ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LV, 5 SECTIO D 2000

Katedra i Klinika Dermatologii Akademii Medycznej w Lublinie
Chair and Department of Dermatology, Medical University of Lublin

GRAŻYNA CHODOROWSKA, DOROTA CZELEJ,
MARIA BOROWIEC, ALDONA PIETRZAK,
DOROTA KRASOWSKA, MAŁGORZATA KOWAL

Study on activity of Interleukin-6, C-reactive protein and α-2 macroglobulin in psoriatic patients during the acute clinical stage and remission

Badania zachowania się Interleukiny-6, białka C-reaktywnego i α-2 makroglobuliny u chorych na łuszczycę w okresie objawowym i w remisji

IL-6 is a pleiotropic cytokine whose activity is of particular interest in psoriasis because of its powerful influence on local and systemic inflammation and also on proliferatory and immune response of various cell types (7,9,15). The wide spectrum of IL-6 activity causes that this cytokine, as a mitogen for keratinocytes and endothelial cells (1,15), activator and chemotactic agent for T cells (9), can partake in initiation of all basical events in the psoriatic patogenesis. Moreover, activation of the acute phase proteins by IL-6 (2,7,9,12) may account for an existence of systemic symptoms in severe forms of psoriasis, such as pustular or arthropathic psoriasis. Healthy people have a constant level of acute phase proteins in pheripheral blood, which reflects a state of balance between synthesis and degradation (11).

Among the large group of acute phase reactants, C-reactive protein (CRP) and α-2 macroglobulin (α-2 MG) may be of interest in psoriasis, because of their relations with cytokines taking part in the development of skin inflammation. CRP is a sensitive, although non-specific indicator of inflammation of various origin, including trauma and cancers (5,6,11,12,14). One of the most important functions of CRP is binding the changed biological material in the peripheral blood, its blocking, detoxication and facilitation of elimination (11,13). Elevated concentration of this protein in the peripheral blood and other body fluids is always a result of interaction between proinflammatory cytokines (especially IL-6, TNF-α, IL-1), their receptors, and inhibitory factors. α-2 Macroglobulin (α-2 MG) is a glycoprotein, whose all physiologic functions have not been fully

recognized yet. Despite its role as a protease inhibitor (8), α-2 MG has also an ability to bind cytokines, toxins, bacterial and viral proteins, zinc and hormones (3,4). Thus, α-2 MG can during inflammation or trauma act as biologically active mediator, capable not only to inhibit activity of proteases, but also to capture various proteins and deliver them to macrophages, hepatocytes and other cells engaged in biological detoxication (3,4,8,13,15). In the last years the regulation of distribution and activity of many cytokines is regarded as the most important role of α-2 MG (8). α-2 MG is a carrier of many mediators of immune response, including various cytokines and growth factors : IL-6, IL-1β, TGF-β, b-FGF, NGF (3,4,8). So, α-2 MG may be regarded as one of the paracrine regulators of growth and differentiation of various cells, among them B and T cells (3,4). Special relation connects α-2 MG with IL-6, because this cytokine is able to induce synthesis of the acute phase proteins (2)]. Since α-2 macroglobulin is carrier for IL-6, both proteins, at least in part, can modulate each ather's activity. Thus, changes of α-2 MG levels in peripheral blood may reflect various complex phenomena happening during inflammation.

Moreover, activity of acute phase proteins in psoriasis may help to recognize better, although indirectly, participation of their major inducer IL-6 in pathogenetic events of this disease.

MATERIAL AND METHODS

175 male patients with medium-severe and severe psoriasis were included into the study. Mean age in the group was 38 years, range 17-60. They suffered from psoriasis for 1 to 45 years, mean duration of disease was 16 years.The clinical activity of disease was evaluated by the same investigator according to the PASI score. It ranged from 18 to 70.8; mean PASI value was 29. The group of 175 patients was subdivided into 3 aproximately equal parts: a) 60 patients with active psoriasis, b) 58 patients with chronic psoriasis, c) 57 patients with arthropathic psoriasis. Remission was achieved after 4 to 12 weeks of treatment with various methods: local treatment, retinoids, PUVA, Re-PUVA, methotrexat, cyclosporin A.

