RESEARCH ARTICLE

Clinical and cytokine profile evaluation in Northeast Brazilian psoriasis plaque-type patients

Pablo Ramon Gualberto Cardoso[1], Emerson Vasconcelos de Andrade Lima[1,2], Mariana Modesto de Andrade Lima[1], Moacyr Jesus Barreto de Melo Rêgo[2], Claudia Diniz Lopes Marques[2], Ivan da Rocha Pitta[1], Angela Luzia Branco Pinto Duarte[1], Maira Galdino da Rocha Pitta[1]

[1] Laboratory of Immunomodulation and Novel Therapeutic Approaches (LINAT), Research Center for Therapeutic Innovation Suely Galdino (NUPIT-SG), UFPE, Recife, Brazil
[2] Clinics Hospital, Federal University of Pernambuco (UFPE), Recife, Brazil

Correspondence: M.G.R. Pitta, Universidade Federal de Pernambuco (UFPE), Centro de Ciências Biológicas, Núcleo de Pesquisa em Inovação Terapêutica Suely Galdino (Nupit SG), Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife – PE, 50670-901, Brazil
<mgrpitta@gmail.com>

To cite this article: Cardoso PRG, Lima EVA, Lima MMA, Rêgo MJBM, Marques CDL, Pitta IR, Duarte ALBP, Pitta MGR. Clinical and cytokine profile evaluation in Northeast Brazilian psoriasis plaque-type patients. *Eur. Cytokine Netw.* 2016; 27(1): 1-5 doi:10.1684/ecn.2016.0371

ABSTRACT. ***Objective and Design:*** **Psoriasis is a common, enigmatic, and recurrent disease. The precise etiology and pathogenesis of psoriasis are still unclear. Psoriasis has been treated as an inflammatory disorder related to an underlying Th1/Th17-dominated immune response. Interleukins are involved in the development of psoriasis lesions through Th-17-associated inflammation. Th1 and Th17 cytokines are found in skin lesions and in the peripheral blood of psoriasis patients. We sought to analyze serum levels of IL-1-β, IL-8, IL-9, IL-27, IL-29, IL-35, IFN-γ, TNF and TGF-β in patients with psoriasis and healthy control volunteers.** ***Material:*** **Blood samples were collected from fifty-three patients with psoriasis and thirty-five healthy controls.** ***Methods:*** **Serum cytokines concentrations were determined using an enzyme-linked immunosorbent assay.** ***Results:*** **Serum IL-8, IL-9, IL-27, IL-29 and TNF levels were statistically significant in psoriasis patients. Detectable serum IL-9 levels were found in 47 patients of the 53 in the psoriasis group.** ***Conclusions:*** **Interleukins-8, 27, 29 and TNF levels measured in the serum of psoriasis patients were slightly elevated as compared to healthy controls in a weakly significant way. On the other hand, there were highly significant differences in IL-9 levels between the two groups.**

Key words: psoriasis, IL-8, IL-9, IL-27, IL-29, Brazilian Patients

Psoriasis is a chronic, T-cell-mediated disorder characterized by histological features such as inflammatory infiltration, hyperproliferation of epidermal cells and dilated microvessels. It affects 1-3% of the world's population, with a considerable impact on quality of life [1, 2]. Patients with psoriasis have been found to be at greater risk of developing metabolic syndrome and cardiovascular complications [3].

The precise pathomechanism of psoriasis remains unclear. The ultimate pathological process that leads to both the skin manifestations and comorbidities is chronic inflammation, with an underlying Th1/Th17-dominated immune response [4, 5]. Cutaneous and systemic overexpression of pro-inflammatory cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor (TNF) and interleukins (IL-1, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, IL-19, IL-20, IL-22, IL-23) has been demonstrated in patients with psoriasis [6, 7]. Th1 cytokines were found both in skin lesions and in patients' peripheral blood [2, 8].

Cytokines are directly involved in the pathogenesis of psoriasis. Some are able to initiate inflammation by signaling keratinocytes, with consequent greater defense cell recruitment, i.e. leukocytes. In this way, the characteristic inflammation associated with psoriasis is established [9]. Some authors have suggested cytokine-blocking treatments, including specific blockers such as anti-TNF currently in clinical use, and more recently, potential IL-12, IL-23p40 and IL-17 blockers that are yielding good results [10-12].

