New aspects of the role of Fas receptor and Fas Ligand in patients with Sjögren's syndrome

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Research article

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Abstract

Background: Sjögren's syndrome patients, like other patients with autoimmune disorders, display dysregulation in the function of their immune system. Fas and Fas Ligand (FasL) belong among dysregulated proteins. Although they have been long studied, there are still several unknown aspects of biological importance of Fas and FasL.

Methods: In patients with Sjögren's syndrome and age-matched healthy individuals, we studied Fas and FasL on IL-2R α ⁺ cells and in serum using flow cytometry and enzyme-linked immunosorbent assay, respectively. We also measured the expression of *Bcl-2* and *Bax* by reverse transcription quantitative real-time PCR (RT-qPCR) and percentage of apoptotic and dead cells using Annexin V and 7-AAD staining in lymphocytes.

Results: FasL was increased in patients' T and B cells and serum while Fas was increased in patients' monocytes, T and B cells. No signs of increased apoptosis were found. We suspect an effect of non-steroidal anti-inflammatory therapy on the percentage of Fas⁺ B cells which decreased with the therapy in our patients. In the healthy individuals, there was a noticeable pattern in the expression of Fas which was mutually correlated between populations of mononuclear cells; this correlation was absent in the patients with Sjögren's syndrome. **Conclusions:** The increased expression of Fas in IL-2R α ⁺ lymphocytes does not increase the apoptosis of lymphocytes. These lymphocytes increase the expression of FasL and may induce apoptosis in other cells. The lack of correlation for the expression of FasL in Sjögren's syndrome patients may suggest a loss of regulation.

Background

Fas-Fas ligand (FasL) pathway has been found implicated in several unrelated autoimmune diseases, such as systemic lupus and multiple sclerosis, and tells us how complex autoimmune diseases are and that a patient's body undergoes many changes when getting from physiological state to harming autoimmune responses [1, 2]. Our study on Fas and FasL is another small piece of information that these proteins are involved in pathogenesis of Sjögren's syndrome and their function goes beyond well-known role in apoptosis. Indeed, Fas-FasL interaction which leads to cell proliferation or autoantigen generation, and reverse signalling pathway by FasL has been described [3–5]. Although apoptosis might be one of mechanisms which can damp exocrine glands of patients with Sjögren's syndrome, results of studies are conflicting [6, 7]. Mononuclear cells, primarily lymphocytes, infiltrate the exocrine glands and do express FasL that, in a common setting, induces apoptosis in cells carrying Fas. Both Fas and FasL have been observed abnormally expressed by various cells of patients with Sjögren's syndrome [8, 9]. Many cells constitutively express Fas, whereas the expression of FasL is due to its effect more restricted. In activated T cells, the upregulation of transcription factor IRF-1 as well as NF- κ B plays a role in the expression of FasL [10, 11]. Because a binding site for NF- κ B is present in the Fas gene, too, the upregulation of both FasL and Fas in activated cells is unsurprising [12].

Question remains whether activated T and B cells in the patients with Sjögren's syndrome undergo apoptosis after executing their function. When comes to Fas and FasL, there are many check points in which apoptosis can be suspended and diverted. Beginning with soluble forms of Fas and FasL (sFas and sFasL) that may not only interfere with apoptosis but even support cell proliferation, neat interplay between downstream proteins determines the result of Fas signalling [13–16]. sFas and sFasL were found dysregulated in the patients with Sjögren's syndrome; in some studies, their abnormal level was even correlated to clinical symptoms and progress of the disease [17–19].

FasL-induced apoptotic pathway commonly involves death-inducing signalling complex (DISC) formation and caspase 8/10 activation. cFLIP (cellular FLICE-like inhibitory protein) is a regulatory protein that can be recruited along with caspase-8 to the DISC. In T cells, cFLIP is capable of both inhibition of apoptosis and support of proliferation [20]. Following the activation of TLRs, cFLIP is also an essential protein in proliferation of B cells [21]. Other regulatory proteins include Bcl-2 and Bax. If upregulated, Bcl-2 can prevent Bax oligomerization [22, 23]. Only oligomerised Bax anchors in the mitochondrial membrane and induces the formation of pores through which cytochrome c and Diablo (SMAC) escape. Thus, Bax works as the pro-apoptotic protein while Bcl2 as the anti-apoptotic protein [24, 25]. As such, they were used in this study.

