studied. Patient characteristics are summarized in Table S1.

IL24 mRNA expression is increased in spontaneous wheals as compared to nonlesional skin from CSU patients ($P < 0.0002$), or skin from healthy controls ($P = 0.0072$). Conversely, *IL24* mRNA was not detected in wheals induced by ASST (Figure 1A). Previous studies have detected cytokine expression in ASST biopsies, suggesting this test may not be a too "early" model for cytokine expression in general.[8] Instead, these data suggest that IL-24 appears later, when inflammatory process has already been initiated. The cellular source of IL-24 may be keratinocytes responding to inflammatory process, since some cytokines (IL-1β, TNFα) can up-regulate expression of IL-24 by keratinocytes.[5,9] IL-24 may also be produced by infiltrating cells. In the same line, using immunohistochemistry and flow cytometry with homemade and commercial antibodies, we were unable to characterize IL-24-secreting cells in spontaneous wheals biopsies (data not shown).

IL24 mRNA was also increased in cultured-PBMCs from 38 of 64 CSU patients, as compared to 4 of 23 HCs ($P = 0.0040$) (Figure 1B). Stimulation of PBMCs by CD3/CD28/IL-2 increased *IL24* expression in the majority of CSU patients ($P < 0.0001$) (not shown). IL-24 protein was also higher in supernatants from CSU cultured-PBMCs as compared to HC ($P = 0.0112$) (Figure 1C). As expected, expression of IL-24 mRNA and protein was positively correlated (Figure 1D). In contrast, IL-24 was not detected in sera suggesting that a population of PBMCs, perhaps of skin-homing cells, is more prone to produce IL-24 in some conditions. As only part of the CSU patient PBMCs (only 60%) expressed IL-24, we asked whether its expression correlates with ASST positivity, disease severity, or autoimmune status.

IL-24 expression in cultured-PBMCs of CSU patients did not correlate with autoimmune status or ASST positivity (Figure 2A,B), but did correlate significantly with disease severity (Figure 2C). In addition, expression of *IL24* correlated with an inflammatory marker: *IL6*, the sole cytokine described as a biomarker of CSU activity (Figure 2D)[10*] In line with this, neutrophil and basophil blood counts correlated positively with *IL24* expression by PBMCs stimulated with CD3/CD28/IL-2. In addition, neutrophil infiltrate in the skin also correlated with local *IL24* expression (Figure S1). A positive correlation was also observed between monocyte blood count and IL-24 protein level in supernatants. In contrast, IL-24 mRNA and protein were not correlated with CRP serum level, total IgE serum level, or D-dimer plasma level (Table S2).

In all, these results demonstrate that IL-24 expression correlates with inflammatory processes rather than with autoimmune status.

*Additional references 10-15 are provided in Supporting information.

