Acta Derm Venereol 2003; 83: 262–265

Taylor & Francis
healthsciences

INVESTIGATIVE REPORT

Interleukin-18 Levels in the Plasma of Psoriatic Patients Correlate with the Extent of Skin Lesions and the PASI Score

ALDONA PIETRZAK[1], BARBARA LECEWICZ-TORUN[1], GRAŻYNA CHODOROWSKA[1] and JACEK ROLINSKI[2]

Departments of [1]Dermatology and [2]Clinical Immunology, Medical University, Lublin, Poland

Interleukin-18 is a cytokine with a possible role in the pathogenesis of psoriasis. We examined subpopulations of peripheral blood lymphocytes and their expression of activation markers and correlated this with plasma levels of IL-18 and clinical disease severity in patients with psoriasis. We included 12 patients with psoriasis who had a PASI score from 15 to 48 and compared them to controls. IL-18 plasma concentrations were determined with an enzyme-linked immunosorbent assay. We observed a significant correlation between the IL-18 levels and the area of skin affected with psoriasis and the PASI score. We also observed an increase in NK cells and memory helper CD45RO+/CD4+cells. ***Key words: blood lymphocytes; cytokines; psoriasis.***

(Accepted February 11, 2002.)

Acta Derm Venereol 2003; 83: 262–265.

Aldona Pietrzak, Dermatology Department, Medical University, 13 Radziwiłłowska str., 20-080 Lublin, Poland. E-mail: patrycjamikulska@wp.pl

Interleukin-18 is a proinflammatory cytokine that stimulates natural killer cells (NK) and T cells and enhances innate immunity as well as specific Th1 immune responses. Human keratinocytes produce IL-18, like monocytes and macrophages do, being two major sources of this molecule (1, 2). IL-18 acts directly on NK cells to stimulate INF-γ synthesis and upregulate their killing capacity (1, 3). It is believed that IL-18 derived from keratinocytes might be involved in the cutaneous Th1-type immune response (1). Recent publications have shown that IL-18 expression in psoriatic lesional skin is higher than in normal skin (1, 4). McKenzie et al. (4) assessed the expression of IL-18 and IL-18 receptor (R) mRNA in biopsies of lesional and non-lesional psoriatic skin. A significant 2.7-fold increase in the expression of IL-18 mRNA in lesional psoriatic skin compared to both normal ($p=0.04$) and non-lesional skin ($p=0.004$) was observed. Moreover, IL-18R mRNA was 5.2-fold greater in lesional skin compared to normal ($p=0.01$) and non-lesional psoriatic skin ($p=0.001$). No difference in IL-18 and IL-18R mRNA was noted between normal skin and non-lesional one. Western blotting combined with ELISA for IL-18 demonstrated that the immunoreactive IL-18 in extracts of psoriatic scales contained the mature form of IL-18, but most of the IL-18 was pro-IL-18 (1).

As a whole, these findings indicate that keratinocyte-derived IL-18 participates in the development of Th1 responses in psoriatic lesions, and that its bioactivity appears to be tightly regulated in cutaneous inflammation. We investigated the correlations between IL-18 in peripheral blood and the subpopulations of blood lymphocytes in patients who underwent local therapy for psoriasis vulgaris.

MATERIAL AND METHODS

We examined 12 patients (6 women and 6 men) ranging in age from 18 to 57 years (mean 29.9 ± 11.2) and compared them with 10 healthy subjects (5 women and 5 men) matched for age. The patients were hospitalized in the Department of Dermatology, Lublin Medical University. They had active psoriasis vulgaris needing admission, but did not receive any medication including systemic treatment. Appearance of present lesions on the skin was from 15 to 180 days (mean 62.5 ± 51.4). Duration of the disease varied from 1 to 46 years (mean 13.0 ± 11.7 years) and size of the plaques from 2 to 6 cm. The extent of psoriasis was evaluated by the same investigator using the Psoriasis Area and Severity Index (PASI) score, which ranged from 15 to 48. We evaluated the percentage of affected skin based on formulas used in surgery to assess the affected body surface in burns (5). Peripheral blood was collected before the administration of any form of treatment.

