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Activity of Tumor Necrosis Factor-α (TNF-α) and its soluble type I receptor (p55TNF-R) in some drug-induced cutaneous reactions

Adverse drug-induced cutaneous reactions are the heterogenic group of disorders connected with immune response of various types. Cytokines are believed to be the crucial mediators engaged in immune pathogenic events leading to development of these skin reactions. TNF-α is a pleiotropic cytokine playing protective or damaging role in inflammatory and immune reaction, depending on target cell and intensity of inflammation (12). TNF-α is released by various types of cells: monocytes/macrophages, T cells, NK cells, keratinocytes, Langerhans' cells, mast cells, neutrophiles and fibroblasts (2,4,9,11,12,14). Apart from many internal and external stimuli, the main factors enhancing TNF-α expression are other cytokines present in skin environment: IL-2, IFN-γ, GM-CSF (4,7,11,15). Moreover, TNF-α itself acting autocrinally is also capable of increasing its production by cells (4,7,11). On the other hand, TNF-α can induce the release of many proinflammatory cytokines in the skin, including IL-1, IL-6, IL-8, G-CSF, GM-CSF, LIF, TGF-α (2,3,10,14). TNF-α belongs to "primary cytokines", due to its ability to activate sufficient numbers of effector mechanisms to induce skin inflammation independently of other proinflammatory factors (15). As an inflammatory promotor, TNF-α can act both directly and by inducing the "secondary cytokines" release (15). In skin diseases, TNF-α is regarded as a mediator of disorders connected with lymphocyte infiltrations, including psoriasis, atopic dermatitis, toxic epidermal necrolysis (2,10). In drug-induced cutaneous diseases, the partake of this cytokine in recruitment of effector cells into inflammatory foci seems to be of special importance. TNF-α effects the early stage of cell migration which is the adhesion of neutrophiles, T cells and monocytes to endothelium. This powerful influence occurs via increasing expression of ICAM-1, ELAM-1 and VCAM-1 on the endothelial cells (2,6,10,14). TNF-α takes part in generating systemic symptoms of inflammation, such as the acute phase response (2,10). Being a multifunctional cytokine, TNF-α can act both as a positive and negative regulator of proliferation and differentiation concerning various cell types. The final effect of TNF-α influence upon cells depends on a complex interaction between this cytokine and other synergetic and antagonistic cytokines coming from various cell types (10). TNF-α also exerts a powerful influence on other inflammatory mediators, including prostaglandines, adhesion molecules, colagenase, chemoatractants and angiogenic factors (10).

TNF-α performs its biological function through binding with the specific receptors on the cell surface: p55TNF-R (type I TNF receptor) and p75TNF-R (type II TNF receptor) (3,4,6,8,10,12). Both types of receptor can also bind TNF-β with similar affinity (13). Studies concerning the presence and localization of TNF receptors in the skin revealed their presence on

epithelial cells, endothelial cells and cutaneous fibroblasts (12). p55TNF-R can be found in human normal and transformed keratinocytes in tissue cultures (6,12). In normal skin this receptor is present in the whole alive layer of epidermis (6,12). Common presence of TNF receptors on various types of cells indicates their important biological role. TNF receptors constitute natural inhibitors of the cytokine blocking its biological activity (3,8,13). TNF-α and its receptor can modulate each other's activity, because the expression of membrane receptor is induced by TNF-α (8). Elevated levels of soluble TNF-α receptors were found in the peripheral blood and urine of patients in many various diseases, especially including infections, immune disorders, and malignancies (3,4,8,12).

The research data indicate the partake of p55TNF-R not only in antimicrobial defense but also in the induction of necrosis or apoptosis in the course of various diseases (4,8,12). It was found that p55TNF-R had similar cytoplasmic sequencies as Fas antigen (13). p55 TNF-R and other proteins creating the TNF-receptors family are engaged in transmitting death signals into cells and due to this ability described as "death receptors", although it is not entirely elucidated yet whether the function of p55 and p75 receptors in apoptosis is not different (5,13). It is believed now that TNF-α, its natural inhibitor p55TNF-R and Fas antigen create a functional system consisting of the elements affecting each other and modulating their activity (5,13).

MATERIAL AND METHODS

126 patients with drug-induced cutaneous reactions were included into the study. Among them there were 61 women and 65 men, aged from 18 to 77 years, mean age 41.5 years. The control group consisted of 30 healthy volunteers of appropriate age.

All patients were subdivided into 6 following groups: 1) maculopapular eruptions (ME) – 40 patients, 2) drug-induced urticatia (DU) – 33 patients, 3) erythema multiforme (EM) - 24 patients, 4) erythema multiforme + erythema nodosum (EMN) – 6 patients, 5) hyperergic vasculitis (HV) – 14 patients, 6) Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) – 9 patients.