There has been much study of the Th17 pathway in the pathogenesis of psoriasis [13-15], but in addition to the cytokines involved in this pathway, other cytokines have been found in the serum of patients with psoriasis [13, 16, 17], and there may be interactions that exacerbate the immune response. Consequently, this study has focused on searching for other cytokines that may be involved in the pathogenesis of psoriasis such as IL-1β, IL-8, IL-9, IL-27, IL-29, IL-35 IFN-γ, TNF and TGF-β; trying to find a link between them and the severity of disease as measured by the PASI.

PATIENTS AND METHODS

Clinical assessment and patients

A sample population consisting of 53 patients (31 men and 22 women, age range 18-80 years), with plaque-type psoriasis attending the rheumatology outpatient clinic at the Universidade Federal de Pernambuco (UFPE),

doi: 10.1684/ecn.2016.0371

Table 1
Clinical parameters of controls

Characteristics	Mean (Range or SD) Patients (n = 53)	Mean (Range) Controls (n = 35)
Male	31	19
Female	22	16
Age (years)	49 (18-80)	46 (22-64)
PASI	17.15 (7-41)	
Moderate (n = 22)	9.2 (± 1,8)	-
Severe (n = 31)	22.8 (± 7,1)	-
Erythrocyte sedimentation rate	17.8 (± 13,2)	-
Immunosuppressive treatment	None	None

Recife-Brazil, and 35 healthy controls (*table 1*), were recruited for the study.

A diagnosis of plaque psoriasis was made in strict accordance with the diagnostic criteria of Nestle *et al.* [18]. The severity of disease in each patients was assessed using the PASI score [19]. Psoriasis was classified as moderate (PASI 8-12) or severe (PASI>12) [20]. According to the PASI score, 22 patients had moderate and 31 patients, severe psoriasis. At diagnosis, blood samples were collected from all patients. The diagnose of psoriatic arthritis was excluded in all 53 patients by a rheumatologist. The patients included in this study had no other autoimmune disorders, acute or chronic infections or any malignancies. They had not received any systemic treatment involving immunosuppressive drugs or phototherapy, and they had been off topical treatment for four weeks prior to the PASI score evaluation and blood sample collection. Healthy, volunteer controls were matched by age and sex. Formal written consent was obtained from all patients and healthy volunteers enrolled in the study. The ethics committee of the UFPE approved the research (CEP: 528/11).

Blood samples were collected by venipuncture (8 mL), and processed after clotting for 20 minutes at room temperature. Serum samples were obtained for further measurements by centrifugation at 2000 rpm for 10 min, and were stored at -80°C for subsequent assay.

ELISA

Serum IL-1 β, IL-8, IL-9, IL-27, IL-29, IL-35, IFN-γ, TNF and TGF-β levels, for patients and healthy volunteers, were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBiosciences) according to the manufacturer's instructions. The standard substances and serum samples were incubated in a 96-well polystyrene microplate with the corresponding cytokine antibody. The plate was read at 450-570 nm. The sensitivity of the ELISA was 0.78 pg/mL for IL-9 and IL-35; 1.95 pg/mL for IL-8; 3.91 pg/mL for IL-1β; 4.68 pg/mL for IFN-γ. For IL-29 and TNF, the limit was 7.81 pg/mL; for TGF-β, 15.62 pg/mL, and finally, 62.5 pg/mL for IL-27.

Statistical analysis

The statistical analysis used the average and range for nonparametric data analysis, and the mean and standard error of the mean (SEM) for normal distribution. The D'Agostino test verified the normality of samples. Differences in serum cytokine levels between patients with psoriasis and healthy controls were analyzed using the Mann-Whitney test. For two independent groups, the Fisher test was used. Pearson's coefficient was used to measure the linear relationship between two variables. The Spearman correlation coefficient (ρ) was used to measure the linear relationship between two variables at the ordinal level. The correlation classification followed the consideration r = 0.10 to 0.29 (weak); r = 0.30 to 0.49 (moderate); r = 0.50 to 1 (strong). The statistical significance is represented by stars as follow: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. Statistical analysis was performed using GraphPad Prism® version 6.0.

