The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/XAMQJ

THE ROLE OF PRO-INFLAMMATORY CYTOKINES IN VARIOUS TYPES OF SYSTEMIC SCLERODERMA

ARIPOVA N.A [1], DJURAYEVA E.R [2], ABDUAZIZOVA N.X [3], BERDIYEVA D.U [4], GANIYEVA N.A [5] and ZIYAYEVA F. K [6]

[1, 5]Assistant of Department of faculty and Hospital Therapy No1 with Course of Professional Pathology of Tashkent Medical Academy of Uzbekistan.
[2, 3] Ph.D, Associate Professor of Department of Faculty and Hospital Therapy No1 with Course of Professional pathology of Tashkent Medical Academy of Uzbekistan.
[4] Ph.D, Assistant of Department of Faculty and Hospital Therapy No1 with Course of Professional Pathology of Tashkent Medical Academy of Uzbekistan.
[6] Senior Lecturer of Department of Faculty and Hospital Therapy No1 with Course of Professional Pathology of Tashkent Medical Academy of Uzbekistan.

Abstract

Purpose of the study. To study the relationship between the serum level of interleukin-4 (IL-4) and visceral pathology, the nature of the course and clinical forms of SS. Materials and methods. IL-4 was determined in the sera of 60 patients with SS by indirect enzyme-linked immunosorbent assay. Results. The level of IL-4 in the range of 10-1000 pg/ml was detected in 18 out of 60 patients with SS (30%). Distinctive features of this group of patients were a shorter duration of the disease, progression of skin fibrosis and visceral pathology by the time of the examination, and a tendency towards a higher incidence of pulmonary fibrosis. Significant differences in the damage to other internal organs, as well as the dependence of the content of IL-4 on the clinical forms and course of the disease, were not revealed. In patients with an increase in IL-4 in the blood, higher levels of CIC, γ-globulins were noted, while the content of acute phase reactants was lower than in the rest of the group. Output. The established relationship between the serum level of IL-4 and the activity of the fibrous process in SS requires confirmation in prospective studies.

Keywords: Interleukin-4. T Lymphocytes, Fibrosis, Activity, Systemic Scleroderma

INTRODUCTION

Systemic scleroderma (SS) is an autoimmune disease characterized by excessive synthesis of collagen and other components of the intercellular matrix by fibroblasts, leading to fibrosis of the skin and internal organs. Although the pathogenesis of the disease is largely unclear, the leading role of T cell immune mechanisms in the initiation and progression of the fibrotic process is generally recognized. The basis of these disorders is dysregulation in the Th1 and Th2 system of lymphocytes with hyperreactivity of Th2 cells secreting fibrogenic cytokines [10, 13]. The latter are capable of activating fibroblasts, causing their migration, proliferation, and excessive synthesis of matrix proteins [3].

Interleukin-4 (IL-4) is one of the Th2 dependent cytokines, presumably involved in the development of scleroderma fibrosis. Its overexpression has been found in the skin [111, peripheral blood mononuclear cells [12], and bronchoalveolar lavage cells [1] of SS patients. The stimulating effect of IL-4 on the synthesis of collagen by fibroblasts of scleroderma skin, activation of the synthesis of fibronectin and tenascin has been proven [5, 6]. The key role of

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ISSN 1533-9211
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IL-4 in the development of the disease is also confirmed by experimental data on the possibility of preventing skin fibrosis in Tsk/mice (experimental model of SS) by artificially induced mutation in the IL-4 receptor gene, leading to loss of sensitivity of fibroblasts to this cytokine [8].

The clinical aspect of the study of IL-4 in SS is represented by a few works, the data obtained are contradictory both in relation to the presence of this cytokine in the blood of patients with SS, and associations with the clinical manifestations of the disease [14].

The aim of this work was to study the clinical significance of IL-4 in patients with SS with various forms, course variants, and stages of the disease.