Plasma samples were collected using EDTA, centrifuged for 10 min at 1000 *g* and stored at -80°C. The presence of IL-18 was determined with the human IL-18 ELISA kit (MBL, Japan; sensitivity 12.5 pg/ml). The average coefficient of variance was 5–10% for both inter- and intra-assay reproducibility. The assay uses two monoclonal antibodies against two different epitopes on human IL-18.

The following cells were studied using flow cytometry: CD3+ (T lymphocytes), CD19+ (B lymphocytes), CD4+8+ (double-positive lymphocytes), CD4+ (T helper lymphocytes), CD8+ (T cytotoxic lymphocytes), CD3−/CD16+ CD56+ (natural killer cells), CD3+25+ (T regulatory lymphocytes with the alpha chain of IL-2R), CD25+ (alpha chain of IL-2R on all lymphocytes), CD45 RO+ (memory cells on all lymphocytes), CD45 RO+/CD4+ (memory T helper lymphocytes). The cell surface antigens were determined on fresh cells at the time of sampling. Mononuclear cells were isolated by density centrifugation on Lymphoprep (Nycomed, Norway) and washed twice in phosphate-buffered saline (PBS) containing 1% bovine serum albumin. Double-colour immunofluorescence studies were performed using combinations of phycoerythrin (PE) and fluorescein isothyocyanate (FITC) conjugated monoclonal antibodies. The antibody

 ISSN 0001-5555

Table I. *Details of monoclonal antibodies used for definition of lymphocyte subsets*

Antibody	Fluorochrome	Clone
CD3+	PE	IgG2a
CD19+	PE	Id 01,clone 407
CD45+/CD4+	FITC/PE	IMMU 19
CD45+RA/CD4+	FITC/PE	2/RM 052
CD45+/CD14+	FITC/PE	IgGl/IgG2a, Clone IMMU 19.2/RM052
CD45+RA	FITC	IgG,IgG1
CD4+/CD8+	FITC/PE	Isotype IgG2a/IgG2a
CD45+RO	FITC	Isotype IgG2a
CD4+	PE	IgG2a
CD8+	PE	IgG2a
CD25+	FITC	IgG2a
CD3+/CD19+	FITC/PE	IgG2/IgG1
CD56+	PE	IgG1
CD3−/CD16+CD56+	FITC/PE	SK7/B73 1+
IgG1FITC/IgG2PE	FITC/PE	Negative control
Goat Anti-Mouse	FITC	

All the antibodies were from the Ortho Diagnostics System except CD19+ and CD3−/CD16+CD56+, which were from Becton-Dickinson.
FITC = fluorescein isothyocyanate; PE = phycoerythrin.

combinations used are presented in Table I. 10^6 cells were incubated with monoclonal antibodies for 30 min at 4°C and washed twice with PBS afterwards.

All samples were measured on a cytoron flow cytometer (Ortho Diagnostic Systems) and 10,000 cells were analysed per test. In order to quantify the levels of fluorescence, the mean fluorescence intensity of the studied antigens was calculated. The data of the IL-18 level and the percentage of lymphocytes were analysed using the Mann-Whitney U test; the correlations were estimated by Spearman test using the Statistica software, and $p<0.05$ was considered significant.

RESULTS

IL-18 levels were significantly increased in the patients with psoriasis compared with healthy controls (312.3 ± 137.0 vs. 175.7 ± 78.90 pg/ml; $p<0.03$) (Fig. 1). We observed a significant correlation between the level of IL-18 and the area of psoriatic lesions ($p<0.04$, $R=0.6070$) and the PASI score ($p<0.03$, $R=0.6130$) (Fig. 2). There was a significant correlation between IL-18 and NK cells expression (CD3−/CD 16+ CD56+ cells ($p<0.02$, $R=0.8182$), and between IL-18 and the percentage of memory helper CD45RO+/CD4+cells ($p<0.04$, $R=0.5884$). Other correlations were not statistically significant.

