ACTA MEDICA LITUANICA. 2008. Vol. 15. No. 4. P. 211–215

Serum levels of transforming growth factor β1 (TGF-β1) in patients with rheumatoid arthritis and Sjögren's syndrome

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Background. Autoimmune diseases, including rheumatoid arthritis (RA) and Sjögren's syndrome, are characterized by loss of immunological tolerance to self-antigens. Transforming growth factor (TGF-β1) is a pleiotropic cytokine that regulates cell growth, adhesion, and differentiation in a wide variety of cell types. Studies of animal models and human patients have revealed a critical function for TGF-β1 in regulating leukocyte functions in autoimmune diseases. Measurement of TGF-β1 is becoming an important screening tool for identifying biomarkers of autoimmune diseases.

The purpose of this study was to assess and to compare serum levels of TGF-β1 in patients with rheumatoid arthritis (RA), Sjögren's syndrome secondary to rheumatoid arthritis (sSS + RA) and primary Sjögren's syndrome (pSS).

Material and methods. Sera were obtained from 23 patients with RA, 23 patients with sSS + RA, 23 patients with pSS and 20 healthy individuals. Serum levels of TGF-β1 were measured using the respective commercially available BIOSOURCE Immunoassay kit (BioSource International, Inc., USA).

Results. Comparison of the serum levels of TGF-β1 in patients with RA, sSS + RA, pSS and healthy individuals showed significant differences of TGF-β1 concentration between four groups (respectively, 51736 ± 12483 pg/ml vs 32953 ± 3090 pg/ml vs 17500 ± 3773 pg/ml and vs 31600 ± 2040 pg/ml, $p < 0.001$).

Conclusion. The appropriate serum levels of TGF-β1 may reflect an ongoing autoimmune inflammation in the different target organs.

Key words: transforming growth factor-β1, rheumatoid arthritis, Sjögren's syndrome

INTRODUCTION

Autoimmune diseases, including rheumatoid arthritis (RA) and Sjögren's syndrome, are characterized by loss of immunological tolerance to self-antigens. Rheumatoid arthritis is a T cell-dependent, antibody-mediated inflammatory disease that targets the joints (1). Sjögren's syndrome (SS) is an "autoimmune epithelitis" characterized by sicca syndrome as well as by extraglandular manifestations that reveal the severity of this disorder. SS can occur alone as primary SS (pSS) or accompany other autoimmune disorders as secondary SS (sSS). Sjögren's syndrome secondary to rheumatoid arthritis seems to be a complication of this disorder, and the evolution of Sjögren's syndrome is closely linked to that of RA. Sjögren's syndrome secondary to rheumatoid arthritis usually is subclinical and required specific tests for its diagnosis (2–4).

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Transforming growth factor β1 (TGF-β1) is a secreted protein that regulates proliferation, differentiation and death of various cell types. TGF-β1 is a potent immunosuppressor, and perturbation of TGF-β1 signalling is linked to autoimmunity, inflammation and cancer (5). In addition, TGF-β1 controls the initiation and resolution of inflammatory responses through the regulation of chemotaxis, activation, and survival of lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes. The regulatory activity of TGF-β1 is modulated by the cell differentiation state and by the presence of inflammatory cytokines and costimulatory molecules. Collectively, TGF-β1 inhibits the development of immunopathology to self or non-harmful antigens without compromising immune responses to pathogens. Studies of animal models and human patients have revealed a critical function for TGF-β1 in regulating leukocyte functions in autoimmune diseases. (1). Thus, measurement of TGF-β1 is becoming an important screening tool for identifying biomarkers of autoimmune diseases.

The purpose of this study was to assess and to compare serum levels of TGF-β1 in patients with rheumatoid arthritis (RA), Sjögren's syndrome secondary to rheumatoid arthritis (sSS + RA) and primary Sjögren's syndrome (pSS) .

