

## Letter to the Editor

**Increased serum CRH levels with decreased skin *CRHR-1* gene expression in psoriasis and atopic dermatitis**

To the Editor:

Autoimmune skin diseases, such as psoriasis and atopic dermatitis (AD), are chronic inflammatory skin disorders mediated primarily by T cells,<sup>1</sup> but mast cells are also implicated.<sup>2</sup> Symptoms of both psoriasis and AD worsen with stress.<sup>3</sup> Acute stress leads to increased vascular permeability and inflammation of skin, through mast-cell activation by corticotropin-releasing hormone (CRH) both in rodents<sup>4</sup> and in humans.<sup>5</sup> CRH and CRH receptor 1 (CRHR-1) are both expressed in human skin,<sup>6</sup> leading to the hypothesis that CRH may be involved in the pathophysiology of skin diseases.<sup>7</sup>

Full-length 3-mm<sup>3</sup> punch skin biopsies were collected for diagnostic purposes from subjects with psoriasis and AD who had not received any medication for 15 days prior to biopsy and were free from any systemic allergic or inflammatory disease. Serum was also collected from patients and controls. The Medical Ethics Committee approved this protocol. The Psoriasis Area and Severity Index (PASI) score was recorded for some of the patients with psoriasis; no severity index score was obtained from the patients with AD. Also, some patients with psoriasis and AD filled the State-Trait Anxiety Inventory (STAI), which has been validated for the Greek population,<sup>8</sup> to investigate the extent of stress. The STAI measures separate constructs of psychosocial stress and clearly differentiates the temporary condition of "state anxiety" (STATE now) from the more general and long-standing quality of "trait anxiety" (STATE trait).

Serum level and skin gene expression results from patients were compared with those of controls by using the Mann-Whitney nonparametric *U* test. There was no statistical difference in the mean age between controls and patients. All subject characteristics and the summary of the results are presented in Table I.

For CRH serum measurements, the mean age for patients with psoriasis (*n* = 48; 23 women and 25 men, 27 of whom had a PASI score of 10-50) was  $46 \pm 17$  years, while the mean age for controls (*n* = 19; 10 women and 9 men) was  $47 \pm 16$  years. Serum CRH levels were higher (*P* = .0001) in patients with psoriasis ( $22.5 \pm 13.7$  pg/mL) than in controls ( $9.7 \pm 4.2$  pg/mL) (Fig 1, A). When patients with psoriasis were separated into those with PASI scores of less than 10 and those with PASI scores of more than 10, there was a statistically significant increase (*P* = .0001) in patients with psoriasis with PASI scores of more than 10 (Fig 1, A) but not in those with PASI scores of less than 10 (data not shown). For CRH serum measurements, the mean age for patients with AD (*n* = 15; 9 women and 6 men) was  $36 \pm 18$  years, while the mean age for controls (*n* = 19; 10 women and 9 men) was  $47 \pm 16$  years. Serum CRH levels were higher (*P* = .0001) in patients with AD ( $31.0 \pm 19.5$  pg/mL) than in controls ( $9.7 \pm 4.2$  pg/mL) (Fig 1, A).

For cytokine serum measurements, the mean age for patients with psoriasis (*n* = 56; 26 women and 30 men, 31 of whom had PASI scores of 10-50) was  $40 \pm 14$  years, while the mean age for controls (*n* = 33; 14 women and 19 men) was  $48 \pm 18$  years. Serum cytokines and vascular endothelial growth factor (VEGF)

were evaluated by using Milliplex microbead technology, and the measurements were performed by Millipore (St Charles, Mich). Serum levels of IL-6, IL-9, IL-33, TNF $\beta$ , and thymic stromal lymphopoietin in patients with psoriasis were undetectable, except for VEGF and IL-8. Serum VEGF levels were not different in patients with psoriasis ( $411.9 \pm 280$  pg/mL) as compared with those in controls ( $257 \pm 123.8$  pg/mL) (Fig 1, B). However, serum VEGF levels in patients with psoriasis with PASI scores of more than 10 were significantly increased (*P* = .0286) (Fig 1, B), while in patients with psoriasis with PASI scores of less than 10 were not (data not shown). There was no difference between serum IL-8 levels in patients with psoriasis ( $25.7 \pm 27.5$  pg/mL) and those in controls ( $31.3 \pm 31.2$  pg/mL).