DISCUSSION

IL-18 has been found to have a variety of biological actions. As a pleiotropic cytokine, it can play an immunoregulatory role in the human defence system, especially in inflammatory, infectious and autoimmune diseases (6). IL-18 promotes angiogenesis and tumour suppression (6), and has been found increased in almost

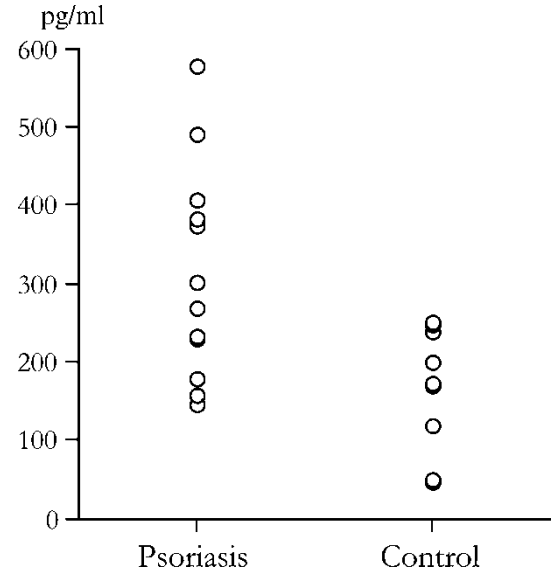

Fig. 1. The range of IL-18 plasma concentration in patients with psoriasis and healthy donors.

every inflammatory disease – from psoriasis and atopic eczema to pancreatitis and infections (5, 6–12, 15–17).

Naik et al. (19) observed that among non-bone marrow derived skin cells, IL-18 mRNA is constitutively expressed by human keratinocytes, but not by dermal microvascular endothelial cells, dermal fibroblasts or melanocytes. IL-18 mRNA and intracellular protein levels are not influenced by pro-inflammatory stimuli neither for human keratinocytes nor by dermal cells. In immunohistochemistry, IL-18 protein is detected in the basal keratinocytes of normal human skin, but its expression is markedly upregulated in suprabasal keratinocytes in psoriasis (4, 18).

Both Ohta et al. (1) and McKenzie et al. (4) state that in psoriasis there is an increased concentration of IL-18 and its receptor in lesional and non-lesional skin, indicating its involvement in the psoriatic process. The IL-18 elevated plasma levels found in our study may indicate a systemic activation of this cytokine production. This may also be connected with an increased neutrophil activation and an over-production of adhesion molecules, VCAM, ICAM-1, and E-selectin in psoriasis (19).

Neutrophils are an important participant in psoriasis. Jablonska et al. (21) investigated the effect of IL-18 on the production of IL-1β, IL-1Ra and sIL-1R2 in human neutrophils and found that rhIL-18 induces IL-1β and, to a lesser extent, IL-1Ra and sIL-1R2 production by human LPS-stimulated neutrophils. The capacity of IL-18 to serve as an effective modulator for IL-1β and its regulatory proteins may be significant in the inflammatory and immune reactions mediated by IL-1β.

Our finding of increased plasma levels of IL-18 which correlate with the extent of skin lesions and the PASI score suggest that IL-18 influence immune cells not