RESULTS

All psoriasis patients had squamous plaques characteristic of the plaque-type disease. The patients that were chosen presented with active disease, but were not receiving any biological or immunosuppressive drugs and/or phototherapy. From the classification of their psoriatic lesions, patients were grouped by PASI scale into moderate and severe groups [20]. *Table 1* shows clinical and laboratory parameters of volunteers.

The sandwich type ELISA detects IFN-γ, IL-1β, IL-8, IL-9, IL-27, IL-29, IL-35, TGF-β and TNF serum levels. The values obtained can be seen in *figure 1*.

Age correlation analysis shows a weak statistical significance with IL-8 and TNF (data not shown). Of all the cytokines, IL-9 was the most common in patients with psoriasis. Forty-seven out of fifty-three patients showed positive values for IL-9 (4 ± 2.2 pg/mL). If we compare them to the controls, just two out of thirty-five showed positive values for IL-9 (1.2 ± 2.5 pg/mL). Consequently, we decided to analyze sensitivity and specificity. The IL-9 assay had a high specificity (94%), and quite good sensitivity (88.6%).

DISCUSSION

We undertook this study in order to find other cytokines in the serum of psoriasis patients. We found statistical significances for IL-8, IL-9, IL-27, IL-29 and TNF cytokines, and we can conclude that these cytokines are present in serum of Brazilian psoriasis patients.