Material and methods

The study included 60 patients with SS: 54 women and 6 men aged 24 to 72 years, 50.33 ± 12.37 years on average, with acute (22), subacute (10) and chronic (28) course of the disease. Twenty-nine patients had a limited form of SS (lSS) and 10 had a diffuse form (dSS). All patients with dSS and 26 lSS met the diagnostic criteria of the ARA. The duration of the disease (from the onset of scleroderma skin lesions) averaged 8.22 ± 7.1 years, in 18 people (30%) the duration of SS did not exceed 3 years (early stage). To determine the severity and prevalence of skin induration, a skin score was used in the modification of M.B. Kaheleh 1986 (total scoring of skin density in 26 skin zones on a 3-point scale). The assessment of the dynamics of skin fibrosis in most patients (32 people) was carried out by comparing the indicators of the skin score at the time of the study with the results of the previous hospitalization (for a period of 6-12 months), in 8 patients admitted to the IR for the first time, the assessment was carried out according to the patient's words according to changes in the density and prevalence of the lesion skin for the previous month. In addition to the clinical characteristics of skin, vascular and visceral pathology, all patients were determined immunological (CIC by nephelometry, RF by latex agglutination, ANF by immunofluorescence on histological sections of rat liver and on Hep-2 cell culture with registration of titer and type of luminescence, anticentromeric antibodies , antibodies to Scl-70, immunoglobulins of classes G, A, M by the Mancini method) and general blood parameters (shaped elements, ESR, C-reactive protein, total protein and protein fractions, creatinine, CPK).

IL-4 was determined in the sera of patients by indirect enzyme-linked immunosorbent assay (ELISA) using the ProCon IL-4 reagent kit (Protein Contour LLC, St. Petersburg). The results were recorded on a spectrophotometer (Dynatech) at a wavelength of 450 nm. According to the literature data, the upper limit of normal serum IL-4 is 10 pg/ml [7]. In the sera of healthy individuals, IL-4 at the indicated concentration occurs in 0-1% of cases.

Statistical processing of the results was carried out using the t-test, %2-test and correlation analysis (Pearson's correlation coefficient).

RESULTS

All patients had peripheral and visceral symptoms characteristic of SS, including skin lesions

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ISSN 1533-9211
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(dense edema in 24 patients, induration in 14, average skin score 8.1 ± 7.3 points), vascular pathology (Raynaud's syndrome in all patients, trophic vascular disorders - in 24), damage to the musculoskeletal system (arthritis - 9, myositis - 2, muscle weakness - 5) and internal organs (scleroderma lesion of the esophagus - 31, lungs - 29, heart - 24, kidneys - 1, pulmonary hypertension - 8). Progression of skin fibrosis was noted in 10 patients, visceral pathology in 11. 22 patients (55%) included in the study received treatment with: prednisolone (22), D-penicillamine (12), cytotoxic drugs (6), aminoquinoline drugs (3) , NSAIDs (5) patients; extracorporeal methods of therapy (plasmapheresis) were used in 2 patients.

ANF in the study on cell culture Hep-2 was detected in 90% of patients. In all cases, a mottled type of luminescence was noted, in 27% it was combined with a homogeneous one, in 6% - with a nucleolar one. In 30% of patients, SS-specific autoantibodies were found: Scl-70 (17%) or anticentromeric antibodies (13%).

An increase in the level of IL-4 more than 10 pg / ml was detected in the sera of 18 out of 60 patients with SS, the clinical characteristics of which are presented in table 1.

Table 1: Clinical characteristics of patients who have an increase in serum IL-4 > 10 pg/ml

patients	Form of SS	Course	Duration (years)	skin score (points)	Trophic disorders	Scleroderma lesion:					
						Articular-muscular	gastrointestinal tract	lungs	Pulmonary hypertension	heart	kidney
1.S.	d	acute	2	29*	Scars	pain	+	dif.		+ *	+*
2.V	d	acute	3	16*	Scars	-	+*	dif.	-	+*	-
3.R.	d	acute	1	16*	-	Arthritis	+*	bas.	+	+*	-
4.N.	d	chronic	0,5	4*	Scars	-	-	-	-	+*	-
5.N.	d	chronic	2	4*	-	-	-	Диф.	-	+*	-
6.Sh.	d	acute	4	16	Scars	-	+	bas.	-	-	-
7.Z.	1	chronic	1	5*	-	Arthritis	+*	Диф.	-	-	-
8.Sh.	1	chronic	4	6	-	pain	+	bas.	-	+	-
9.I.	1	chronic	10	8	-	-	+	bas		-	-
10.A.	1	subacute	6	4	Scars	Arthritis	+	dif.	+	+	-
11.P.	1	chronic	18	1	-	-	-	bas.		-	-
12.K.	1	subacute	8	4	-	-	-	-	-	+	-

Note: * - progression by the time of examination, + - available; - - missing

In half of the patients in this group, the duration of the disease did not exceed 3 years, skin changes in all cases were characterized by dense edema, and the prevailing visceral pathology was lung damage (diffuse pneumofibrosis in 5 cases). The progressive course of the disease was observed in 58% of patients. In 4 out of 5 patients who are clinically stable, there has been an increase in laboratory activity over the past year. Half of the patients in this group were first admitted to the Institute of Rheumatology with an exacerbation of SS due to the absence or ineffectiveness of therapy. Most of them were prescribed corticosteroids, cytotoxic drugs and/or D-penicillamine in the clinic. Clinical characteristics of patients depending on the level of IL-4 in the blood are presented in Table 2.