Blood samples were taken from all the patients: a) during the acute stage of disease, before the treatment was administered; b) after clearing of skin lesions following effective treatment.

Measurement of TNF-α and soluble p55TNF-receptor concentrations: An enzyme-linked immunosorbent assay (ELISA) was used to detect and quantify the presence of TNF-α and p55TNF-R in plasma. The kits for ELISA were provided by Endogen Inc. USA. The measurements were performed in duplicates according to the instructions included in the assays. 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 average values was tested by the Student's t-test and Mann-Whitney's test.

RESULTS

TNF-α PLASMA CONCENTRATIONS

In the acute stage of drug-induced skin reactions highly significantly ($p<0.001$: ME, EM, DU, HV, SJS/TEN) or significantly ($p<0.05$: EMN) elevated TNF-α mean level in comparison with healthy control was found in the peripheral blood (Tab. 1). After clearing of skin lesions, TNF-α mean concentrations, despite a decrease, still remained highly significantly or

Table 1. Plasma concentrations of TNF-α (pg/ml) in 126 patients with drug-induced skin reactions

Patients	Group	Statistical characteristics						Comparison		
		n	M	SD	Min	Max	V%	with control		after vs before
								p	lg%	p
ME	C	30	2.57	3.03	0.00	10.20	117.80			
	P_1	40	24.01	11.29	8.30	64.60	47.03	p < 0.001	2.97	p < 0.001
	P_2	40	7.47	4.97	0.00	17.30	66.55	p < 0.001	2.46	
EM	C	30	2.57	3.03	0.00	10.20	117.80			
	P_1	24	25.96	12.33	8.60	48.30	47.50	p < 0.001	3.00	p < 0.001
	P_2	24	7.92	5.53	0.00	20.80	69.97	p < 0.001	2.49	
EMN	C	30	2.57	3.03	0.00	10.20	117.80			
	P_1	6	30.77	12.40	12.60	44.90	40.31	p < 0.01	3.07	p < 0.05
	P_2	6	7.48	4.89	0.90	16.10	65.34	p < 0.01	2.46	
DU	C	30	2.57	3.03	0.00	10.20	117.80			
	P_1	33	19.59	10.32	0.00	38.70	52.70	p < 0.001	2.88	p < 0.001
	P_2	33	6.46	4.66	0.00	14.30	72.10	p < 0.001	2.40	
HV	C	30	2.57	3.03	0.00	10.20	117.80			
	P_1	14	28.47	12.50	13.90	64.60	43.89	p < 0.001	3.04	p < 0.001
	P_2	14	11.66	5.54	0.90	18.50	47,51	p < 0.01	2.65	
SJS/TEN	C	30	2.57	3.03	0.00	10.20	117.80			
	P_1	9	26.64	6.79	14.60	37.60	25.49	p < 0.001	3.01	p < 0.01
	P_2	9	8.17	4.10	3.70	13.70	50.18	p < 0.001	2.50	
All patients	C	30	2.57	3.03	0.00	10.20	117.80			
	P_1	126	24.23	11.45	0.00	64.60	42.27	p < 0.001	2.97	p < 0.001
	P_2	126	7.81	5.13	0.00	20.80	65.73	p < 0.001	2.48	

C – control, P1 – patients before treatment, P2 – patients after treatment

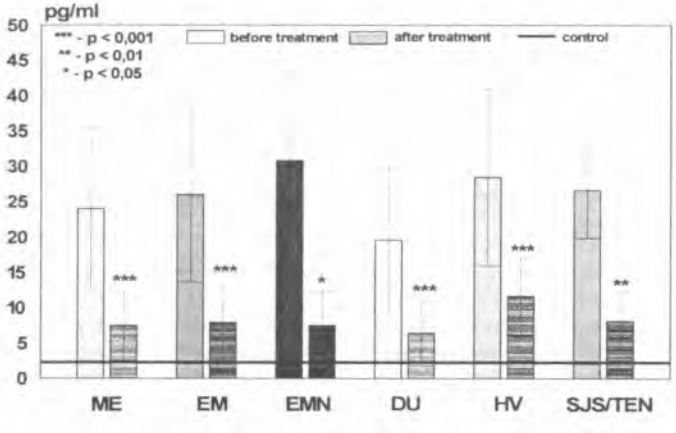

Fig. 1. Plasma concentrations of TNF-α (pg/ml) in 126 patients with drug-induced skin reactions before and after treatment

significantly (EMN) elevated when compared with the control. Recovery of clinical symptoms was connected with highly significant (ME, EM, HV) or significant (EMN, DU, SJS/TEN) decrease of TNF-α mean plasma concentrations in comparison with the before treatment values (Fig. 1). The lowest plasma TNF-α level before and after treatment was found in DU patients. The highest before treatment cytokine concentration appeared in EMN patients, but after treatment – in HV group.