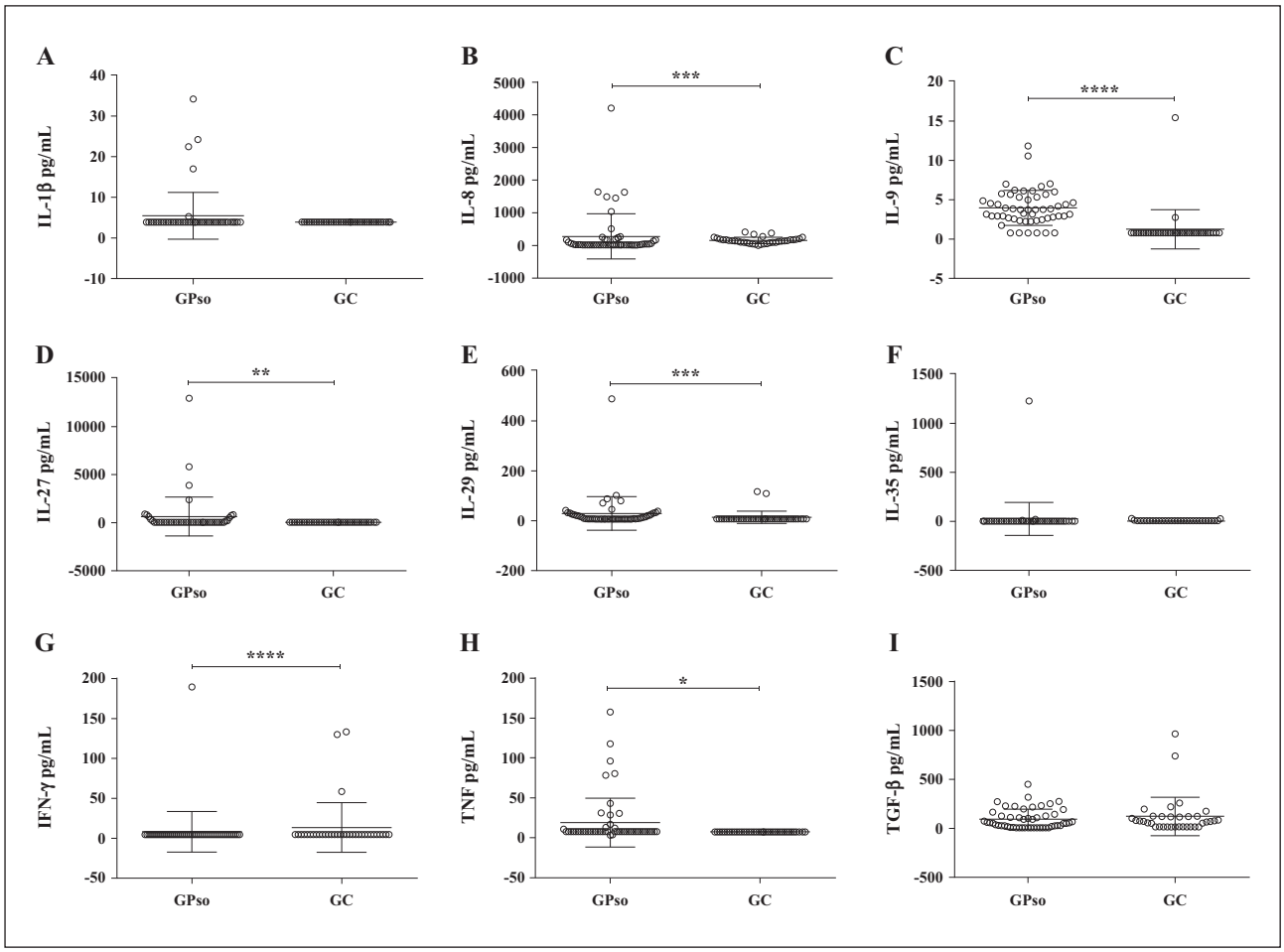

Figure 1

Brazilian psoriasis patients pytokine profiles. In this image, it is possible to observe IL-1β, IL-8, IL-9, IL-27, IL-29, IL-35, IFN-γ, TNF and TGF-β (pg/mL) cytokine dosages in a; b; c; d; e; f; g; h; i; respectively. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$.

These results showed a positive statistical significance for IL-8 levels in patients with psoriasis compared to healthy controls. This cytokine was detected in 51 of the 53 patients. The discovery of IL-8 at such statistically significance levels in patients with active disease may indicate its participation in the development of psoriasis [16, 21]. Nograles and Kruger [22] conducted an important study of IL-8 in psoriasis in a review article where they showed an improved picture of the disease when IL-8 was blocked [23]. The participation of IL-8 is thus obvious within the framework of psoriasis. In addition, results show a tendency towards the higher the IL-8 levels, the higher the IL-27 and TNF levels. IL-8 can be closely involved in inflammation. In this case, when produced by keratinocytes and the psoriasis is aggravated by the Th1 cell activation pathway, in conjunction with IL-27, it could be an important factor in the induction of the disease. It appears that IL-27 plays an important role in the relationship between these three cytokines, serving as a pivot for the inflammatory Th1 pathway [24-26]. TNF has been frequently described in inflammatory diseases such as psoriasis, where it is found in the serum and cells of these patients [16, 27, 28]. We also found higher levels of TNF in patients with psoriasis.

IL-27 is a recently discovered cytokine that is produced by antigen-presenting cells (APCs) [29]. Recently, it was identified as a key cytokine involved in the worsening of inflammatory diseases such as lupus erythematosus and psoriasis because of its potential to activate naïve T cells into Th17 [30]. Our results show that IL-27 is present at levels that are statistically significant in the serum of psoriatic patients. This suggests that Il-27 could be involved in the triggering of the disease as it was not detected in the serum of control patients. It is known that IL-27 has pro-and anti-inflammatory functions, but in this study, in correlation with IL-8, we believe that IL-27 is acting in a proinflammatory capacity.

IL-29, also known as IFNλ1, was discovered in 2003, and it initially appeared to be involved in the antiviral response. It is believed that this cytokine might be involved in the Th1 response [31, 32]. Eventually, IL-29 was classified as an IL-10 family member [33]. A recent study suggested the production of IL-29 by Th17 cells in patients with psoriasis [34]. Observation of the importance of IL-29 within the psoriatic framework distinguished this cytokine as an important objective of the study. Serum levels of IL-29 were positively statistically significant in patients with psoriasis compared to controls. Given the number of patients who had detectable levels of IL-29, this suggests that the cytokine is involved in the inflammatory stages of the disease and should be studied further.

