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# ELEVATION OF SERUM EGF AND IL-1RA IN ACTIVE PSORIASIS VULGARIS

**Running head:** Serum levels of cytokines in psoriasis

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**What is already known about this topic:**

Serum levels of cytokines and chemokines in psoriasis patients have been analysed with contradictory results.

**What does this study add:**

We identified markedly increased serum levels of EGF and IL-1Ra in psoriasis patients compared with matched controls. None of these cytokines were correlated to the severity of the disease (PASI) or decreased with phototherapy, suggesting that sources other than lesional skin contribute to the production of these cytokines which provide a potential mechanism linking psoriasis with its extracutaneous co-morbidities.

## Summary

*Background* The psoriatic plaques present a complex expression profile, including high levels of cytokines, chemokines and growth factors. Circulating cytokines has been suggested to reflect the activation status of the inflammatory process.

*Methods* Using a multiplex cytokine assay, 20 cytokines, chemokines and growth factors were analysed in 14 patients with psoriasis vulgaris at the start and during the course of the UVB treatment.

*Results* We identified increased serum levels of EGF (mean 323 vs 36.6 pg/ml,  $p=0.0001$ ), IL-1Ra (mean 39.1 vs.14.6 pg/ml,  $p=0.02$ ) and TNF $\alpha$  (mean 7.5 vs. 4.5 pg/ml,  $p=0.04$ ) at baseline in psoriasis patients compared with matched controls. None of these cytokines were correlated to the severity of the disease (PASI) or decreased with phototherapy, suggesting that sources other than lesional skin contribute to the production of these cytokines. Using cluster analysis, we observed coordinate upregulation of EGF, IL-6, MIP-1 $\beta$ , and VEGF.

*Conclusions* The sustained high expression of inflammatory circulating cytokines is a potential mechanism linking psoriasis with its extracutaneous co-morbidities.

## **Introduction**

Psoriasis is currently regarded as a chronic inflammatory reaction that has an autoimmune basis. The inflammatory infiltrate mostly consists of IFN $\gamma$ -producing (Th1) and IL-17/ IL-22-producing (Th17) helper T cells. A complex expression profile, including cytokines, chemokines and growth factors, has been found to be present within psoriatic plaques. Serum levels of cytokines and chemokines in psoriasis patients have been analysed with contradictory results, reviewed by Pietrzak<sup>1</sup>. Thus, the serum levels of cytokines such as IFN $\gamma$ , TNF $\alpha$ , IL-1, IL-6, IL-8 were reported to be unchanged or elevated in psoriasis patients compared with normals<sup>2-3</sup>. We have analysed 20 cytokines in serum from psoriatic patients and report elevated serum levels of EGF, IL-1Ra, TNF $\alpha$ , MIP-1 $\alpha$ , and IL-6.

## **Material and methods**

### **Patients and controls**

Sera from this cohort of patients had been previously analysed to study the role of psoriasis as a serologic biomarker of psoriasis<sup>4</sup>. Patients had psoriasis vulgaris for which no treatment had been given during the past four weeks. None of the patients had psoriatic arthritis. Fourteen patients were included. Individual baseline PASI scores ranged from 2.0-25.3 (mean 8.5). Patients received standard narrow-band UVB therapy and peripheral blood serum samples were collected at weeks 0, 2, 4 and 6 and at follow-up, 10 weeks. Eighty-two percent (9/11 evaluable patients) experienced an improvement in PASI score of at least 75% ( $p < 0.003$ ). Fourteen age-, sex- and ethnicity-matched healthy controls were enrolled in the study.

## **Multiplex cytokine assay**

Multiplex cytokine analysis was performed using xMAP technology (Luminex Corporation, Austin, TX). The Milliplex MAP multiplex assay was conducted in a 96-well microplate format according to the manufacturer's recommendations (Millipore, Billerica, MA). The cytokines analysed are shown in Table 1. Briefly, 60 µl of each of the bead solutions were pipetted into a mixing vial and brought up to 3 ml with bead diluent. Internal controls and standards ranging from 0 to 10000 pg/ml for each cytokine were included with every assay. After the addition of sera and beads, the plate was incubated overnight at 4°C. Detection antibodies and streptavidin-phycoerythrin were sequentially added at room temperature for 30 min and the plate was analysed on a Luminex 200 machine.