P55 TNF-R PLASMA CONCENTRATIONS

In the acute stage of drug-induced cutaneous reactions highly significant elevation of p55TNF-R mean plasma concentrations ($p<0.001$) was found in the whole group of 126 patients in comparison with the control group (Tab. 2). When clinical recovery was achieved,

Table 2. Plasma concentrations of p55TNF-R (pg/ml) in 126 patients with drug-induced skin reactions

Patients	Group	Statistical characteristics						Comparison		
		n	M	SD	Min	Max	V%	with control		after vs before
								p	lg%	p
ME	C	30	210.50	73.97	52.00	352.00	35.14			
	P_1	40	324.00	124.97	156.00	742.00	38.57	< 0.001	2.18	p < 0.001
	P_2	40	211.43	72.14	62.00	408.00	34.12	> 0.05	2.00	
EM	C	30	210.50	73.97	52.00	352.00	35.14			
	P_1	24	362.33	289.56	214.00	1670.00	79.92	< 0.001	2.24	p < 0.001
	P_2	24	249.50	183.17	106.00	1080.00	73.41	> 0.05	2.07	
EMN	C	30	210.50	73.97	52.00	352.00	35.14			
	P_1	6	884.33	758.12	366.00	2270.00	85.73	< 0.001	2.62	p < 0.05
	P_2	6	435.33	375.21	220.00	1162.00	86.19	< 0.001	2.31	
DU	C	30	210.50	73.97	52.00	352.00	35.14			
	P_1	33	322.85	183.75	168.00	1170.00	56.92	< 0.001	2.18	p < 0.001
	P_2	33	208.70	82.17	48.00	472.00	39.37	> 0.05	1.99	
HV	C	30	210.50	73.97	52.00	352.00	35.14			
	P_1	14	646.71	470.12	218.00	1730.00	72.69	< 0.001	2.48	p < 0.001
	P_2	14	349.14	205.60	104.00	840.00	58.89	< 0.001	2.22	
SJS/TEN	C	30	210.50	73.97	52.00	352.00	35.14			
	P_1	9	566.89	749.68	204.00	2550.00	132.25	< 0.001	2.43	p < 0.01
	P_2	9	466.22	716.09	140.00	2370.00	153.59	> 0.05	2.34	
All patients	C	30	210.50	73.97	52.00	352.00	35.14			
	P_1	126	410.89	366.80	156.00	2250.00	89.27	< 0.001	2.29	p < 0.001
	P_2	126	262.13	243.44	48.00	2370.00	92.87	> 0.05	2.09	

C – control, P1 – patients before treatment, P2 – patients after treatment

mean concentrations of the receptor, were in ME, EM, DU, SJS/TEN groups not different from the control values ($p>0.05$), but in EMN and HV groups, although considerably decreased, remained still significantly ($p<0.05$) or highly significantly ($p<0.001$) elevated than in the healthy control. Clinical recovery due to the treatment was connected with highly significant (ME, EM, DU, HV) or significant (EMN, SJS/TEN) decrease of p55TNF-R mean concentrations in comparison with the before treatment values. The lowest plasma level of p55TNF-R before treatment was found in DU patients and the highest in EMN group, and after treatment the lowest plasma level was still found in DU group, but the highest in SJS/TEN group (Fig. 2).

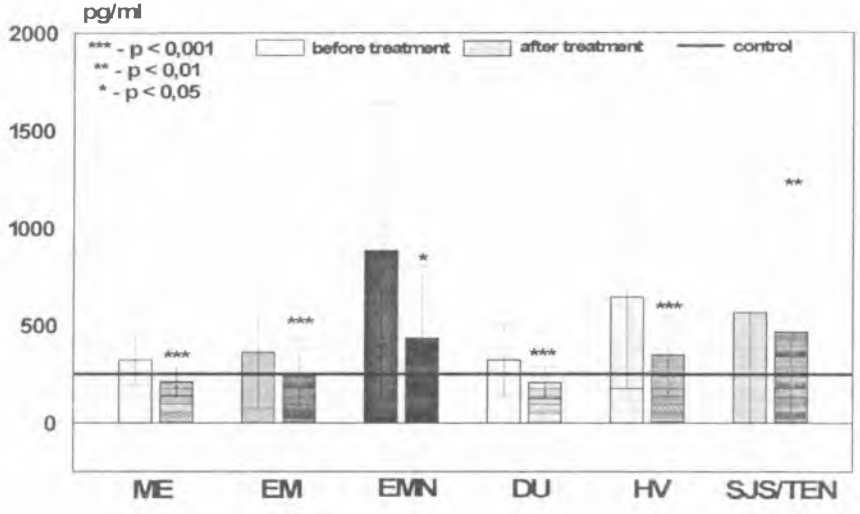

Fig. 2. Plasma concentrations of p55TNF-R (pg/ml) in 126 patients with drug-induced skin reactions before and after treatment

DISCUSSION

Many authors stress central role of TNF-α in cytokine network, because this protein can influence cytokine production by various cells involved in cutaneous inflammation (2,7,6,11). Moreover, being essentially a proinflammatory mediator, TNF-α takes part in compensatory anti-inflammatory response through stimulation of IL-10 production by monocytes/ macrophages (11). The activity of cytokine is usually accompanied by activation of cytokine receptors, both present on the surface of appropriate cells and their soluble form. Soluble receptors released from the cell membrane constitute an essential element of immune homeostasis, because they can modulate both positively and negatively the response of specific cytokines.