Downstream activated caspase-3 is an executioner caspase in apoptosis but also represents another regulatory point as it can participate in the generation of an excessive number of autoantigens, such as α -fodrin and cleaved poly signalling (ADP-ribose) polymerase, which both accompany autoimmune diseases, including Sjögren's syndrome [26, 4].

As mentioned before, upregulation of (unmutated) Fas and FasL does not necessary lead to apoptosis and the Fas signalling pathway can not only result in the increased survival and proliferation of autoreactive immune cells but also autoantigen generation. Rather than mutations and polymorphisms, epigenetic modulation of gene expression of proteins participating throughout the pathway may take part in various outcomes of the signalling.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been found interfering with proliferation and apoptosis via several mechanisms [27]. These involve activation of caspases by intrinsic as well as extrinsic death receptor mediated pathway. Both induced pathways are likely to converge in mitochondria [28]. The fact that NSAIDs can increase apoptosis of lymphocytes with autoimmune features may be beneficial for patients with Sjögren's syndrome.

Methods

Patients and Controls

A group of 16 patients with primary Sjögren's syndrome participated in this study. All patients fulfilled the European-American consensus group criteria (AECC). The diagnosis of Sjögren's syndrome was based on a routine evaluation of the patient's symptoms and laboratory results (auto-antibody analysis, test of

salivary flow rate, Schirmer’s test, medical records, etc.) performed at the Departments of Dentistry, Immunology and Allergy, Rheumatology and Ophthalmology at the University Hospital in Hradec Kralove, CR, Table 1.

The control group consisted of 16 healthy individuals who matched the patients in gender and age. The participants in the control group did not use medication.

Patients and controls were recruited from the same geographical area.

Table 1
Demographic and clinical data

	Controls (16)	Patients (16)		Controls (16)	Patients (16)
Men/Women	1/15	2/14	Autoantibodies	0	15
Age (years)	53.5	56	Anti-Ro	0	13
Leukocytes (x10 ⁹ /L)	6.8	4.85	Anti-La	0	11
Xerophthalmia	0	16	Decreased salivary flow	N/A	8
Xerostomia	0	15	Schirmer’s test	N/A	16
Dysphagia	0	10	MSG biopsy	N/A	11
TMJ disorders	0	8			
Thyroid dysfunction	0	4			
Arthritis	0	4			
Fatigue	0	11			
Weight loss	0	4			
Raynaud’s phenom.	0	3			
NSAIDs	0	5			
Cyclosporin A	0	4			
Corticosteroids	0	5			
Antimalarials	0	2			

Age and number of leukocytes are displayed as median values. All other parameters are described by the number of positive cases. Xerophthalmia and xerostomia were characterised as subjective perception of eye and mouth dryness, respectively. **Abbreviations:** MSG biopsy: Minor salivary gland biopsy; NSAIDs:

Non-steroidal anti-Inflammatory drugs; TMJ disorder: Temporomandibular joint disorder; AUTOANTIBODIES: Anti-Ro, Anti-La: autoantibodies against ribonucleoproteins. **Positive test of decreased salivary flow:** saliva volume less than 8 ml/30 min (basal salivation (15 min)/stimulated salivation (15 min)). **Positive Schirmer's test:** tear production less than 5 mm of the testing paper in 5 min. **Positive minor salivary gland biopsy:** focus score (FS) more or equal 1 (FS is defined as a count of lymphocytic foci containing more than 50 mononuclear cells per 4 mm² tissue biopsy); these three tests were not applied in the control group.

Samples

Blood samples were collected into Vacutainer tubes with heparin, tubes with EDTA, and tubes containing a thrombin additive (BD, UK). Anti-coagulated blood was used for flow cytometry and RT-qPCR immediately after the collection, while the serum was separated from blood elements by centrifugation at 1000g for 15 min and frozen at -70°C prior to analysis.