## **Statistical analysis**

Correlation analysis was assessed using Spearman's rank correlation test (r). The statistical significance of the differences between patients and normal subjects in the Luminex assay was analysed using a paired Wilcoxon's signed-rank test. Heat maps were generated using MultiExperiment Viewer<sup>5</sup>.

## **Results**

### **Increased serum levels of IL-1Ra, EGF and TNFα at baseline in psoriasis patients compared with normals**

The serum levels of 20 cytokines and chemokines were analysed at baseline prior to narrow-band UVB treatment in 14 psoriasis patients and 14 age-, and sex- matched controls. The level of TNFα (7.46 vs 4.53 pg/ml, p=0,0419) was only slightly increased in psoriasis patients

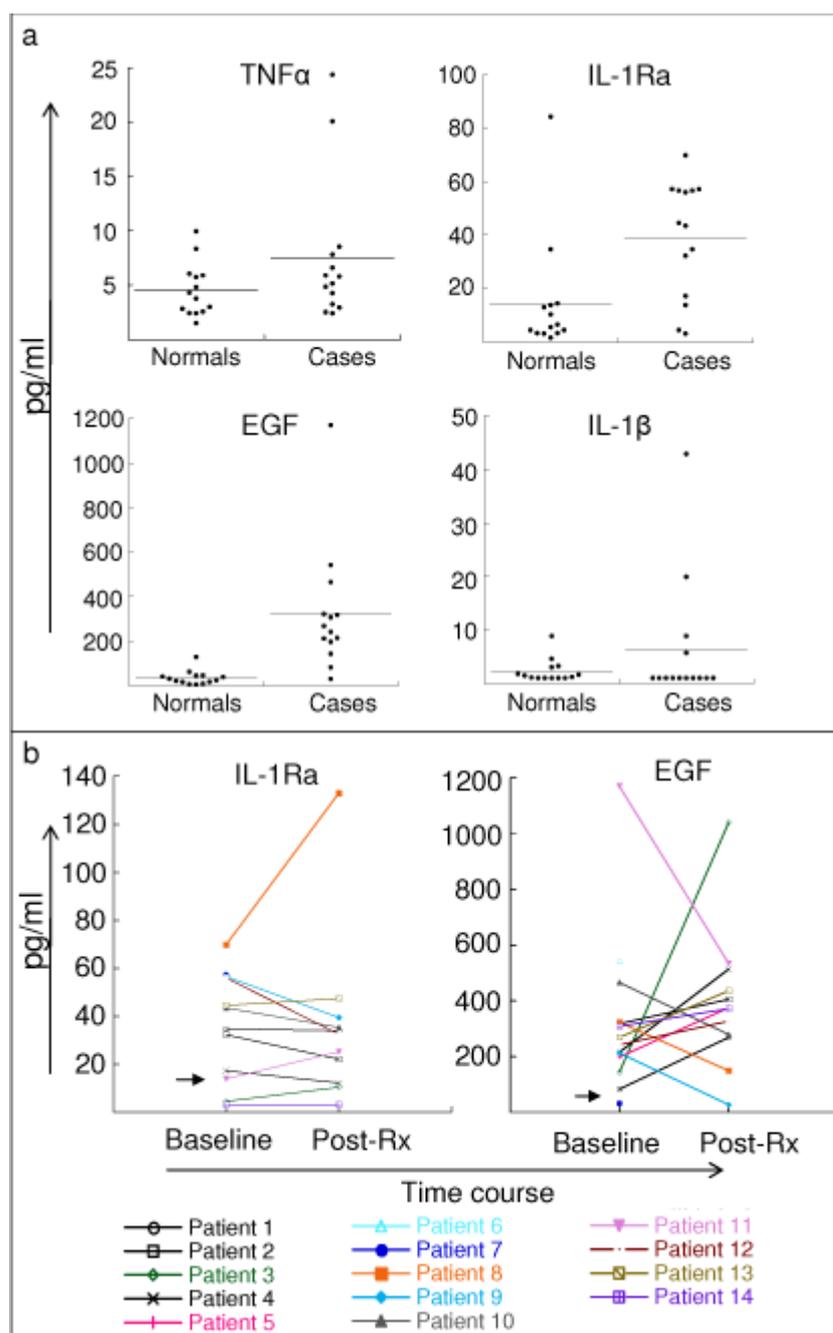
and at the lower detection limit of the assay (Fig. 1a). IL-1Ra, an inhibitory marker of IL-1 activation, was increased in psoriasis patients (mean 39.1 vs.14.6 pg/ml,  $p=0.0175$ ). In contrast, no change was seen in IL-1 $\beta$  levels. Most strikingly, EGF was almost 10 fold increased in patients compared with normals (mean 323 vs 36.6 pg/ml,  $p=0.0001$ ). ROC curves of each of the three cytokines were performed, with the area under the curve (AUC) for EGF (0.959), IL-1Ra (0.804), and TNF $\alpha$  (0.643) (data not shown).