Finally, IL-9 was detected in 47 of our 53 patients, but in only two control patients. The present study indicated that patients with plaque-type psoriasis showed positive statistical significance for serum IL-9 levels as compared to healthy controls. The sensitivity of the ELSA kit used

in the experiment was 0.78 pg/ml, which is very precise. In this context, the detection of serum IL-9 levels in 47 of the 53 individuals in the psoriasis group, compared to only two in the healthy control group of 35, becomes very interesting, suggesting that this cytokine may be of greater importance in the setting of psoriasis than was previously thought. In this study, the data point to a sensitivity of 88.6% and a specificity of 94%, suggesting that IL-9 might be a good serum biomarker for plaque-type psoriasis.

Inflammatory and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and psoriasis, associated with the Th17 pathway, showed high serum levels of IL-9 [35]. A recent finding has indicated a link between IL-9, a Th2 and Th9 cytokine, and the Th17 pathway in psoriasis, demonstrating a markedly higher expression of IL-9R in psoriatic skin lesions. This cytokine can also be expressed by Th17 cells [36, 37]. In addition, IL-9 associated with IL-6 and TGF-β1, increased the production of IL-17 by peripheral blood mononuclear cells or CD4+ T cells, especially in cells isolated from individuals with psoriasis [14]. Accordingly, Th17 cells are known to express the receptor for IL-9, which can contribute directly to Th17 differentiation, and behave in such a way in the context of psoriasis [38].

Recently, Singh *et al*. demonstrated that IL-9 had no *ex vivo* effects on a number of IFN-γ-secreting CD4+ T cells on ELISPOT assay using cells from individuals with psoriasis, suggesting that IL-9 does not contribute to the Th1 component of the disease [14, 39]. This observation is consistent with other, similar reports [40]. Indeed, the finding that IL-9 has a pathogenic role in psoriasis was clearly seen in K5.HTGFβ1 transgenic mice, which exhibit a phenotype similar to human psoriasis. Intradermal injection of IL-9 in these animals induced Th-17-associated skin inflammation, including expression of IL-17A. Moreover, when anti-IL-9 antibody was injected, not only did it diminish the psoriasis-like morphological changes, including cellular infiltration and neo-vascularization of the skin, it also reduced the expression of IL-17A [14]. In addition, the injection of anti-IL-17 in mice decreased skin IL-9 m RNA and serum IL-9 protein levels, suggesting a positive feedback loop between IL-9 and IL-17A [14]. We found that the serum levels of IL-9 were increased in patients with plaque psoriasis compared to healthy controls. The specific mechanism of the high expression of IL-9 in psoriasis remains unknown, but with these results from clinical trials, we are able to refine our understanding of the pathogenesis of psoriasis, which may provide a new therapeutic approach for this debilitating disease.

Finally, interleukin-35 is the newest member of the IL-12 cytokine family. It is involved in Treg cell regulation. Studies suggest that it may reduce the proliferation of CD4 + cells, thus being a potential treatment for autoimmune diseases [41-44]. To date there had been no data available concerning the possible role of IL-35 in psoriasis. We decided to include IL-35 in our study because of its supposed anti-inflammatory characteristics. We did not find IL-35 in the serum of Brazilian psoriasis patients.

CONCLUSION

The results indicate that cytokines IL-8, IL-9, IL-27, IL-29, IFN-γ and TNF are present in the serum of northeast Brazilian, plaque-type psoriasis patients. The findings are important for further study of diseases mediated by immune cells.