For serum measurements, the mean age for patients with AD (*n* = 18; 12 women and 6 men) was  $38 \pm 20$  years, while the mean age for controls (*n* = 33; 14 women and 19 men) was  $48 \pm 18$  years. Serum levels of IL-6, IL-9, IL-33, TNF $\beta$ , and thymic stromal lymphopoietin were undetectable, except for VEGF and IL-8. Serum VEGF levels were not different in patients with AD ( $291.4 \pm 280.1$  pg/mL) as compared with those in controls ( $257.2 \pm 123.8$  pg/mL) (Fig 1, B). Serum IL-8 levels were lower (*P* = .0171) in patients with AD ( $19.7 \pm 17.2$  pg/mL) than in controls ( $31.3 \pm 32.2$  pg/mL) (data not shown). There was a reasonable correlation between serum CRH levels and STAI (STATE now) scores (Pearson *r* = 0.55; *P* = .04; *n* = 14) for patients with psoriasis with PASI scores of more than 10, but there was no correlation with STAI (STATE trait). There was no correlation between STAI scores and CRH serum measurements or VEGF gene expression for the patients with AD (Fig 1, C).

For gene expression assays, the mean age for patients with psoriasis (*n* = 40; 19 women and 21 men) was  $46 \pm 17$  years, while the mean age for controls (*n* = 30; 22 women and 8 men) was  $43 \pm 15$  years. Skin *CRHR-1* gene expression was lower (*P* < .0001) in affected skin samples from patients with psoriasis ( $0.1 \pm .01$ ) than in those from controls ( $1 \pm 0.7$ ) (Fig 2, A). *CRHR-1* expression was documented in lesional psoriasis skin by immunohistochemistry and appeared to colocalize with mast cells (Fig 2, B). There was also statistically significant lower (*P* = .0001) *CRHR-1* gene expression in the samples obtained from affected versus unaffected psoriasis skin, while there was no statistically significant difference in *CRHR-1* gene expression between the control and the nonlesion samples (data not shown). Lesional skin *CRH* gene expression was undetectable in psoriasis (data not shown). For *CRHR-1* gene expression assays, the mean age for patients with AD (*n* = 16; 10 women and 6 men) was  $35 \pm 21$  years, while the mean age for controls (*n* = 30; 22 women and 8 men) was  $43 \pm 15$  years. Skin *CRHR-1* gene expression was lower (*P* = .0001) in affected samples from patients with AD ( $0.2 \pm 0.1$ ) than in those from controls ( $1 \pm 0.7$ ) (Fig 2, A).

Skin VEGF gene expression was lower (*P* = .0121) in affected samples from patients with psoriasis ( $0.6 \pm 0.4$ ) than in those from controls ( $1 \pm 0.7$ ) (Fig 2, C). There was statistically significant lower VEGF gene expression (*P* = .0009) among the samples obtained from affected and from unaffected psoriatic skin (data not shown). Skin VEGF gene expression was also lower (*P* = .0001) in affected samples from patients with AD ( $0.2 \pm 0.1$ ) than in those from controls ( $1.0 \pm 0.7$ ) (Fig 2, C).

**TABLE I.** Summary of subject characteristics and results

|           | Sex; no.; age (y)                        | Serum CRH                                 | Serum VEGF                               | Skin CRHR-1                              | Skin VEGF                                |
|-----------|------------------------------------------|-------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| AD        | F; n = 13; 41 ± 23<br>M; n = 7; 28 ± 9   | F; n = 8; 42 ± 23<br>M; n = 5; 28 ± 11    | F; n = 12; 43 ± 22<br>M; n = 6; 27 ± 9   | F; n = 10; 40 ± 24<br>M; n = 6; 27 ± 9   | F; n = 10; 40 ± 24<br>M; n = 6; 27 ± 9   |
|           | <b>Results</b>                           | 31 ± 19.5 pg/mL<br>( <i>P</i> < .0001)    | 291.4 ± 280.1 pg/mL                      | Lower than control ( <i>P</i> < .0001)   | Lower than control ( <i>P</i> < .0001)   |
| Psoriasis | F; n = 30; 40 ± 15<br>M; n = 36; 49 ± 18 | F; n = 23; 41 ± 14<br>M; n = 25; 42 ± 15  | F; n = 26; 40 ± 14<br>M; n = 30; 48 ± 19 | F; n = 19; 42 ± 16<br>M; n = 21; 48 ± 18 | F; n = 19; 42 ± 16<br>M; n = 21; 48 ± 18 |
|           | <b>Results</b>                           | 22.5 ± 13.7 pg/mL<br>( <i>P</i> < .0001)* | 411.9 ± 280 pg/mL†                       | Lower than control ( <i>P</i> < .0001)   | Lower than control ( <i>P</i> = .121)    |
| Control   | F; n = 22; 49 ± 16<br>M; n = 26; 54 ± 14 | F; n = 10; 10 ± 5<br>M; n = 9; 9 ± 3      | F; n = 14; 53 ± 16<br>M; n = 19; 49 ± 19 | F; n = 22; 52 ± 18<br>M; n = 8; 40 ± 16  | F; n = 22; 52 ± 18<br>M; n = 8; 40 ± 16  |
|           | <b>Results</b>                           | 9.7 ± 4.2 pg/mL                           | 257.2 ± 123.8 pg/mL                      |                                          |                                          |

Values represent mean ± SD.