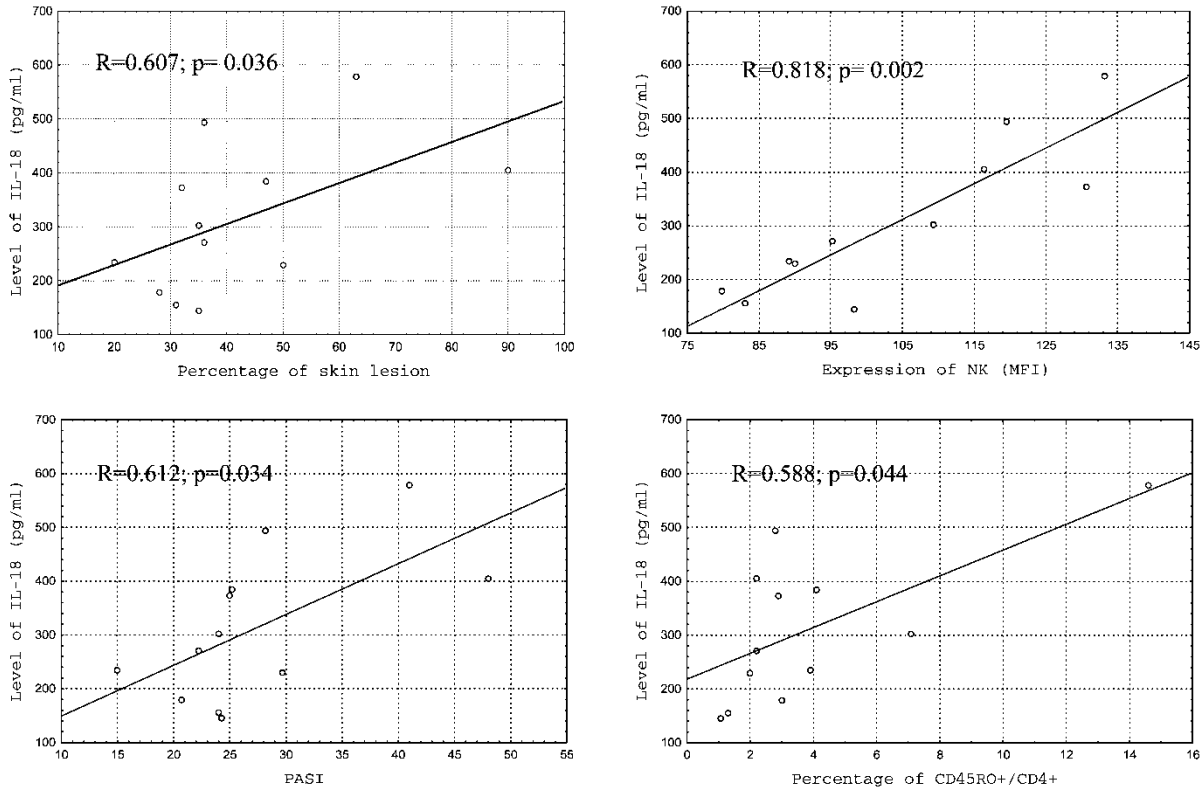

Fig. 2. The significant correlations between plasma IL-18 values the extent of skin lesions and PASI, the percentage of CD45RO+/CD4+ cells and expression of natural killer cells (NK).

only in the skin, but also in peripheral blood. IL-18 may affect the expression of selected receptors on NK cells, e.g. CD16, and augment the percentage of memory helper CD45RO+/CD4+cells supporting a systemic influence on the immune system. However, as IL-18 is augmented in many inflammatory diseases, it is not a specific event for psoriasis (19, 22, 23).