Lymphocyte separation and RT-qPCR

We used negative separation (RosetteSep, StemCells Technologies, USA) with 1.081 g/cm³ density gradient medium (DM-L, StemCells Technologies, USA) to isolate the lymphocytes from EDTA-treated samples. In this population, the Th cells and B cells were further separated using CD4- and CD19-specific microbeads (PluriSelect, Germany). The expression levels of *Bax*, and *Bcl-2* mRNA were quantified in Th cells and B cells as described below. To standardise the results, the expression was compared to those of two housekeeping genes expression, *GAPDH* and *HPRT*. The total RNA was extracted from cells in TRIZOL using phenol-chloroform extraction. Approximately 1 µg of RNA was transcribed into cDNA using random primers and M-MLV Reverse Transcriptase, Rnase H Minus, Point Mutant (Promega, USA) in a total volume of 25 µl. Briefly, the samples and random primers were heated to 70°C for 5 min and quickly chilled on ice. Five microlitres of 5xRT buffer, 5 µl of dNTPs (10 mM), 1 µl of RNAsin, and 1 µl of MLV H-RT were added to the samples. The mixtures were incubated for 10 min at room temperature and then heated to 47°C for 50 min and 75°C for 15 min in a standard thermal cycler (GeneAmp 9700, Applied Biosystems). TaqMan Gene Expression Master Mix and TaqMan Gene Expression Assays were used for amplification (Life Technologies, USA). Each reaction consisted of 45 cycles in a Rotor-Gene 6000 instrument (Corbett Live Science). The parameters for PCR were as follows: 50°C for 2 min, 90°C for 10 min and 45 cycles at 95°C for 15 seconds and at 60°C for 60 seconds.

Mononuclear cell separation, flow cytometry, and ELISA

Mononuclear cells were collected from blood samples treated with EDTA by density gradient centrifugation. We used 1.077 g/cm³ medium (PBS-diluted 1.124 g/cm³ Easycoll, Biochrom, Germany) for the separation. The platelets were further removed on 1.063 g/cm³ Easycoll at 400g for 15 min. The cells were washed with pre-binding buffer (140 mM NaCl, 4 mM KCl, 0.75 mM MgCl₂, 10 mM HEPES; pH 7.2 – 7.4) and re-suspended in binding buffer (pre-binding buffer recipe plus 2.5 mM CaCl₂). The cells were stained with Annexin V FITC (NeXins Research, Netherlands) and 7-AAD (BD, USA) for 15 min at room temperature in the dark. We measured the samples on a FACSCalibur flow cytometer (BD, USA).

The expression of Fas and FasL in various cell populations was measured in whole blood samples treated with heparin. The peripheral blood samples were stained with the following combination of antibodies: anti-Fas FITC/IL-2R α PE/CD19 PerCP/CD4 APC and anti-IL-2R α FITC/Fas Ligand PE/CD19 PerCP/CD4 APC. Fluorescence minus one (FMO) control determined the positive expression of the aforementioned markers, but we also used isotype controls to exclude non-specific binding. Cells expressing markers at a low density were also considered positive. All antibodies were purchased from Exbio, CR. Following staining, the red blood cells were lysed using an isotonic solution of NH₄Cl, centrifuged, and immediately measured on the flow cytometer. The adequate performance of the flow cytometer was regularly verified using CaliBrite beads and FACSCComp (BD, USA). CellQuest (BD) was used to acquire data. The collected data were batch-analysed with the FlowJo 8.7 software (TreeStar, USA). The expression was characterised with the median fluorescence intensity, MFI. We used flow cytometry as a semi-quantitative method. The expression was measured using the same instrument setting, which enabled us to compare patients and controls. Therefore, all charts display relative values and do not allow inter-laboratory comparisons.

An enzyme-linked immunosorbent assay (ELISA) was performed using the sFas ELISA kit and the sFasL ELISA kit (R&D Systems, USA). The detection range of the assay specific for sFas was 15.6 – 2000 pg/ml. The manufacturer declared no cross-reactivity between anti-sFas antibodies and other members of the TNFR family. The detection range of the assay specific for sFasL was 7.8 – 1000 pg/ml. The manufacturer observed no cross-reactivity between anti-sFasL antibodies and members of the TNF family other than FasL.

Statistical analyses

The normality of data samples was tested using the Shapiro-Wilk test. The homogeneity of variances was determined with Levene's test. Normally distributed and homoscedastic data were compared using a t-test; otherwise, a Mann-Whitney U Test or Kolmogorov-Smirnov Test was used. The relationship between variables was assessed using the Pearson correlation coefficient. Categorical variables were evaluated with Fisher's exact test. The data were tested at a significance level of 5%. We used Statistica 10 (StatSoft, USA) to perform the tests. Statistica 10, GraphPad Prism 5 (GraphPad Software, USA) and Microsoft Excel (version 14, 2010 Microsoft Corporation, USA) were used to plot graphs. Graphs that show individual cases only display median values. All other graphs display median values, quartiles, and non-outliers.