MATERIAL AND METHODS

Sera were obtained from 23 patients with RA (2 males and 21 females, mean age 58.4 ± 13.4, range 28–79 years), 23 patients with sSS + RA (2 males and 21 females, mean age 63.1 ± 10.2, range 49–80 years), 23 patients with pSS (1 male and 22 females, mean age 59.6 ± 11.9, range 33–79 years) and 20 healthy controls (2 males and 18 females, mean age 48.1 ± 12.0, range 27–66 years). All the RA patients fulfilled 1987 revised criteria of the American College of Rheumatology (6), while all the primary Sjögren's syndrome and Sjögren's syndrome secondary to rheumatoid arthritis patients met the American-European Consensus Group classification criteria for Sjögren's syndrome (7). The mean disease duration of rheumatoid arthritis in patients with RA and sSS + RA was similar (14.93 ± 9.49 and 14.27 ± 9.55, respectively, $p > 0.05$). Furthermore, the mean disease duration of Sjögren's syndrome in patients with pSS and sSS + RA was similar (13.87 ± 8.84 and 13.04 ± 8.05, respectively, $p > 0.05$).

Immunological tests were performed in all the patients and healthy controls and included the determination of the following auto-antibodies: ANA were determined by indirect immunofluorescence using Hep-2 cells as substrate (DACO, USA), precipitating antibodies to the extractable nuclear antigens Ro / SSA and La / SSB were detected by ELISA (R & D systems, USA, Minneapolis), rheumatoid factor –IgM (RF-IgM) was detected by ELISA (The binding site, UK).

Serum levels of TGF-β1 were measured using the respective commercially available BIOSOURCE Immunoassay kit (BioSource International, Inc., USA). Measurements of TGF-β1 were performed after activation to transform latent TGF-β1 to immunoreactive TGF-β1 by BioSource assay. The activation procedure was carried out according to the instructions of the manufacturer. All the measurements were performed in duplicate. The detection limits of this assay ranged from 0 to 2000 pg/mL for TGF-β1.

The data were analyzed using SPSS 12.0 software and are expressed as mean ± SD. Comparisons among three or more groups were made by a Kruskal-Wallis test, and those between two groups were made by a Mann-Whitney test. Correlations were determined by a Spearman rank correlation test. A value of $p < 0.05$ was considered statistically significant. The study has been approved by the Lithuanian Bioethics Committee.

RESULTS

Assessment of the serum levels of TGF-β1 in patients with rheumatoid arthritis (n = 23), with Sjögren's syndrome secondary to rheumatoid arthritis (n = 23), with primary Sjögren's syndrome (n = 23), and healthy controls (n = 20) and comparison of the mean serum concentration of TGF-β1 between the four groups showed significant differences ($p < 0.001$) (Figure). We found the highest serum TGF-β1 concentration in RA patients, intermediate in sSS + RA patients and healthy controls, and the lowest in pSS patients (51735.5 ± 12482.9 pg/ml vs 32953 ± 3089.5 pg/ml vs 31599.8 ± 2039.4 pg/ml and vs 17500.3 ± 3773.4 pg/ml, respectively, $p < 0.001$). Significant differences showed the comparison of the mean concentration of TGF-β1 between two groups: RA group compared with sSS + RA group, pSS group and healthy controls ($p < 0.001$). Levels of TGF-β1 in the serum of sSS + RA patients and healthy controls were comparable ($p = 0.134$) (Table 1). No correlations were found between serum levels of TGF-β1 and disease duration of rheumatoid arthritis and Sjögren's syndrome in the composite groups of patients with RA and sSS + RA and patients with pSS and sSS + RA ($r = 0.245$, $r = -0.245$, respectively).