### **Correlation of serum cytokine level and disease severity**

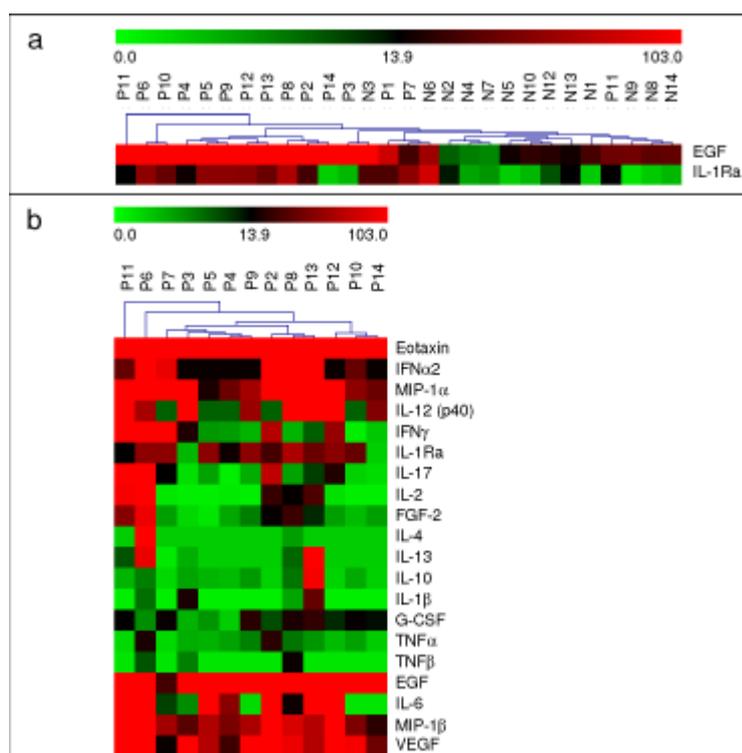
We divided the patients into two severity groups (PASI < 7; PASI > 7). IL-1Ra and EGF levels were significantly elevated in psoriasis patients compared with normals in both severity groups (IL-1Ra: normal vs PASI < 7,  $p=0,02243$ ; normal vs PASI > 7,  $p=0,03155$  and EGF: normal vs PASI < 7,  $p=0,00052$ ; normal vs PASI > 7,  $p=0,00003$ ). IL-6 was significantly increased in mild psoriasis (normal vs PASI < 7,  $p=0,024$ ), whereas MIP-1 $\alpha$  increased with disease severity (normal vs PASI > 7,  $p=0,027$ ). Interestingly, the cytokines that were upregulated at baseline showed sustained elevated expression levels throughout the phototherapy (Fig. 1b).

### **Hierarchical clustering of cytokine expression**

We performed unsupervised hierarchical cluster analysis to identify coordinate regulation of cytokines. Heat map analysis of EGF and IL-1Ra between cases (P) and normals (N) are shown in figure 2a. The heat map in figure 2b shows the coordinate levels of EGF, IL-6, MIP-1 $\beta$ , and VEGF. Of the cytokines elevated in psoriasis patients, only IL-6 and EGF levels were correlated with each other.