REFERENCES

1. Ohta Y, Hamada Y, Katsuoka K. Expression of IL-18 in psoriasis. Arch Dermatol Res 2001; 293: 334–342.
2. Mee JB, Alam Y, Groves RW. Human keratinocytes constitutively produce, but do not process interleukin-18. Br J Dermatol 2000; 143: 330–336.
3. Modrzyński M, Rapiejko P. The place of IL-18 in allergic diseases. Terapia 2001; 1: 43–44.
4. McKenzie RC, Boyce F, Forsey R, Gracie A, Szepietowski J, Weller R, et al. Psoriatic skin expresses high levels of interleukin-18 (IL-18) and IL-18 receptor (IL-18R). Br J Dermatol 2000; 142: 618 (abstract).
5. Rutkowski R, Moniuszko T. Immunoregulatory functions of a novel cytokine-interleukin-18 IFN-gamma inducing factor. Cent Eur J Immunol 1999; 24: 299–305.
6. Shafer KN, Sawyer JR, McCluskey AM, Beck EL, Phipps W. Medical-surgical nursing. St Louis: C.V. Mosby Company, 1975: 792.
7. Nicoletti F, Conget I, Di-Marco R, Speciale AM, Morinigo R, Bendtzen K, et al. Serum levels of the interferon-gamma-inducing cytokine interleukin-18 are increased in individuals at high risk of developing type I diabetes. Diabetologia 2001; 44: 309–311.
8. Wong CK, Ho CY, Ko FW, Chan CH, Ho AS, Hui DS, et al. Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-gamma, IL-4, IL-10 and IL-13) in patients with allergic asthma. Clin Exp Immunol 2001; 125: 177–183.
9. Tanaka H, Miyazaki N, Oashi K, Teramoto S, Shiratori M, Hashimoto M, et al. IL-18 might reflect disease activity in mild and moderate asthma exacerbation. J Allergy Clin Immunol 2001; 107: 331–336.
10. Tanaka T, Tsutsui H, Yoshimoto T, Kotani M, Matsumoto M, Fujita A, et al. Interleukin-18 is elevated in the sera from patients with atopic dermatitis and from atopic dermatitis model mice, NC/Nga. Int Arch Allergy Immunol 2001; 125: 236–240.
11. Rau B, Baumgart K, Paszkowski AS, Mayer JM, Beger HG. Clinical relevance of caspase-1 activated cytokines in acute pancreatitis: high correlation of serum interleukin-18 with pancreatic necrosis and systemic complications. Crit Care Med 2001; 29: 1556–1562.
12. Takada H, Nomura A, Ohga S, Hara T. Interleukin-18 in hemophagocytic lymphohistiocytosis. Leuk Lymphoma 2001; 42: 21–28.
13. Esfandiari E, McInnes I, Lindop G, Huang FP, Field M, Komai-Koma M, et al. A proinflammatory role of IL-18 in the development of spontaneous autoimmune disease. J Immunol 2001; 167: 5338–5347.
14. Lissoni P, Brivio F, Rovelli F, Fumagalli G, Malugani F, Vaghi M, et al. Serum concentrations of interleukin-18 in early and advanced cancer patients: enhanced secretion in metastatic disease. J Biol Regul Homeostatic Agents 2000; 14: 275–277.
15. Kawashima M, Yamamura M, Taniai M, Yamauchi H, Tanimoto T, Kurimoto M, et al. Levels of interleukin-18 and its binding inhibitors in the blood circulation of

patients with adult-onset Still's disease. Arthritis Rheum 2001; 44: 550 – 560.
16. Novick D, Schwartsburd B, Pinkus R, Suissa D, Belzer I, Sthoeger Z, et al. A novel IL-18BP ELISA shows elevated serum IL-18BP in sepsis and extensive decrease of free IL-8. Cytokine 2001; 14: 334 – 342.
17. Mustafa AS, Elbishbishi EA, Agarwal R, Chaturvedi UC. Elevated levels of interleukin-13 and IL-18 in patients with dengue hemorrhagic fever. Fems Immunol Med Microbiol 2001; 30: 229 – 233.
18. Torre D, Giola M, Speranza F, Matteelli A, Basilico C, Biondi G. Serum levels of interleukin-18 in patients with uncomplicated Plasmodium falciparum malaria. Eur Cytokine Network 2001; 12: 361 – 364.
19. Naik SM, Cannon G, Burbach GJ, Singh SR, Swerlick RA, Wilcox JN, et al. Human keratinocytes constitutively express interleukin-18 and secrete biologically active interleukin-18 after treatment with pro-inflammatory mediators and dinitrochlorobenzene. J Invest Dermatol 1999; 5: 766 – 772.
20. Szepietowski JC, Bielicka E, Nockowski P, Noworolska A, Wasik F. Increased interleukin-7 levels in the sera of psoriatic patients: lack of correlations with interleukin-6 levels and disease intensity. Clin Exp Dermatol 2000; 25: 643 – 647.
21. Jablonska E, Izycka A, Jablonski J, Wawrusiewicz N, Piecuch J. Role of IL-18 in the secretion of IL-1 < beta >, sIL-1RII, and IL-1Ra by human neutrophils. Immunol Invest 2001; 30: 221 – 229.
22. Pietrzak A, Lecewiez-Torun B, Chodorowska G, Rolinski J. Interleukin 18 serum concentration in patients with psoriasis vulgaris. JEADV 2002; 16: 1286 – 1287.
23. Pietrzak A, Lecewiez-Torun B, Rolinski J. Interleukin-18 levels in the sera of psoriatic patients; correlates with flow cytometry analysis. Ann Dermatol Venereol 2002; 129: 1766.