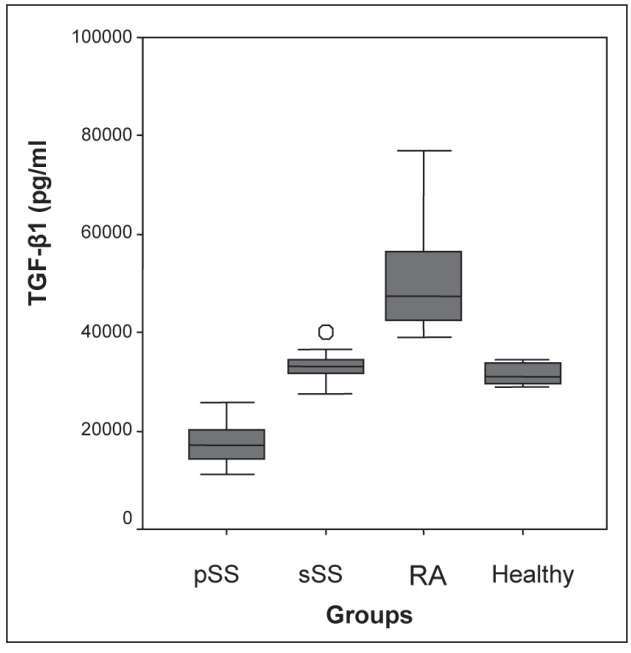

Fig. 1. Comparison of serum levels of TGF-β1 between patients with rheumatoid arthritis, Sjögren's syndrome secondary to rheumatoid arthritis, primary Sjögren's syndrome and healthy controls ($p < 0.001$)

Table. **Serum levels (and range) of TGF-β1 in the groups of rheumatoid arthritis, Sjögren's syndrome secondary to rheumatoid arthritis, primary Sjögren's syndrome and healthy controls**

Group	TGF-β1 (pg/ml)
RA (n = 23)	51735.5 ± 12482.9*
	(39071.0–76926.2)
sSS + RA (n = 23)	32953.6 ± 3089.5
	(27578.8–39962.0)
pSS (n = 23)	17500.3 ± 3773.4**
	(11275.6–25615.4)
Healthy controls (n = 20)	31599.8 ± 2039.4
	(28761.8–34566.4)

* $p < 0.001$, compared with sSS + RA, pSS patients groups and healthy controls;
** $p < 0.001$, compared with RA, sSS + RA patients groups and healthy controls;
$p = 0.134$, sSS + RA patients group compared with healthy controls.

DISCUSSION

Rheumatoid arthritis is a T cell-dependent, antibody-mediated inflammatory disease that targets the joints (1). The development of Sjögren's syndrome secondary to rheumatoid arthritis occurs on a different genetic background (HLA DR4) compared with primary Sjögren's syndrome; ocular symptoms are more prevalent than the oral symptoms, and a distinct set of therapeutic responses suggests a different pathogenetic process. When diagnostic criteria were applied to large patient populations, prevalence figures for secondary Sjögren's syndrome of 31 percent were reported in patients with rheumatoid arthritis. Studies of positive lip biopsies in the same patient groups suggest even higher figure, 35 percent. SS secondary to RA is usually subclinical and required specific tests for its diagnosis (2–4). Classification criteria for rheumatic diseases often serve as diagnostic criteria. This is particularly true when the sensitivity and specificity of classification criteria are both close to 100%. In this case, classification criteria could be used as diagnostic criteria. Classification criteria are not perfect for use in diagnosis, and a certain proportion of patients may be misclassified. Thus, classification cannot be considered to be the medical standard for diagnosis. Most of the rheumatic diseases lack a single distinguishing feature, and each disease is usually identified by the presence of a combination of clinical and laboratory manifestations (7). The results of investigations on new markers promise to provide a wealth of information on candidate biomarkers and possible aetiopathogenetic mechanisms.

Transforming grow factor β1 is a secreted protein that regulates proliferation, differentiation and death of various cell types. All immune cell lineages, including B, T and dendritic cells as well as macrophages, secrete TGF-β1, which negatively regulates their proliferation, differentiation and activation by other cytokines. Thus, TGF-β1 is a potent immunosuppressor, and the perturbation of TGF-β1 signalling is linked to autoimmunity, inflammation and cancer. Therefore, a most critical function of TGF-β1 in the immune system is the suppression of lymphocyte proliferation and differentiation that prevents inappropriate autoimmune responses and balances the requirements of proper immune cell levels during pathologic states that trigger the immune response. Studies of animal models and human patients have revealed a critical function for TGF-β1 in regulating leukocyte functions in autoimmune diseases (1, 8–10).