The blood samples were taken from all the patients: a) during the active phase of disease, before the treatment was administered; b) after clearing of psoriatic lesions, following the treatment. Control group consisted of 30 healthy male volunteers in apropriate age.

MEASUREMENTS OF PROTEIN CONCENTRATIONS

An enzyme-linked immunosorbent assay (ELISA) was used to detect and quantify the presence of selected proteins in plasma.The kits for ELISA were provided by Endogen

Inc.USA (IL-6); Eucardio Laboratory Inc. USA (CRP); Immunodiagnostik GmbH, Germany (α-2 MG). The measurements were done in duplicates according to the instructions included in the assays. Microplate ELISA Reader Model E 960 Metertech Inc. Austria was used for the assay. The obtained data were put to statistical analysis. Average (M), median (Me), standard deviation (SD), the mean error of the average (SE) and variation coefficient (V%) were evaluated. Significance of differences between the averages was tested by the Student's t-test or the Cochran's and Cox's test.

RESULTS AND DISCUSSION

In 175 patients mean plasma levels of IL-6 , CRP and α-2 MG examined in the active stage of psoriasis were highly significantly elevated ($p<0.001$) when compared with

Tab. 1. Plasma concentrations of IL-6, and α-2MG in 175 psoriatic patients in the acute clinical stage and remission in comparison with the control group

Protein	Group	Statistical characteristics							p	Ig%
		n	min	max	M	SD	SE	V%		
IL-6	acute stage	175	0	72	31.68	20.26	1.53	64.0	< 0.001	2.70
	remission	175	0	18	2.43	4.53	0.34	186.2	< 0.001	1.59
	control	30	0	16	6.27	5.43	0.99	86.6	-	
CRP	acute stage	175	0	26.34	7.067	6.111	0.462	86.5	< 0.001	3.39
	remission	175	0	2.65	0.861	0.552	0.042	64.2	< 0.001	2.47
	control	30	0	0.86	0.289	0.296	0.054	102.5	-	
α-2MG	acute stage	175	106	1200	366.7	254.9	19.3	69.5	< 0.001	2.45
	remission	175	22	210	118.4	43.6	3.3	36.9	> 0.15	1.96
	control	30	30	190	129.4	42.9	7.8	33.1	-	

n - number of patients, M - average, SD - standard deviation, SE - standard error of the average, V% - variation coefficient, p - level of significance

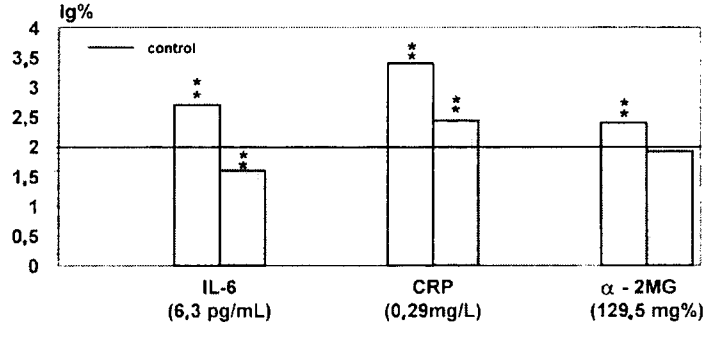

Fig. 1. Plasma concentrations of IL-6, CRP and α-2MG in 175 psoriatic patients in the acute clinical stage and remission expressed as Ig% of the control; 1) control values are expressed below the respective bars, 2) Significance of differences in comparison with control expressed as ⁑($p<0.001$), *($p<0.01$)

Tab. 2. Plasma concentrations of IL-6, CRP and α2MG in the acute stage and remission in 175 patients with relation to the clinical form of psoriasis