Acknowledgment. *Instituto Nacional de Ciência e Tecnologia para Inovação Farmacêutica – INCT_if; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Capes; Fundação de Amparo à Ciência e Tecnologia de Pernambuco – FACEPE; Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq.*

Disclosure. *Financial support: none. Conflict of interest: none.*

REFERENCES

1. Ariza ME, Williams MV, Wong HK. Targeting IL-17 in psoriasis: from cutaneous immunobiology to clinical application. *Clin Immunol* 2013; 146: 131-9.
2. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet* 2007; 370: 263-71.
3. Gisondi P, Tessari G, Conti A, *et al*. Prevalence of metabolic syndrome in patients with psoriasis: a hospital-based case-control study. *Br J Dermatol* 2007; 157: 68-73.
4. Johnston A, Arnadottir S, Gudjonsson JE, *et al*. Obesity in psoriasis: leptin and resistin as mediators of cutaneous inflammation. *Br J Dermatol* 2008; 159: 342-50.
5. Nickoloff BJ, Qin JZ, Nestle FO. Immunopathogenesis of psoriasis. *Clin Rev Allergy Immunol* 2007; 33: 45-56.
6. Killeen ME, Ferris L, Kupetsky EA, Falo L, Mathers AR. Signaling through purinergic receptors for ATP induces human cutaneous innate and adaptive Th17 responses: implications in the pathogenesis of psoriasis. *J Immunol* 2013; 190: 4324-36.
7. Dardalhon V, Awasthi A, Kwon H, *et al*. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. *Nat Immunol* 2008; 9: 1347-55.
8. Elloso MM, Gomez-Angelats M, Fourie AM. Targeting the Th17 pathway in psoriasis. *J Leukoc Biol* 2012; 92: 1187-97.
9. Tokura Y, Mori T, Hino R. Psoriasis and other Th17-mediated skin diseases. *J UOEH* 2010; 32: 317-28.
10. Kupetsky EA, Mathers AR, Ferris LK. Anti-cytokine therapy in the treatment of psoriasis. *Cytokine* 2013; 61: 704-12.
11. Mease PJ. Adalimumab: an anti-TNF agent for the treatment of psoriatic arthritis. *Expert Opin Biol Ther* 2005; 5: 1491-504.
12. Tobin AM, Kirby B. TNF alpha inhibitors in the treatment of psoriasis and psoriatic arthritis. *BioDrugs* 2005; 19: 47-57.
13. Michalak-Stoma A, Pietrzak A, Szepietowski JC, Zalewska-Janowska A, Paszkowski T, Chodorowska G. Cytokine network in psoriasis revisited. *Eur Cytokine Netw* 2011; 22: 160-8.
14. Singh TP, Schön MP, Wallbrecht K, Gruber-Wackernagel A, Wang XJ, Wolf P. Involvement of IL-9 in Th17-associated inflammation and angiogenesis of psoriasis. *PLoS One* 2013; 8: e51752.
15. Lynde CW, Poulin Y, Vender R, Bourcier M, Khalil S. Interleukin 17A: toward a new understanding of psoriasis pathogenesis. *J Am Acad Dermatol* 2014.
16. Arican O, Aral M, Sasmaz S, Ciragil P. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm* 2005; 2005: 273-9.

17. Takahashi H, Tsuji H, Hashimoto Y, Ishida-Yamamoto A, Iizuka H. Serum cytokines and growth factor levels in Japanese patients with psoriasis. *Clin Exp Dermatol* 2010; 35: 645-9.

18. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med* 2009; 361: 496-509.

19. van de Kerkhof PC. The Psoriasis Area and Severity Index and alternative approaches for the assessment of severity: persisting areas of confusion. *Br J Dermatol* 1997; 137: 661-2.

20. Schmitt J, Wozel G. The psoriasis area and severity index is the adequate criterion to define severity in chronic plaque-type psoriasis. *Dermatology* 2005; 210: 194-9.

21. Abdel-Hamid MF, Aly DG, Saad NE, Emam HM, Ayoub DF. Serum levels of interleukin-8, tumor necrosis factor-α and γ-interferon in Egyptian psoriatic patients and correlation with disease severity. *J Dermatol* 2011; 38: 442-6.

22. Nograles KE, Davidovici B, Krueger JG. New insights in the immunologic basis of psoriasis. *Semin Cutan Med Surg* 2010; 29: 3-9.

23. Duan H, Koga T, Kohda F, Hara H, Urabe K, Furue M. Interleukin-8-positive neutrophils in psoriasis. *J Dermatol Sci* 2001; 26: 119-24.

24. Pflanz S, Timans JC, Cheung J, *et al*. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. *Immunity* 2002; 16: 779-90.