Results

Apoptosis

Expression of *Bcl-2* and *Bax*, as well as the ratio of *Bax* to *Bcl-2* did not differ between patients and controls, Table 2.

Table 2
Ratio of *Bax* to *Bcl-2* (normalised to *HPRT*)

	Controls		Patients	
	Median	(Min – Max)	Median	(Min – Max)
Th cells	0.62	(0.53 – 0.95)	0.6	(0.55 – 1.06)
B cells	0.69	(0.62 – 0.78)	0.69	(0.67 – 0.84)
Number of repeats: 3				

Annexin V and 7-AAD staining revealed a low frequency of apoptotic and dead cells in both patients and controls. The percentage of apoptotic lymphocytes (1.17% in controls, 0.87% in patients) and percentage of apoptotic monocytes (4.35% in controls, 4.57% in patients) did not significantly differ between groups. The results were similar for the percentage of late apoptotic and dead lymphocytes (0.27% in controls, 0.24% in patients) and monocytes (0.38% in controls, 0.37% in patients), Fig. 1.

We measured the expression of Fas and FasL in mononuclear cells, which included monocytes, IL-2Rα⁺ B cells, and IL-2Rα⁺ Th cells. Prior to analysing the Fas and FasL expression, we first evaluated differences in the percentage of monocytes, IL-2Rα⁺ B cells, and IL-2Rα⁺ Th cells between controls and patients.

We used forward versus side scatter gating and the expression of cell-surface antigens (CD19, CD4) to distinguish between monocytes, B cells, and Th cells.

Monocytes and expression of alpha chain of IL-2 receptor in Th cells and B cells

The percentage of monocytes and IL-2Rα⁺ B cells did not differ between controls and patients ($P = 0.133$ and $P = 0.769$, respectively, data not shown). The percentage of IL-2Rα⁺ Th cells did not significantly differ between patients and controls ($P < 0.1$), however, this population showed distinct distributions for patients and controls (Fig. 2a and b).

Expression of Fas ligand

The expression of FasL in monocytes was not different when comparing patients and controls (data not shown) while the expression in IL-2Rα⁺ Th cells differed. The percentage of IL-2Rα⁺ **Th cells** expressing FasL was increased in patients ($P < 0.005$) and moreover, FasL was found in greater abundance on these cells in patients ($P = 0.001$), Fig. 2c and d.

Similar to FasL⁺ IL-2Rα⁺ Th cells, the percentage of FasL⁺ IL-2Rα⁺ **B cells** was higher in patients ($P = 0.021$), although the difference between controls and patients was not as distinct as for Th cells.

Expression of FasL measured as a density on IL-2Rα⁺ B cells was again higher in patients than controls ($P = 0.008$), Fig. 2e and f.

We found a relationship between the expression of FasL on the cell membrane of lymphocytes and monocytes in controls but not in patients. The expression of FasL correlated between the following three cell populations: FasL⁺ IL-2Rα⁺ B cells and FasL⁺ IL-2Rα⁺ Th cells ($R = 0.98$, $P < 0.001$), FasL⁺ IL-2Rα⁺ Th cells and monocytes ($R = 0.85$, $P < 0.001$), monocytes and FasL⁺ IL-2Rα⁺ B cells ($R = 0.9$, $P < 0.001$), Fig. 3a, b, c.

Expression of Fas

The expression of Fas (MFI) on the surfaces of IL-2Rα⁺ Th and IL-2Rα⁺ B cell was higher in patients than in controls ($P < 0.001$ and $P = 0.037$, respectively), Fig. 4a and b. Furthermore, the percentage of Fas⁺ IL-2Rα⁺ B cells was significantly increased in patients ($P = 0.003$), Fig. 4c. Strikingly, all five patients that used NSAIDs had lower percentage of Fas⁺ IL-2Rα⁺ B cells than the other eleven patients who did not use NSAIDs (median value of 18.8% vs. 49.6%, $P < 0.001$). As a result, the percentage of Fas⁺ IL-2Rα⁺ B cells in patients using NSAIDs was similar to the percentage of these cells in controls (median value of 23.5%), Fig. 4c.

The expression of Fas on the cell membrane of monocytes was significantly higher in patients ($P = 0.04$), Fig. 4d and e. This finding is in contrast to the expression of FasL on monocytes, which did not differ between controls and patients.