Protein	Clinical stage	Form of psoriasis	Statistical characteristics						
			n	nim	max	M	SD	SE	p
IL6	acute stage	active	60	0	72	31.07	18.84	2.43	a b
		chronic	58	0	72	25.93	20.17	2.65	a
		arthropathic	57	0	72	38.18	20.25	2.68	b
	remission	active	60	0	14	2.27	4.41	0.57	A B
		chronic	58	0	12	1.66	3.50	0.46	A
		arthropathic	57	0	18	3.40	5.41	0.72	B
CRP	acute stage	active	60	0	26.34	7.593	4.914	0.634	b
		chronic	58	0	12.19	2.826	2.079	0.273	a
		arthropathic	57	2.28	25.41	10.828	7.271	0.693	
	remission	active	60	0	2.61	0.942	0.433	0.056	b
		chronic	58	0	1.40	0.427	0.383	0.050	a
		arthropathic	57	0.24	2.65	1.218	0.519	0.069	C
α2MG	acute stage	active	60	130	1200	375.6	240.0	31.0	b
		chronic	58	106	1000	279.0	202.2	26.6	a
		arthropathic	57	116	1200	448.5	290.5	38.5	b
	remission	active	60	30	202	121.9	42.0	5.4	A
		chronic	58	22	210	110.4	41.0	5.4	A
		arthropathic	57	22	190	122.8	47.4	6.3	A

n - number of patients, M - average, SD - standard deviation, SE - standard error of the average, p - level of significance. Differences between groups are significant ($p \leq 0.05$) if are not marked with the same letter

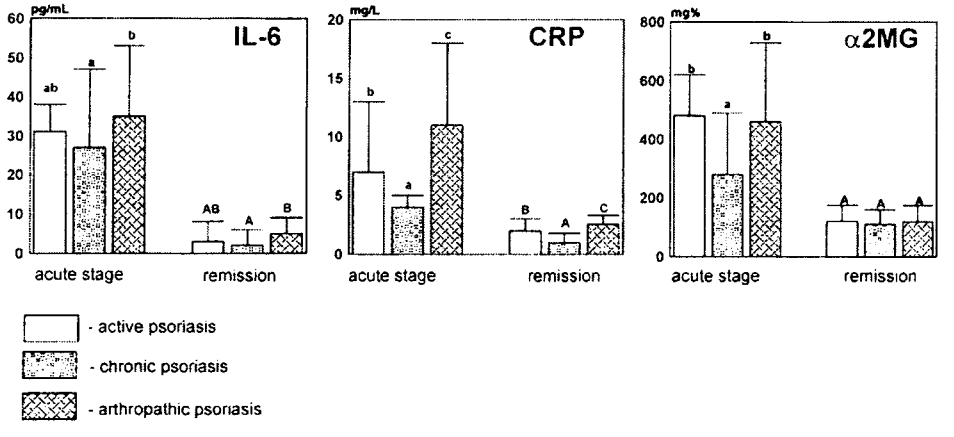

Fig. 2. Plasma concentrations of IL-6, CRP and α-2MG in the acute clinical stage and remission in 175 patients with relation to clinical form of psoriasis; small letters - acute stage of psoriasis, capital letters - remission. Differences between groups are significant ($p \leq 0.05$) if are not marked with same letter

healthy control (Tab.1, Fig.1). In remission the plasma concentrations of these proteins lowered towards the control values. The CRP level, however, despite its deep decrease following all methods of treatment was still significantly higher in comparison with the

control, α-2 MG did not differ from the control ($p>0.15$), and IL-6 after treatment level was even significantly lower than control values (Fig.1).

Concerning the differences in plasma levels of the selected proteins between the three examined groups of patients (Tab.2, Fig.2), the highest IL-6 mean plasma concentration was found in the arthropathic psoriasis group, and the lowest in the chronic psoriasis group. The difference was significant ($p<0.05$). There was no significant difference however, between the groups of active and arthropathic psoriasis (Fig.2).

CRP plasma concentrations examined in three groups of patients differed significantly ($p<0.05$), in the acute clinical stage as well as in remission. The highest mean plasma level was observed in the arthropathic psoriasis group and the lowest in the patients with chronic psoriasis.