25. Wolk K, Haugen HS, Xu W, *et al*. IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. *J Mol Med (Berl)* 2009; 87: 523-36.

26. Cao Y, Doodes PD, Glant TT, Finnegan A. IL-27 induces a Th1 immune response and susceptibility to experimental arthritis. *J Immunol* 2008; 180: 922-30.

27. Guilloteau K, Paris I, Pedretti N, *et al*. Skin inflammation induced by the synergistic action of IL-17A, IL-22, oncostatin M, IL-1{alpha}, and TNF-{alpha} recapitulates some features of psoriasis. *J Immunol* 2010.

28. Kouris A, Pistiki A, Katoulis A, *et al*. Proinflammatory cytokine responses in patients with psoriasis. *Eur Cytokine Netw* 2014; 25: 63-8.

29. Shibata S, Tada Y, Kanda N, *et al*. Possible roles of IL-27 in the pathogenesis of psoriasis. *J Invest Dermatol* 2010; 130: 1034-9.

30. Spadaro A, Scrivo R, Rinaldi T, *et al*. [The role of interleukin-12 in immune-mediated rheumatic diseases]. *Reumatismo* 2002; 54: 113-21.

31. Sheppard P, Kindsvogel W, Xu W, *et al*. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat Immunol* 2003; 4: 63-8.

32. Jordan WJ, Eskdale J, Srinivas S, *et al*. Human interferon lambda-1 (IFN-lambda1/IL-29) modulates the Th1/Th2 response. *Genes Immun* 2007; 8: 254-61.

33. Uzé G, Monneron D. IL-28 and IL-29: newcomers to the interferon family. *Biochimie* 2007; 89: 729-34.

34. Wolk K, Witte K, Witte E, *et al*. IL-29 is produced by T(H)17 cells and mediates the cutaneous antiviral competence in psoriasis. *Sci Transl Med* 2013; 5: 204ra129.

35. Dantas AT, Marques CD, da Rocha Junior LF, *et al*. Increased serum interleukin-9 levels in rheumatoid arthritis and systemic lupus erythematosus: pathogenic role or just an epiphenomenon? *Dis Markers* 2015; 2015: 519638.

36. Nowak EC, Weaver CT, Turner H, *et al*. IL-9 as a mediator of Th17-driven inflammatory disease. *J Exp Med* 2009; 206: 1653-60.

37. Friberg C, Björck K, Nilsson S, Inerot A, Wahlström J, Samuelsson L. Analysis of chromosome 5q31-32 and psoriasis: confirmation of a susceptibility locus but no association with SNPs within SLC22A4 and SLC22A5. *J Invest Dermatol* 2006; 126: 998-1002.

38. Elyaman W, Bradshaw EM, Uyttenhove C, *et al*. IL-9 induces differentiation of TH17 cells and enhances function of FoxP3+ natural regulatory T cells. *Proc Natl Acad Sci U S A* 2009; 106: 12885-90.

39. Singh TP, Huettner B, Koefeler H, *et al*. Platelet-activating factor blockade inhibits the T-helper type 17 cell pathway and suppresses psoriasis-like skin disease in K5.hTGF-β1 transgenic mice. *Am J Pathol* 2011; 178: 699-708.

40. Coimbra S, Figueiredo A, Castro E, Rocha-Pereira P, Santos-Silva A. The roles of cells and cytokines in the pathogenesis of psoriasis. *Int J Dermatol* 2012; 51: 389-95, quiz 95-8.

41. Collison LW, Workman CJ, Kuo TT, *et al*. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 2007; 450: 566-9.

42. Niedbala W, Wei XQ, Cai B, *et al*. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. *Eur J Immunol* 2007; 37: 3021-9.

43. Collison LW, Chaturvedi V, Henderson AL, *et al*. IL-35-mediated induction of a potent regulatory T cell population. *Nat Immunol* 2010; 11: 1093-101.

44. Choi J, Leung PS, Bowlus C, Gershwin ME. IL-35 and autoimmunity: a comprehensive perspective. *Clin Rev Allergy Immunol* 2015.