**Fig. 1 Elevation of serum levels of TNF $\alpha$ , IL-1Ra, and EGF in patients with psoriasis were not affected by narrow-band UVB therapy.** **a.** Of twenty different cytokines, the mean values for TNF $\alpha$  (SD = 2.44 in normals and 6.59 in cases), IL-1Ra (SD = 21.0 in normals and 22.1 in cases), and EGF (SD = 31.8 in normals and 278.7 in cases), were significantly higher in psoriasis than in the normal group (paired Wilcoxon's t-test,  $p < 0.05$ ). For IL-1 $\beta$ , SD = 2.2 in normals and 11.5 in cases. **b.** Patients received narrow-band UVB therapy and peripheral blood serum samples were collected prior to therapy (baseline) and after 6 weeks of therapy (post-Rx). No consistent change was observed after UVB therapy.



**Fig. 2 Hierarchical cluster analysis of cytokines in the sera of normal and psoriasis patients. a,** The serum levels of EGF and IL-1Ra of normal (N) and psoriasis patients (P) are compared using unsupervised cluster analysis. The image demonstrates the serum level of the cytokines and is represented by the intensity of green color (low) and red color (high expression). Each column represents a patient and each row represents a cytokine. **b,** When analysing only the patients, coordinate upregulation of EGF, IL-6, MIB-1 $\beta$ , and VEGF is shown (bottom).

### Comparison of data from a study of psoriatic arthritis

We directly compared our results with a recent study from PsA using the same xMAP technology<sup>6</sup>. The study population consisted of 43 Norwegian patients with PsA of polyarticular type. EGF was only slightly upregulated in PsA. There was a limited change in the expression of TNF $\alpha$  in both conditions. Moreover, in PsA, there was an upregulation of VEGF, IL-10, IL-13, IFN $\alpha$ , FGF, MIP-1 $\beta$  and eotaxin<sup>6</sup> that we did not observe for psoriasis in this cohort. These results suggest that there may be differential systemic cytokine dysregulation between patients with psoriasis and psoriatic arthritis.

## Discussion

In psoriasis, the cutaneous overexpression of various pro-inflammatory cytokines, such as interleukins has been demonstrated. These cytokines influence the cellular composition of the inflammatory infiltrate within the psoriatic plaques, as well as mediating the hyperproliferation of keratinocytes<sup>7</sup>. In addition, it has been suggested that cytokines produced in psoriatic skin lesions also play a role in the pathogenesis of the systemic comorbidities, including diabetes mellitus, hypertension, coronary artery disease and the metabolic syndrome. For instance, TNF $\alpha$  has been linked to obesity-induced insulin resistance and its receptor has been detected on various cell types in the central nervous system<sup>8</sup>.

In the present study, the most pronounced upregulation was observed for EGF. An earlier study has suggested an increase in EGF by the finding of a slight, but not statistically significant, increase in circulating EGF in chronic but not in acute psoriasis<sup>9</sup>. Several reports indicate that epidermal growth factor receptor (EGFR) and its endogenous ligands are overexpressed in psoriatic lesions<sup>10</sup>. A putative role for disturbed EGFR-mediated signalling in psoriatic keratinocytes has been proposed<sup>10</sup>. The high expression of EGFR and its ligands in lesional skin, together with the high expression of EGF in the sera, suggests that this pathway contribute to the pathophysiology of the disease. Our data suggests that combination therapy of EGFR inhibition with TNF $\alpha$  inhibition may be more effective than either alone. This is supported by the fact that vitamin D, a commonly used drug in the treatment of psoriasis, downregulates EGFR<sup>11</sup>.