α-2 MG mean plasma level before treatment was significantly lower in the chronic psoriasis group ($p<0.05$) in comparison with both arthropathic and active psoriasis groups. There were no significant differences between the three groups after treatment.

Increased levels of IL-6 in the peripheral blood were previously observed in psoriatic patients (7,9,10). It is stressed that activity of this cytokine may not be detected in every case, but only in severe psoriasis (9,10). Some investigators believe that the observed IL-6 increase in circulation is the most probably connected with the enhanced production of this cytokine in psoriatic lesions (10).

Induced by Interleukin-6, C-reactive protein is the most sensitive indicator of inflammation, whose concentration can be increased 100 and more times even in first twenty--four hours and can be lowered to almost normal level during several days after effective treatment or decrease spontaneously with clearing of disease (11,14). In the examined group of psoriatic patients the 25-fold increase in plasma in comparison with the control values was found, but the complete reduction to normal values was not observed. In remission the CRP mean concentrations in plasma were still about 3 times higher than the control values. It means clearly that despite the lack of clinical symptoms of psoriasis there is a persistent inflammatory process in skin.

Some authors stress that there is a direct connection between the CRP level in blood and activity of inflammation (6,11). Kozioł-Montewka et al. (6) observed a considerable increase of serum CRP in dialized patients during peritonitis. Sarnecka--Wysokińska (11) examining the infants with acute infections found out the close relation between decrease of previously elevated CRP and clinical improvement of patients.

It is worth to stress that in the examined psoriatic patients, not only highly elevated concentrations of CRP in the active stage and decrease of this protein in clinical remission were found out, but despite the efficient treatment of psoriasis the CRP plasma levels were still highly significantly higher than those in healthy control. Moreover, the highest CRP concentrations were observed in the group of the most severe suffered psoriatic patients with arthropathic psoriasis. The results relative to arthropathic psoriasis are parallel with the data of other authors (5,14). The connection between the CRP activity in peripheral blood and clinical severity of arthropathic psoriasis was observed previ-

ously (5). Some investigators believe that CRP can be regarded as a very useful parameter in clinical evaluation of arthropathic psoriasis (5). Literature data indicate that CRP activity may be important not only in evaluation of the somatic diseases, for elevation of IL-6 and CRP during the acute stage of depression, and consecutive decrease parallel to clinical improvement was also observed in patients with depressive disorders (12).

In the examined three groups of patients, α-2 macroglobulin mean levels in peripheral blood were also highly elevated in the acute stage of psoriasis, but when remission was achieved as a result of treatment, the levels of this protein decreased to the control values.

In this study the significant difference between elevated concentrations of α-2 MG between arthropatic and active psoriasis was not found. On the other hand, Toruniowa et al. (14), examining the protease inhitors in psoriatic patients observed the significant increase in serum α-2 MG concentrations, but mostly in active type, and not in arthropathic psoriasis. The observed changes in activity of α-2 MG additionally confirm the belief that in the course of severe psoriasis the acute phase response can be mobilized.

CONCLUSIONS

1. In the active stage of psoriasis, before the treatment was administered, highly elevated expression of IL-6, CRP and α-2 MG in plasma of 175 psoriatic patients was found out. Remission achieved as a result of treatment with various methods, was connected with considerable decrease of examined proteins' levels in plasma.

2. Results of this study indicate that in the pathogenic process in psoriasis the acute phase response becomes initiated.

3. Acute phase response is not extinguished completely with achievement of remission, which is evidenced by the presence of elevated concentrations of CRP remaining in the peripheral blood.

4. Persistent elevated plasma levels of CRP indicate that in clinical remission some symptoms of immune preactivation anticipating future relapse of psoriasis can be found.

5. The presence of elevated levels of IL-6, CRP and α-2 MG in peripheral blood and also their changes due to efficient treatment indicate that these proteins are the active participants of pathogenic events in psoriasis.