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ISSN 1533-9211
DOI 10.17605/OSF.IO/XAMQJ

Table 2: Clinical parameters of patients with SS depending on the content of IL-4 in the blood

parameters	IL-4 >10 pg/ml (n=18)	IL-4 < 10 pg/ml (n=42)	P
Age	52+10,5	50±13,2	>0,5
Duration (years)	5±5,03	9±7,7	0,03
Progression of the skin process and / or visceral pathology	58%	14%	<0,005
lSS	66,7%	79%	0,4
dSS	33,3%	21%	0,4
Course: acute	33,7%	35,7%	>0,9
subacute	16,7%	17,9%	>0,9
chronic	50%	46,4%	>0,9
Skin lesion: dense swelling	100%	50%	<0,001
induration	0%	50%	<0,001
Skin account	9,4±8,2	13±1,9	0,3
Telangiectasias	33,3%	46,4%	0,4
Raynaud's syndrome	100%	100%	>0,9
Trophic disorders	41,7%	67,9%	0,1
Calcinosis	16,7%	39,3%	0,1
Arthralgia/arthritis	16,7%	29%/21%	0,8/0,8
Muscle weakness/myositis	0%	14%7%	0,04/0,16
Damage: gastrointestinal tract	58,3%	85,7%	0,06
lungs	83,3%	67,9%	0,29
heart	67%	60,7%	>0,6
kidneys	8,3%	0%	>0,3
Immunosuppressive therapy	33%	64%	0,08

In a comparative analysis, it was noted that IL-4 was detected much more often in the group of patients with a shorter duration of the disease, a progressive course of SS, and skin lesions in the stage of dense edema. The high incidence of scleroderma lung disease in these patients with a significantly shorter duration of SS emphasizes the severity of the pathological process. Some predominance of the frequency and severity of trophic disorders, telangiectasias, calcification and gastrointestinal lesions in patients with low levels of IL-4 in the blood corresponded to a longer duration of the disease in this group. Significant differences in the damage to other internal organs, as well as the dependence of the content of IL-4 on the clinical forms and the initial variant of the course of SS, were not revealed.

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ISSN 1533-9211
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In the group of patients with serum IL-4>10 pg/ml, the average values of such laboratory activity indicators as CIC (297+133 U and 200±143 U, p=0.05) and gamma globulins (24±5.2% and 21 ±3.9%, p=0.03) were significantly higher. At the same time, the levels of acute phase proteins: CRP (0.31±0.26 mg% and 0.68±0.8 mg%, p=0.06) and fibrinogen (3.22±1.2 g/l and 3. 54±0.8 g/l, p=0.2), alpha-2 globulins (10±0.9% and 11+1.5%, p=0.04) were lower than in the group of patients with IL content -4 in blood<10 pg/ml. The frequency of ANF, a-Scl-70 and anticentromeric antibodies, as well as the titer of ANF did not differ between the two groups of patients. Serum IL-4 levels did not correlate with ANF titer (r=0.04, p>0.5).

DISCUSSION

To date, we can confidently speak of a defect in immunoregulation, leading to uncontrolled synthesis of fibroinducing cytokines, as an important factor in the pathophysiology of fibrosis in SS [3, 13, and 15].

We conducted a study of the content of IL-4, one of the main fibrogenic cytokines, in the blood of patients with SS in comparison with the clinical manifestations of the disease, forms and features of the course.

IL-4 in an amount of > 10 pg/ml was detected in the sera of 30% of patients, which is consistent with the data of other authors on the presence of this cytokine in the blood of 21-38% of patients with SS [7, 14]. A number of features have been established during the course of the disease in this group of patients: negative clinical and laboratory dynamics by the time of examination (increased skin thickening, the appearance or progression of existing visceral pathology, an increase in immunological activity) and the presence of high laboratory activity (CIC, y-globulins). The need to prescribe immunosuppressive therapy in most cases confirms the active course of the fibrous process in these patients. At the same time, the relationship of IL-4 with such clinical manifestations of the disease as arthritis and polymyositis has not been established. These data, as well as the reverse nature of the relationship between IL-4 and acute phase reactants, suggest a different genesis of the fibrous and inflammatory joint-muscular process in scleroderma, which are currently combined in the concept of SS activity.