\*For patients with psoriasis with PASI scores of more than 10, serum CRH levels were statistically significantly increased compared to control (27.8 ± 10.4; *P* = .0001).

†For patients with psoriasis with PASI scores of more than 10, serum VEGF levels were statistically significantly increased compared to control (369.8 ± 197.5; *P* = .0286).

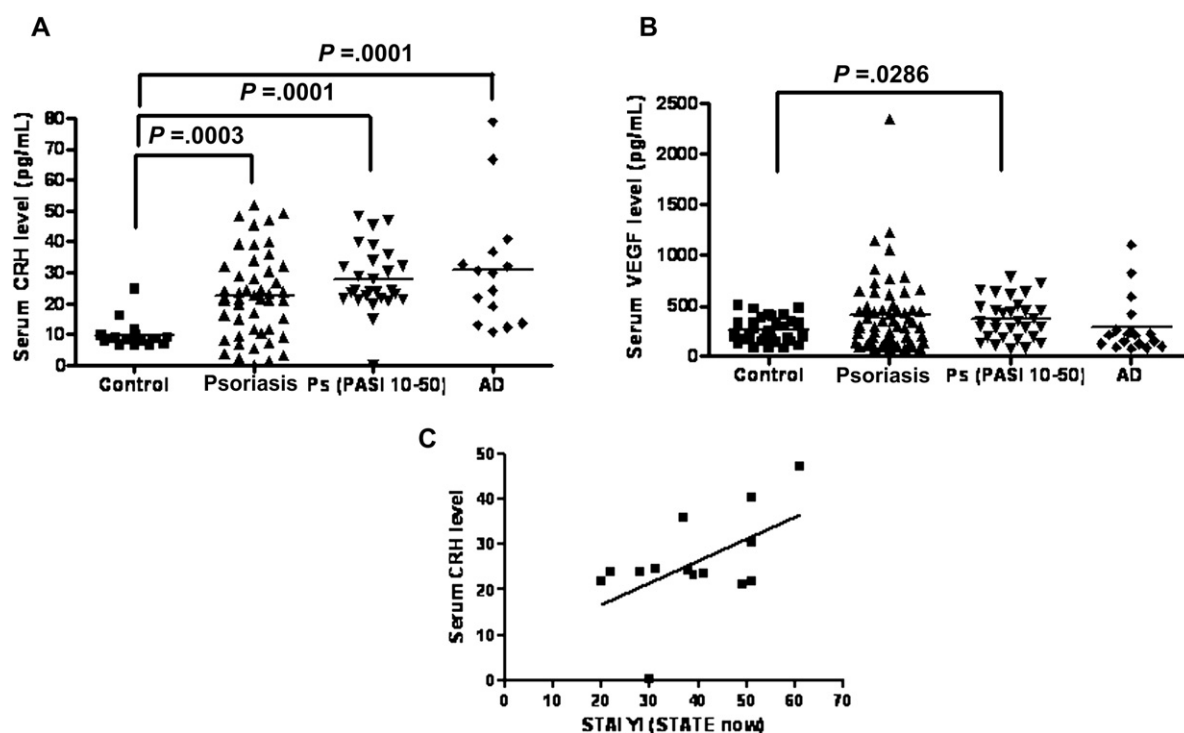


**FIG 1.** CRH and VEGF serum levels in patients with psoriasis and AD and controls (**A** and **B**), as well as correlation of serum CRH levels in patients with severe psoriasis with STAI (*STATE now*) (**C**).

There was a positive correlation between CRH serum levels and VEGF gene expression in patients with psoriasis with PASI scores of more than 10 (Pearson *r* = 0.44; *n* = 22; *P* = .04; Fig 2, D), which is known to be associated with increased skin vascularization. This finding implies that high serum CRH level correlates best only in patients with severe psoriasis.

Our findings suggest that overstimulation by the increased serum levels of CRH and VEGF, possibly in response to stress, leads to decreased gene expression of skin CRH and CRHR-1, as well as skin VEGF gene expression, respectively. The positive correlation between serum CRH levels and the STAI-now scores in patients with severe psoriasis supports this possibility. The lack of a similar correlation in patients with AD may be because we did

not have access to severity index scores for these patients, unlike in patients with psoriasis, in order to carry out a subgroup analysis.

The present findings suggest that high serum CRH level, possibly in response to stress, stimulates skin mast cells to release VEGF and contribute to skin inflammation evident in patients with severe psoriasis. Continuous or repeated stimulation may lead to decreased expression of skin CRHR-1, as we showed recently with cultured mast cells,<sup>9</sup> and hence decreased VEGF, as was evident in patients with mild disease. CRH and CRHR-1 may therefore participate in the pathogenesis of psoriasis and AD, especially when worsened with stress, through mast-cell activation to release VEGF. Mast-cell blockers may provide novel treatment approaches.