REFERENCES

1. Ballaun C.,Werninger W., Ulthman A. et al.: Human keratinocytes express the three major splice forms of vascular endothelial growth factor. J. Invest. Dermatol., 104, 7-10, 1995.
2. Borden E. C., Chin P.: Interleukin-6: a cytokine with potential diagnostic and therapeutic roles. J. Lab. Clin. Med., 123, 824-829, 1994.
3. Borth W.: α-2 Macroglobulin, a multifunctional binding protein with targeting characteristics. FASEB J. 6: 3345-3353, 1992.
4. Gonias S. L.: α-2 Macroglobulin: a protein at the interface of fibrinolysis and cellular growth regulation. Exp. Hematol., 20, 302-311, 1992.
5. Helliwell P. S., Marchesoni A., Peters M. et al.: Cytidine deaminase activity, C-reactive protein, histidine and erythrocyte sedimentation rate as measures of disease activity in psoriatic arthritis. Ann. Rheum. Dis., 50, 362-365, 1991.
6. Kozioł-Montewka M., Książek A., Janicka L. et al.: Serial cytokine changes in peritoneal effluent and plasma during peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD). Inflamm. Res., 46, 132-136, 1997.
7. Krasowska D., Pietrzak A., Lecewicz-Toruniowa B.: Badanie stężenia IL-6 w surowicy krwi pacjentów z łuszczycą i liszajem płaskim. Przeg. Dermatol., 83,3, 251-256, 1996.
8. LaMarre J., Wollenberg G. K.,Gonias S. L. et al.: Cytokine binding and clearance properties of proteinase-activated α-2 macroglobulin. Lab. Invest., 65,1, 3-14, 1991.
9. Neuner P., Urbański A., Trautinger F. et al.: Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J. Invest. Dermatol., 97, 27-33, 1991.
10. Prens E.P., Benne K., Van Damme J. et al.: Interleukin-1 and Interleukin-6 in psoriasis. J. Invest. Dermatol., 95, 121-124, 1990.
11. Sarnicka-Wysokińska B.: Wartość diagnostyczna białka C-reaktywnego w ostrych zakażeniach u niemowląt. Praca doktorska, Akademia Medyczna w Lublinie, 1996.
12. Seidel A., Arolt V., Hunstiger M. et al.: Cytokine production and serum proteins in depression. Scand. J. Immunol., 41, 534-538, 1995.
13. Steel D.M., Whitehead A.S.: The major acute phase reactants. Immunol. Today, 2, 81-88,1994.
14. Toruniowa B., Pietrzak A., Krasowska D. et al.: Białko C-reaktywne i inhibitory proteaz w różnych postaciach łuszczycy. Post. Dermatol., 11, 55-62, 1994.
15. Williams I.R., Kupper T.S.: Immunity at the surface: homeostatic mechanisms of the skin immune system. Life Sciences, 58,18, 1485-1507, 1996.

Otrz.: 2000.02.17

STRESZCZENIE

Badano stężenia osoczowe Interleukiny-6 (IL-6), białka C-reaktywnego (CRP) i α-2 makroglobuliny (α-2 MG) u 175 chorych z łuszczycą średnio-ciężką i ciężką. W zależności od rodzaju łuszczycy podzielono chorych na trzy grupy: grupę łuszczycy aktywnej, przewlekłej i stawowej. Aktywność wybranych białek oznaczano w osoczu przy pomocy metody ELISA w okresie objawowym łuszczycy przed leczeniem oraz po uzyskaniu remisji klinicznej w następstwie leczenia. Stwierdzono wysoce istotne podwyższenie stężeń IL-6 i wybranych białek ostrej fazy ($p<0.001$) w aktywnym okresie choroby oraz obniżenie poziomu badanych białek w kierunku wartości kontrolnych wraz z uzyskaniem ustąpienia zmian skórnych. Jednak, pomimo znacznego obniżenia, stężenia osoczowe CRP pozostały w stanie klinicznej remisji nadal znacznie podwyższone w porównaniu z kontrolą. Uzyskane wyniki wskazują, że w ciężkiej łuszczycy dochodzi do uruchomienia odpowiedzi ostrej fazy i że ta odpowiedź nie wygasa całkowicie wraz z osiągnięciem remisji zmian skórnych.