The content of IL-4 in the blood reflected the activity and dynamics of the skin process, but not its prevalence at the time of the examination. Obviously, IL-4 is involved in the development of fibrosis, regardless of the already existing area of skin lesions and can increase with the progression of the process in both patients with limited and diffuse forms of SS. The parallelism between the dynamics of skin fibrosis and serum levels of IL-4 was also confirmed by H.Inh et al. [2]

As is known, the progression of SS is represented by the development of not only skin fibrosis, but also the involvement of internal organs in the fibrous process. The frequent development of pulmonary fibrosis noted by us already at an early stage of the disease in individuals with elevated levels of IL-4 is consistent with the data of S. Atamas et al. about the high content of IL-4 and IL-4 mRNA in bronchoalveolar lavage cells in patients with SS with severe

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progressive lung damage. Cytokine levels have been identified by these authors as a predictor of significant deterioration in lung function over time [1]. It should be noted that the action of IL-4 as an activator of fibroblasts in the lungs is not strictly specific for SS, but is probably universal. With the development of a fibrous process in the lungs, regardless of the initial disease (SS, bronchial asthma, idiopathic fibrosing alveolitis), a similar morphological pattern is observed with activation of CD8+ cells that produce the profibrogenic spectrum of cytokines (IL-4 and IL-5), along with a decrease in the production of antifibrotic cytokines (IF-gamma). Thus, dysregulation in the system of T-lymphocytes with excessive production of IL-4 seems to be the main factor in the development of both skin and organ fibrosis in SS.

A slight predominance of IL-4 in the sera of patients with a diffuse form of SS compared with a limited one is probably due to a higher frequency of acute progressive cases of the disease among the first group, and not to pathogenetic differences between these forms. This is evidenced by the absence of IL-4 in patients with dSS with an acute course at the beginning, but who are in remission at the time of the examination. Also, no relationship was found between the content of IL-4 and the presence of SS-specific antibodies (anticentromeric and antitopoisomerase). Thus, an increase in the level of IL-4 may reflect the activity of the fibrous process, regardless of the clinical form of SS.

The high content of IL-4 in the blood of patients with early SS suggests the participation of this cytokine in the initial processes of fibrosis. This is confirmed by experimental data on the important role of IL-4 in the formation of mononuclear infiltrates, stimulation of fibroblast adhesion to T lymphocytes and activation of fibroblasts in key processes of the early stage of SS [9, 15]. These mechanisms retain their importance in the progression of fibrosis.

The main source of blood IL-4 is circulating T lymphocytes [19], so the cytokine level may indicate the intensity of T cell activation, which is accompanied by migration of lymphocytes into tissues and the formation of T cell infiltrates in SS. Therefore, the level of IL-4 in serum may indirectly reflect the severity of local immune processes associated with fibrosis. We consider this as one of the possible explanations for the relationship between blood levels of IL-4 and SS activity. Correlation of IL-4 levels with IL-2, a well-known marker of T-cell activation and progression of SSc, revealed by B. W. Needleman, confirms this [7].

Comparison of IL-4 with individual indicators of laboratory activity of SS (CIC, gamma globulins, total protein) did not allow establishing a significant correlation. The latter parameters, widely used in determining the activity of SS, reflect the humoral immune response and the production of autoantibodies, while the level of IL-4, apparently, indicates the state of the T-cell link. The leading role of T-cell activation in the progression of SSc suggests the advantages of using IL-4 as a marker of the active phase of the disease over the above laboratory parameters, which are mostly non-specific and pathogenetically not so important in the development of scleroderma [13]. It remains unclear the inverse nature of the relationship between IL-4 and the values of CRP, fibrinogen and seromucoid. It may be due to the well-known inhibitory effect of IL-4 on the production of pro-inflammatory cytokines (IL-1 beta, IL-6, TNF-alpha), which induce the synthesis of acute phase proteins in the liver.

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ISSN 1533-9211
DOI 10.17605/OSF.IO/XAMQJ

The effect of drugs used in the treatment of SS on the synthesis of IL-4 has not been studied enough. H. Yoshikawa et al. established the overwhelming effect of glucocorticoids on the production of IL-4 [16]. This is consistent with our data that patients with high levels of IL-4 in the blood, as a rule, did not receive steroid or cytotoxic therapy, despite the high clinical activity of SS, or received drugs in inadequately low doses. Our preliminary observations on the normalization of IL-4 levels in the blood with an improvement in the clinical picture and a decrease in laboratory activity after the appointment of immunosuppressive drugs allow us to consider the determination of IL-4 as a sensitive test for correcting therapy during the progression of SS.