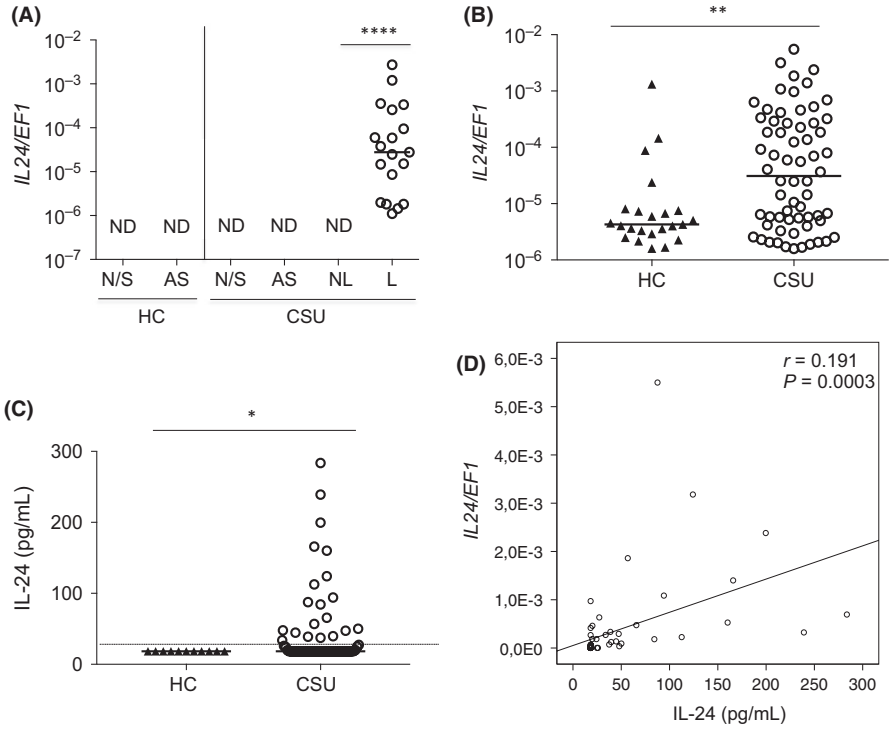

FIGURE 1 A, *IL24* mRNA expression in skin biopsies of HCs (n = 9) and CSU patients (n = 10-19): after injection of normal saline solution (N/S) or autologous serum (AS), nonlesional skin (NL) and lesional (L). IL-24 mRNA (B) and protein (C) expression by cultured-PBMCs from 23 HCs and 64 CSU patients. Correlation of *IL-24* mRNA and IL-24 protein (D) in CSU patients. Each symbol represents one patient. Solid horizontal line represents group medians. *P*-value: **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, *****P* ≤ 0.0001, blank *P* > 0.05. Cutoff for detection of *IL24* mRNA was 10 copies and for IL24 protein was 18 pg/mL (dotted lines)

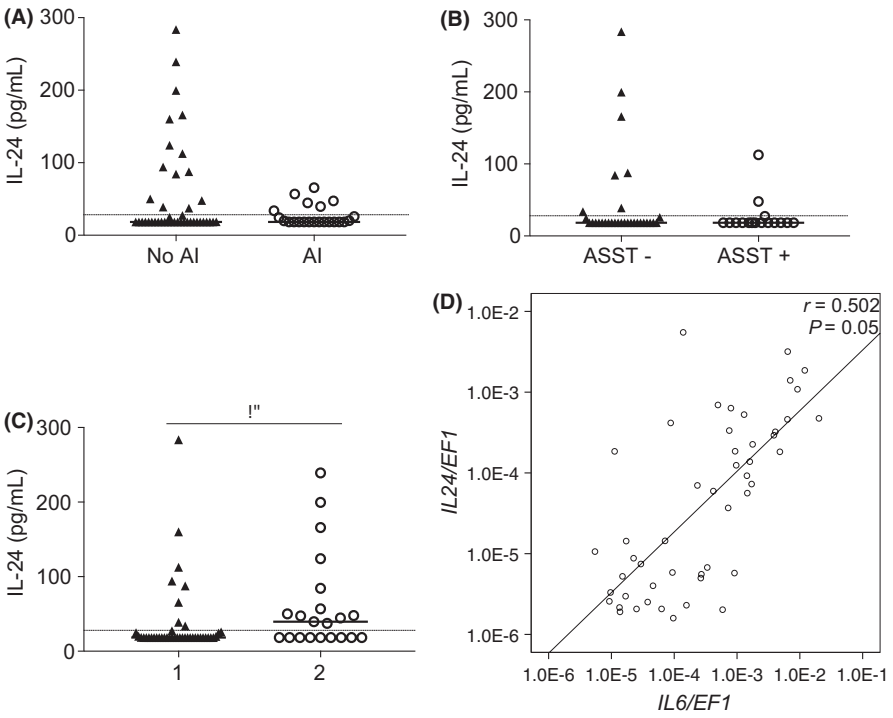

FIGURE 2 Expression of IL-24 protein level in supernatants of cultured-PBMCs from CSU patients: A, according autoimmune status (no A-I n = 42, A-I n = 22). B, according ASST results (ASST − (n = 31), ASST + (n = 16)). C, according disease severity (1 = mild to moderate (n = 43); 2 = moderately severe to severe (n = 21)). D, Correlation between *IL24* and *IL6* mRNA expression in cultured-PBMCs from CSU patients (n = 49). Each symbol represents one patient. Solid horizontal line represents group medians. *P*-value: **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, *****P* ≤ 0.0001, blank *P* > 0.05. Cutoff for detection of IL-24 protein and *IL6*, *IL24* mRNA were, respectively, 18 pg/mL (dotted lines) and 10 copies

We could not, however, exclude that IL-24 expression may also correlate with an autoallergic phenotype, consistent with the presence of IgE against IL-24 in some CSU patients. Anti-IL-24 IgE may cause mast cell degranulation either directly, by IL-24-IgE binding of FcεRIα, or indirectly, by inducing mediators and cytokines that in turn cause histamine release. Indeed, it has been demonstrated that certain highly cytokinergic IgEs can induce production of cytokines and have anti-apoptotic effects on mast cells.[11,12]*

In conclusion, *IL24* gene and protein expression are increased in PBMCs of a subset of CSU patients. Positive correlation of *IL24* expression with both *IL6* expression and disease severity suggests that it participates in the disease. In CSU patients, *IL24* is detected in spontaneous urticarial wheals, but not those induced by autologous serum injection (ASST). Thus, local IL-24 production does not appear to be necessary for wheal generation in CSU. ASST is not, however, a perfect model of CSU urticaria, given its positivity in only ≈50% of

patients.[13*] Future studies will be required to determine the source of IL-24, that is, keratinocytes or infiltrating leukocytes, and whether blocking of IL-24 action in CSU may have any beneficial effect.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

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