Considerable elevation of both TNF-α and its p55 soluble receptor plasma concentrations was found in 126 patients during the acute stage of drug-induced skin reactions. The activity of both these proteins was decreasing towards the control values following the clinical improvement. But despite the deep decrease, the cytokine and its receptor mean levels still remained significantly increased in some groups of patients when compared with healthy control. It is worth to stress that slight presence of TNF-α and p55TNF-R in peripheral blood of

the healthy subjects was also observed by other authors (1,9,12). There was a similarity in the activity changes of the cytokine and its receptor in the course of drug-induced urticaria. The concentration of these two proteins both before and after treatment were the lowest in this group of patients, possibly due to their lesser engagement in type I immune response in urticaria.

The obtained data indicate that the increased activity of the powerful proinflammatory mediator – TNF-α during acute cutaneous inflammation is followed by elevation of its antagonist – p55 TNF-receptor. It can suggest that TNF-α activation can immediately mobilize mechanisms of immune homeostasis leading to increased expression of its natural inhibitor. This finding can also support the belief that TNF-α and p55TNF-R create a specific functional system consisting of two factors crossreacting and modulating each other's biological activity.

CONCLUSIONS

1. Drug-induced skin reactions are connected with increased activity of TNF-α and its type I p55 receptor in the peripheral blood.
2. Changes of TNF-a and p55TNF-R plasma activity are parallel to the clinical course, which indicates the involvement of these proteins in the pathogenic events of drug-induced skin reactions.

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SUMMARY

Plasma concentration of TNF-α and its type I receptor (p55TNF-R) was examined in 126 patients with drug-induced skin reactions using immunoenzymatic ELISA method. Patients were subdivided into 6 groups: maculopapular eruptions (ME), erythema multiforme (EM), erythema multiforme coexisting with erythema nodosum (EMN), hyperergic vasculitis (HV), Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN. In the acute clinical stage highly significant ($p<0.001$) or significant ($p<0.01$) elevation of mean plasma concentrations of the cytokine and its receptor was found in all examined groups in comparison with the control. Clearing of clinical symptoms was connected with considerable decrease ($p<0.001$, $p<0.01$) of mean plasma levels of the both proteins in comparison with the before treatment values. TNF-α concentrations still remained significantly more elevated than those observed in the control. The results indicate that plasma activity of TNF-α and its p55 receptor change with the clinical course of the examined drug-induced skin reactions, which suggests the partake of both proteins in the pathogenesis of these diseases.

Aktywność czynnika martwicy nowotworów -α (TNF-α) i jego rozpuszczalnego receptora typu I (p55TNF-R) w skórnych reakcjach polekowych

Badano osoczowe stężenia Tumor Necrosis Factor-α (TNF-α) i jego receptora typu I (p55TNF-R) u 126 chorych ze skórnymi reakcjami polekowymi, posługując się metodą immunoenzymatyczną ELISA. Wyróżniono 6 badanych grup: osutki plamistogrudkowe (ME), rumień wielopostaciowy (EM), rumień wielopostaciowy współistniejący z rumieniem guzowatym (EMN), ostrą pokrzywkę polekowa (DU), hyperergiczne zapalenie naczyń (HV) oraz zespół Stevens-Johnsona i toksyczną nekrolizę naskórka (SJS/TEN). W ostrym okresie zmian klinicznych stwierdzono wysoce statystycznie istotne ($p<0,001$) lub statystycznie istotne ($p<0,01$) podwyższenie średnich stężeń cytokiny i jej receptora we wszystkich badanych grupach w porównaniu z kontrolą. Ustąpienie zmian klinicznych wiązało się ze znacznym obniżeniem ($p<0,001$, $p<0,01$) średnich osoczowych stężeń obu białek w porównaniu z wartościami przed leczeniem. Stężenia TNF-α pozostały nadal statystycznie istotnie wyższe niż obserwowane w grupie kontrolnej. Uzyskane wyniki świadczą o tym, że aktywność osoczowa TNF-α i jego receptora p55TNF-R zmienia się wraz z klinicznym przebiegiem wybranych polekowych chorób skóry, co może wskazywać na udział obu badanych białek w patogenezie tych chorób.