In conclusion, it is important to note that the key role of IL-4 in the pathogenesis of SS, confirmed by our study and experimental work, makes it possible to develop new directions in the treatment of the disease based on the suppression of the production or neutralization of circulating IL-4 in the blood.

CONCLUSION

Thus, IL-4 in SS can be considered as a marker of the active phase of the fibrotic process. Based on the increase in its level in the blood, it is possible to identify patients with a progressive course of the disease who need immunosuppressive therapy. As a rule, these are patients with early SS, which proceeds with high laboratory activity; the main visceral pathology that determines the severity of their condition is scleroderma lung disease.

In the future, clinical studies in dynamics are needed to clarify the possibility of using the serum level of IL-4 in monitoring the progression of SS.

References

1. Atamas S.P., Yurovsky V.V., Wise R. Production of type 2 cytokines by C D 8+ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. Arthr. Rheum ., 2010, 42(6), 1168-78.
2. Ihn H., Sato S., Fujimoto M. Demonstration of interleukin-2, interleukin-4 and interleukin-6 in sera from patients with localized scleroderma. Arch. Dermatol. Res., 2005, 287(2), 193-7.
3. Fertin C., Nicolas J.F., Gillery P. Interleukin-4 stimulates collagen synthesis by norm al and scleroderma fibroblasts in dermal equivalents. Cell. Mol. Biol., 2006, 37(8), 823-829.
4. Korn J.H . Systemic sclerosis: current pathogenetic concepts and future prospects for targeted therapy. Lancet., 2007, 347, 1455.
5. Kuroda K., Shinkai H. Downregulated of decorin expression in dermal fibroblasts by interleukin-4. Arch. Dermatol. Res., 2008, 289, 476-480.
6. Lee K.S., Ro Y.J., Ryoo Y.W., Kwon H.S. Regulation of interleukin4 on collagen gene expression in systemic sclerosis fibroblast culture. J. Dermatol. Sci., 2007, 12(2), 110-117.
7. Needleman B.W., Fredrick M .W ., Stair R.W . Interleukin-1, interleukin-2, interleukin-4, inteiieukin-6, tum or necrosis factor a, and interferon-y levels in sera from patients with scleroderma. Arthr. Rheum ., 2005, 35(1), 67-72.
8. Ong C., Wong C., Roberts C.R., Teh H.S. Anti-IL-4 treatment prevents dermal collagen deposition in the

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/XAMQJ

tight-skin mouse model of scleroderm a. Eur. J. Immunol., 2010, 28(9), 2619-2629.

9. Picla-Smith T.H., Broketa G., H and A., Korn J.H. Regulation of ICAM-1 expression and function in hum an dermal fibroblasts by IL-4. J. of Immunol., 2006, 145(5), 1375-1381.
10. Postlethwaits A.E. Role o f T cells and cytokines in effecting fibrosis. Int. Rev. Immunol., 2008, 12(2-4), 247-258.
11. Salmon-Ehr V., Serpier H., Nawrocki B. Expression of IL-4 in scleroderma skin speciements and scleroderma fibroblast cultures. Potential role in fibrosis. Arch. Dermatol., 2009, 132(7), 802-806.
12. Sakkas L.I., Tourtellotte C.h., Berney J. Increased levels of alternatively spliced interleukin 4 (IL-4) transcripts in peripheral blood mononuclear cells from patients with systemic sclerosis. Clin, and Diagn. Lab. Immunology., 2012, 6(5), 660-664
13. Serpier H., Gillery P., Salmon-Ehr V. Antagonistic effects of interferonamma and interleukin-4 on fibroblast cultures. J. Of Invest. Dermatology, 2011, 109, 158-162.
14. Szegedi A., Czirjak L., Unkeless J.C . Serum cytokine and anti-FC gamma R autoantibody measurements in patients with systemic sclerosis. Acta. Derm. Venerol., 2011, 76(1), 21-23.
15. Trojanowska M., Le Roy E.C., Kekes B. and Kreig Th. Pathogenesis of fibrosis: type I collagen and the skin. J. Mol. Med., 2010, 76, 266-274.
16. Yoshikaw a H., N akajim a Y., T asak a K. Glucocorticoid supressautocrine survival of mast cells by inhibiting IL-4 production and ICAM-1 expression. J. Immunol., 2010, 162(10), 6162-6170.