Thus, measurement of TGF-β1 is becoming an important screening tool for identifying biomarkers of autoimmune diseases.

Comparison of the mean concentration of TGF-β1 between patients with rheumatoid arthritis, Sjögren's syndrome secondary to rheumatoid arthritis, primary Sjögren's syndrome and healthy controls showed significant differences and a significant increase in the serum level of TGF-β1 in RA patients. Levels of TGF-β1 in the serum of sSS + RA patients and healthy controls were comparable. It is possible that the differences in serum levels of TGF-β1 between RA, sSS + RA, pSS patients might reflect a different genetic background of patients with primary and secondary Sjögren's syndrome and TGF-β1 gene polymorphism. Significantly elevated TGF-β1 levels have been reported in serum of RA patients compared to primary Sjögren's syndrome and in contrast to results of our study no differences were found in serum levels of TGF-β1 between RA patients and healthy control (10). We speculate that these contradictory results might reflect different joint damage or undiagnosed Sjögren's syndrome secondary to RA patients in the above study. Recently, higher TGF-β1 concentrations in serum and synovial fluid were found in patients with rheumatoid arthritis compared with osteoarthritis patients. The foregoing study has suggested that elevated TGF-β1 in rheumatoid synovial tissue may suppress joint inflammation by inhibiting RANTES secretion from synovial fibroblasts, thus blocking the infiltration of immune cells (11, 12). TGF-β1 was reported to increase the expression of pro-inflammatory cytokines and metalloproteinase-1 by synovial fibroblasts. Also, resent findings have supported the hypothesis that the effects of TGF-β1 can potentially promote joint destruction by induction of aggrecanase-1 (13). Some studies demonstrated that systemic administration of TGF-β1 to mice inhibits collagen-induced arthritis (CIA), whereas its local administration to joints induces synovitis and aggravates the disease. Similarly, blocking endogenous TGF-β1 by systemic injection of anti-TGF-β1 antibody exacerbates collagen-induced arthritis in mice, whereas the local blockade of TGF-β1 ameliorates ongoing inflammation. It is speculated that TGF-β1 signalling in T cells regulates CIA because inhibition of TGF-β1 signalling in T cells renders mice more susceptible to the disease; this is associated with increased production of IFN-γ and TNF-α. (1). The above studies can explain the highest serum levels of TGF-β1 in patients with rheumatoid arthritis.

The hypothesis that TGF-β1 polymorphism may determine the progression of joint destruction in rheumatoid arthritis patients has been supported (14). Some researchers have demonstrated TGF-β1 T869C gene polymorphism to be associated with the disease outcome in rheumatoid arthritis (14, 15). Thus, genetic background could explain the lower mean concentration of TGF-β1 in the serum of patients with Sjögren's syndrome secondary to rheumatoid arthritis compared with rheumatoid arthritis patients. It is likely that serum TGF-β1 tends to decrease because of both lymphocytes migration to the epitheliums and genetic determinism and that the immune injury of salivary glands leads to salivary increase of TGF-β1 allowing the process of repair and fibrosis. This study indicates that regulation of rheumatoid arthritis by TGF-β1 is site and context dependent, owing to its pleitropic activities.

We found the lowest mean concentration of TGF-β1 in the serum of patients with pSS compared with RA, sSS+RA patients and healthy controls. Sjögren's syndrome (SS) is an autoimmune epithelitis characterized by sicca syndrome but also extra-glandular manifestations that reveal the severity of this disorder. SS can occur alone as a primary SS (pSS) or accompany another autoimmune disorder as a secondary SS (sSS). Typically, secondary SS is distinguished from pSS. Sjögren's syndrome, secondary to rheumatoid arthritis, seems to be a complication of these disorders: the sicca syndrome is less serious, anti-Ro/SSA and anti-La/SSB are less frequently

present, and the evolution of SS is closely linked to that of RA (2–4). Sjögren's syndrome is a multifactorial disease with genetic and environmental factors, including viruses (16). Some viruses have been shown to directly activate latent TGF-β1 (1). Recent studies indicate that ductual expression of TGF-β1 is increased and associated with lymphoid foci in salivary glands in pSS (16).