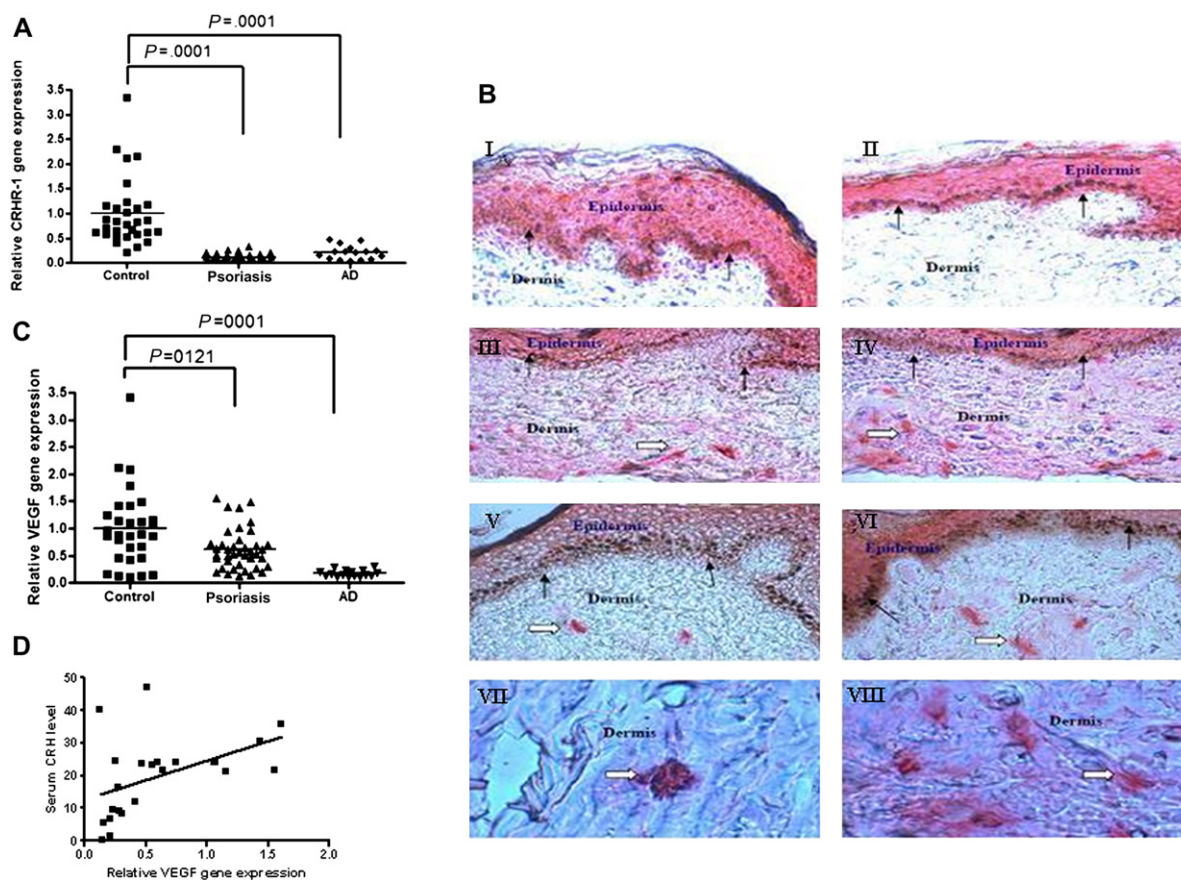


**FIG 2.** CRHR-1 and VEGF skin gene expression in patients with psoriasis and AD and controls (**A** and **C**) and CRHR-1 immunostaining in psoriasis skin (**B**). Cryostat sections were prepared and fixed with acetone for 3 minutes and incubated with normal blocking serum for 20 minutes. Sections were then incubated with primary antibodies (goat polyclonal antibody to CRHR-1; Abcam Inc, Cambridge, Mass, Cat no. ab59023) diluted to 1:100 for 30 minutes and then immunostained with Vectastain ABC AP kit (Vectastain Lab, Burlingame, Calif) and Vector Red Alkaline Phosphatase Substrate kit (Vector Lab) as per the kit's directions. Presence of red color indicates a positive reaction for CRHR-1. Epidermis and mast cells (I and II) in dermis (III-VI) and only mast cells (VII and VIII). Solid arrow shows epidermis, and hollow arrow shows mast cells. Correlation of serum CRH levels with VEGF gene expression in patients with severe psoriasis (**D**).

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