The serum levels of IL-1 $\beta$  was not increased in our patients sample, which confirms a previous study showing higher levels of IL-1 $\beta$  in blister fluid than in serum, supporting the

hypothesis that this cytokine is locally produced in psoriatic lesions<sup>12</sup>. The receptor antagonist IL-1Ra binds to IL-1 receptors without inducing a cellular response, thereby antagonising the pro-inflammatory effects of the receptor agonists IL-1 $\alpha$  and - $\beta$ <sup>13</sup>, in response to activation of the IL-1 signalling cascade. It has anti-inflammatory properties and behaves as an acute-phase reactant protein. IL-1Ra was previously shown to be produced by human keratinocytes<sup>14</sup>. Interestingly, IL-1Ra has shown increased expression in serum in rheumatoid arthritis. IL-1Ra is upregulated by obesity in mouse white adipose tissue (WAT), suggesting that WAT is an important source of IL-1Ra in obesity and possibly also inflammation<sup>15</sup>. Moreover, systemic IL-1Ra levels are increased in patients with impaired glucose tolerance and the metabolic syndrome<sup>16</sup>. Since increased body mass index (BMI) is associated with an increased risk of psoriasis<sup>17</sup>, the increased expression of IL-1Ra in the sera of psoriasis patients may be a pathogenic factor in obesity that may coincide with psoriasis.

We demonstrated that cytokines retained their high expression after successful UVB treatment. Patients with active psoriatic arthritis who responded to the TNF inhibitor infliximab also had sustained high serum TNF $\alpha$  levels<sup>18</sup>. Since the half-life of serum TNF $\alpha$  is measured in minutes<sup>19</sup>, sustained serum levels after adequate treatment of skin lesions and the lack of correlation to the severity of the disease suggest that ongoing production of these cytokines reflects a systemic inflammatory response.

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## References

1. Pietrzak AT, Zalewska A, Chodorowska G, et al. Cytokines and anticytokines in psoriasis. *Clin Chim Acta* 2008;**394**(1-2):7-21.
2. Jacob SE, Nassiri M, Kerdel FA, et al. Simultaneous measurement of multiple Th1 and Th2 serum cytokines in psoriasis and correlation with disease severity. *Mediators Inflamm* 2003;**12**(5):309-13.
3. Gomi T, Shiohara T, Munakata T, et al. Interleukin 1 alpha, tumor necrosis factor alpha, and interferon gamma in psoriasis. *Arch Dermatol* 1991;**127**(6):827-30.
4. Anderson KS, Wong J, Polyak K, et al. Detection of psoriasin/S100A7 in the sera of patients with psoriasis. *Br J Dermatol* 2009;**160**(2):325-32.
5. Saeed AI, Sharov V, White J, et al. TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* 2003;**34**(2):374-8.
6. Szodoray P, Alex P, Chappell-Woodward CM, et al. Circulating cytokines in Norwegian patients with psoriatic arthritis determined by a multiplex cytokine array system. *Rheumatology (Oxford)* 2007;**46**(3):417-25.
7. Nickoloff BJ, Xin H, Nestle FO, et al. The cytokine and chemokine network in psoriasis. *Clin Dermatol* 2007;**25**(6):568-73.
8. Illman J, Corringham R, Robinson D, Jr., et al. Are inflammatory cytokines the common link between cancer-associated cachexia and depression? *J Support Oncol* 2005;**3**(1):37-50.
9. Pietrzak A, Miturski R, Krasowska D, et al. Concentration of an epidermal growth factor in blood serum of males during topical treatment of psoriasis. *J Eur Acad Dermatol Venereol* 1999;**12**(1):1-5.
10. Nanney LB, Stoscheck CM, Magid M, et al. Altered [125I]epidermal growth factor binding and receptor distribution in psoriasis. *J Invest Dermatol* 1986;**86**(3):260-5.
11. Cordero JB, Cozzolino M, Lu Y, et al. 1,25-Dihydroxyvitamin D down-regulates cell membrane growth- and nuclear growth-promoting signals by the epidermal growth factor receptor. *J Biol Chem* 2002;**277**(41):38965-71.
12. Bonifati C, Ameglio F, Carducci M, et al. Interleukin-1-beta, interleukin-6, and interferon-gamma in suction blister fluids of involved and uninvolved skin and in sera of psoriatic patients. *Acta Derm Venereol Suppl (Stockh)* 1994;**186**:23-4.
13. Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol* 1998;**16**(5-6):457-99.
14. Bigler CF, Norris DA, Weston WL, et al. Interleukin-1 receptor antagonist production by human keratinocytes. *J Invest Dermatol* 1992;**98**(1):38-44.
15. Juge-Aubry CE, Somm E, Giusti V, et al. Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. *Diabetes* 2003;**52**(5):1104-10.
16. Salmenniemi U, Ruotsalainen E, Pihlajamaki J, et al. Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. *Circulation* 2004;**110**(25):3842-8.
17. Naldi L, Addis A, Chimenti S, et al. Impact of body mass index and obesity on clinical response to systemic treatment for psoriasis. Evidence from the Psocare project. *Dermatology* 2008;**217**(4):365-73.
18. Amital H, Barak V, Winkler RE, et al. Impact of treatment with infliximab on serum cytokine profile of patients with rheumatoid and psoriatic arthritis. *Ann N Y Acad Sci* 2007;**1110**:649-60.
19. Oliver JC, Bland LA, Oettinger CW, et al. Cytokine kinetics in an in vitro whole blood model following an endotoxin challenge. *Lymphokine Cytokine Res* 1993;**12**(2):115-20.