Soluble Fas and FasL

We measured the concentration of sFas and sFasL in the sera of our patients and controls. We observed a significantly higher concentration of sFas in patients than in controls ($P = 0.043$), Fig. 4f. Furthermore, the concentration of sFasL was higher in patients than in controls (median value of 60 pg/ml and 49 pg/ml, respectively), but this difference was not significant ($P = 0.213$, results not shown).

Discussion

Most of autoimmune diseases can be hardly ever linked to single gene mutations. This is depicted by the animal model of MRL-Fas^{lpr} mice which, owing to mutational changes of the *Fas* gene, develop Sjögren's-like and systemic lupus erythematosus-like symptoms. However, a little of the correlation between *Fas* polymorphisms or mutations and Sjögren's syndrome or systemic lupus erythematosus in humans has been found. It is more likely that these diseases are associated with epigenetic modifications which enable and increase survival of autoreactive T and B cells and also impact Fas and FasL pathway.

Here we describe a shift in the expression of Fas and FasL in the peripheral blood lymphocytes of patients with primary Sjögren's syndrome compared to healthy controls. The expression of Fas and FasL tended to increase on the membranes of B and T cells that express the α-chain of the IL-2 receptor. However, monocytes also displayed an increase in the expression of Fas on the cell membrane (Fig. 4d and e).

In addition to the higher expression of FasL in our patients, the percentage of IL-2R α ⁺ FasL⁺ cells was significantly increased in B and T cells, thus creating a large pool of executive cells that can induce apoptosis. Because MMP-7 cleaves membrane FasL to generate sFasL, a negative correlation between both forms of FasL can be expected. This correlation was not observed in our patients: they exhibited an increase in both membrane and soluble FasL (even though the increase in sFasL was not statistically significant). Further observations indicated a failure of FasL regulation. Positive correlations in FasL between monocytes and lymphocytes were found only in the healthy individuals and not in the patients (Fig. 3). The strong correlations (R above 0.8 at P < 0.001) between T cells, B cells and monocytes suggest a potent regulatory mechanism that controls FasL expression in the population of mononuclear cells. These correlations also demonstrate a connection between innate and acquired immunity. However, this connection is disrupted in the patients, and the details of the regulatory mechanism remain unknown. The presence of multiple cis-acting regulatory elements in FasL further obfuscates how the corresponding expression of FasL is maintained in different cell types [29, 30]. Moreover, the effect of FasL may be influenced by other molecules such as Decoy receptor 3 [21]. Several studies have investigated apoptosis within the exocrine glands of patients with Sjögren's syndrome [31, 32]. Ohlsson and co-workers considered apoptosis a rare event in these patients [7]. They measured a low rate of apoptosis in populations of CD4⁺ and CD8⁺ T cells as well as in mononuclear cells that infiltrated salivary glands of the patients. Ohlsson's finding was complemented by the research of Busamia and co-workers, who found a high degree of proliferation among infiltrating lymphocytes in salivary glands located in the lower lip of patients with Sjögren's syndrome [33]. Caspase-3 and Ki67 were used as markers of apoptosis and proliferation, respectively. These data seem to conflict other studies that found an increased rate of apoptosis among the infiltrated cells. These previous studies used a different approach: isolated T cells were cultured *in vitro*, and 7-AAD or propidium iodide was then used as a marker of apoptosis [34, 35]. In contrast, Ohlsson's research group worked directly with biopsies of the patients and employed terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labelling analysis (TUNEL) [7]. Ohlsson found that the expression of Fas was high in infiltrated cells, despite a low rate of apoptosis. His observation corresponds with the results we found in peripheral blood lymphocytes, which also highly expressed Fas, but the rate of apoptosis was the same as that in control samples.

Prolonged antigen stimulation ultimately leads to the apoptosis of the stimulated cell, a mechanism called activation-induced cell death (AICD) [36]. AICD requires an increase of FasL and Fas in cells at the beginning of their activation, while the cells remain resistant to Fas-induced apoptosis. Nevertheless, these cells finally undergo apoptosis, thereby preventing an exaggerated immune response in healthy individuals [37]. AICD is necessary for the elimination of autoreactive cells but is often non-functional in cancer cells. Prolonged auto-antigen stimulation could be expected in autoimmune diseases, and Ohlsson's findings and ours indicate that AICD is disrupted in the patients with Sjögren's syndrome.