Our findings as well as other studies demonstrated that the lowest TGF-β1 levels in the serum of patients with pSS might reflect the immune injury of salivary and lachrymal glands and spreading of autoimmunity (17). No correlations were found between the serum levels of TGF-β1 and disease duration of rheumatoid arthritis and Sjögren's syndrome in our study.

Thus, the different serum levels of TGF-β1 in patients with rheumatoid arthritis, Sjögren's syndrome secondary to rheumatoid arthritis and primary Sjögren's syndrome might indicate potential of TGF-β1 for new biomarker in rheumatoid arthritis and Sjögren's syndrome, but this will need confirmation in studies on larger rheumatoid arthritis and Sjögren's syndrome populations.

CONCLUSION

This study revealed the different serum levels of TGF-β1 in patients with rheumatoid arthritis, Sjögren's syndrome secondary to rheumatoid arthritis and primary Sjögren's syndrome. The appropriate serum levels of TGF-β1 may reflect ongoing autoimmune inflammation in the different target organs.

ACKNOWLEDGEMENTS

This study was supported by the State Science and Studies Foundation of the Republic of Lithuania, Contract No. T -07324.

Received 8 October 2008
Accepted 28 November 2008

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TRANSFORMUOJANČIO AUGIMO VEIKSNIO β1 KONCENTRACIJA SERGANČIŲJŲ REUMATOIDINIU ARTRITU IR SJOGRENO SINDROMU KRAUJO SERUME

S a n t r a u k a

Autoimuninėms ligoms, įskaitant reumatoidinį artritą bei Sjögreno sindromą, būdinga silpnesnė imuninė tolerancija saviems antigenams. Transformuojantis augimo veiksnys β1 (TGF-β1) yra sekretuojamas baltymas, reguliuojantis įvairių ląstelių proliferaciją, diferenciaciją ir apoptozę. Transformuojančio augimo veiksnio β1 sutrikimai veikimo trajektorijoje yra siejami su autoimuniniais procesais, uždegimu bei onkologinėmis ligomis. Transformuojančio augimo veiksnio tyrimai β1 gali būti reikšmingi ieškant naujų žymenų autoimuninėms ligoms diagnozuoti. Šio darbo tikslas: ištirti ir palyginti transformuojančio augimo veiksnio β1 koncentraciją sergančiųjų reumatoidiniu artritu, antriniu Sjögreno sindromu dėl reumatoidinio artrito bei pirminiu Sjögreno sindromu kraujo serume. Pagal Amerikos reumatologų asociacijos reumatoidinio artrito kriterijus, Amerikos ir Europos bendrus Sjögreno sindromo klasifikacijos kriterijus bei taisykles sudarytos keturios tiriamųjų grupės: I grupė – sergantieji reumatoidiniu artritu (RA), 23 asmenys; II grupė – sergantieji antriniu Sjögreno sindromu dėl reumatoidinio artrito (aSS + RA), 23 asmenys; III grupė – sergantieji pirminiu Sjögreno sindromu (pSS), 23 asmenų; IV grupė – sveiki asmenys, 20 asmenų. TGF-β1 koncentracija kraujo serume tirta ELISA metodu. Palyginus visų keturių tiriamųjų grupių TGF-β1 koncentraciją kraujo serume rastas statistiškai reikšmingas skirtumas (atitinkamai 51736 ± 12483 pg/ml; 32953 ± 3090 pg/ml; 17500 ± 3773 pg/ml ir 31600 ± 2040 pg/ml, $p < 0,001$).

Taigi TGF-β1 koncentracija kraujo serume gali atspindėti autoimuninį uždegimą „organuose-taikiniuose“.