**Table 1**

Table 1. Cytokines analysed with Milliplex MAP multiplex serum assay.

| Type              | Cytokine       | pg/ml        |                 | p-value<br>case v. nl* |
|-------------------|----------------|--------------|-----------------|------------------------|
|                   |                | Case<br>Mean | Normal<br>Range |                        |
| Inflammatory      | IFN $\alpha$ 2 | 215,99       | 16-1260         | 0,6948                 |
|                   |                | 347,73       | 16-3430         |                        |
|                   | IFN $\gamma$   | 50,56        | 1-173           | 0,5416                 |
|                   |                | 35,92        | 1-311           |                        |
|                   | IL-13          | 19,34        | 1-124           | 0,5541                 |
|                   |                | 23,67        | 3-171           |                        |
|                   | IL-17          | 31,38        | 1-157           | 0,8077                 |
|                   |                | 21,50        | 1-70            |                        |
|                   | IL-1 $\beta$   | 6,24         | 1-43            | 0,9057                 |
|                   |                | 2,27         | 1-9             |                        |
| IL-2              | 24,03          | 1-146        | 0,7298          |                        |
|                   | 19,82          | 1-209        |                 |                        |
| IL-4              | 11,18          | 3-112        | 0,3711          |                        |
|                   | 4,07           | 3-15         |                 |                        |
| IL-6              | 138,31         | 2-764        | 0,0935          |                        |
|                   | 30,59          | 2-222        |                 |                        |
| TNF $\alpha$      | 7,46           | 2-24         | <b>0,0419</b>   |                        |
|                   | 4,53           | 2-10         |                 |                        |
| TNF $\beta$       | 3,71           | 2-16         | 1,0000          |                        |
|                   | 3,06           | 2-13         |                 |                        |
| Anti-inflammatory | IL-10          | 11,50        | 2-100           | 0,1353                 |
|                   |                | 3,90         | 1-10            |                        |
|                   | IL-12 (p40)    | 73,26        | 10-188          | 0,1531                 |
|                   |                | 168,64       | 5-787           |                        |
| IL-1Ra            | 39,10          | 3-70         | <b>0,0175</b>   |                        |
|                   | 14,56          | 2-84         |                 |                        |
| Chemokines        | MIP-1 $\alpha$ | 161,72       | 20-391          | 0,0580                 |
|                   |                | 75,44        | 14-198          |                        |
| MIP-1 $\beta$     | 187,49         | 24-1160      | 0,3258          |                        |
|                   | 84,94          | 20-391       |                 |                        |
| Growth factors    | EGF            | 323,04       | 32-1170         | <b>0,0001</b>          |
|                   |                | 36,57        | 7-130           |                        |
|                   | Eotaxin        | 595,21       | 247-1250        | 0,1189                 |
|                   |                | 472,37       | 67-930          |                        |
|                   | FGF-2          | 18,75        | 2-96            | 0,3279                 |
| 11,80             |                | 2-53         |                 |                        |
| G-CSF             | 13,08          | 3-26         | 0,3575          |                        |
|                   |                | 18,09        | 9-35            |                        |
| VEGF              | 275,45         | 16-1420      | 0,4846          |                        |
|                   | 140,85         | 16-467       |                 |                        |

\* paired wilcoxon test