In concordance with Nozawa's group [18], which studied the soluble forms of Fas and FasL in the patients with Sjögren's syndrome, we detected a significant increase in serum sFas in our patients (P =

0.04). In healthy individuals, the activation of Fas promotes the expression of full-length Fas via the activation of kinase FAST K, which indirectly controls the inclusion of exon 6 in the pre-mRNA [38, 39]. Only Fas isoforms that include exon 6 are expressed on the cell membrane. This mechanism, which leads to the expression of membrane Fas, can be diverted by repressors of the inclusion of exon 6. As a result, a soluble isoform of Fas is generated. Therefore, the expression of membrane Fas should negatively correlate with the expression of sFas. Although the activation of Fas may be responsible for the increase in Fas expression on the cell membranes in our patients, it does not explain the increase of sFas that was also found in the sera of our patients. The manner in which the increased levels of both isoforms of Fas are maintained and why the cells that abundantly express Fas are not removed in Sjögren's syndrome remain to be identified. We presume that signalling that promotes proliferation takes place after the ligation of Fas. Nevertheless, the regulation of this signalling in Sjögren's syndrome remains unknown.

Therapy affected the Fas-FasL system and Nozawa and co-workers described a connection between steroid therapy and the decrease in serum sFas and sFasL in patients with systemic lupus erythematosus [18]. We observed a similar connection between NSAIDs and the decrease in the percentage of $^+ \text{Fas}^+ \text{IL-2R}\alpha$ B cells. However, the impact of decreased $\text{Fas}^+ \text{IL-2R}\alpha^+$ B cells on the clinical manifestation of the disease (such as salivary secretions, arthritis, and thyroid dysfunction) is unknown. Moreover, only five patients used NSAIDs to suppress joint pain. Therefore, the validity of our findings is not easily extrapolated, and the use of NSAIDS should be investigated in a larger group of patients with Sjögren's syndrome.

Conclusions

The increased expression of Fas in $\text{IL-2R}\alpha^+$ lymphocytes does not increase the apoptosis of lymphocytes. These lymphocytes increase the expression of FasL and may induce apoptosis in other cells. The lack of correlation for the expression of FasL in Sjögren's syndrome patients may suggest a loss of regulation.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the University Hospital and Faculty of Medicine Hradec Kralove, Charles University, CR.

Consent for publication

All participants confirmed their enrolment in the study by signing a written consent form.

Availability of data and material

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Author contribution

JK and RS designed the study. MS, RS, and IL classified the patients. MS, RS, and IL organized the collection of samples. MK, RV, TS, and MC acquired data and analysed them. MK performed statistical analyses. IL, MK, and MR wrote the manuscript while JK and TS proofread it. All authors have read and approved the manuscript.

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Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Early apoptotic and late apoptotic or dead cells

Figure 2

IL-2R α ⁺ Th cells and B cells.

Comparison of the percentage of IL-2R α ⁺ Th cells where Th cells represent 100%, Kolmogorov-Smirnov Test (a). Comparison of the expression (MFI) of IL-2R alpha in IL-2R α ⁺ Th cells, Kolmogorov-Smirnov Test (b). Comparison of the percentage of FasL⁺ IL-2R α ⁺ Th cells, where IL-2R α ⁺ Th cells represent 100%,

Kolmogorov-Smirnov Test (c). Comparison of the expression of FasL (MFI) in IL-2Rα⁺ Th cells, Mann-Whitney U Test (d). Comparison of the percentage of FasL⁺ IL-2Rα⁺ B cells, where IL-2Rα⁺ B cells represent 100%, t-test (e). Comparison of the expression of FasL (MFI) in IL-2Rα⁺ B cells, Mann-Whitney U Test (f).

Figure 3

Correlation of the expression of FasL. Correlations in the group of controls (a, b, c)

Figure 4

Fas expression. Comparison of the expression of Fas in IL-2R α ⁺ Th cells, t-test (a). Comparison of the expression of Fas in IL-2R α ⁺ B cells, t-test (b). Comparison of the percentage of Fas⁺ IL-2R α ⁺ B cells when IL-2R α ⁺ B cells represent 100%, patients using NSAIDs are denoted by empty squares, Mann-Whitney U

Test (c). Comparison of the expression of Fas in monocytes, t-test (d). Expression of Fas in monocytes in control sample and a patient as an example of the output of flow cytometry data (e). Comparison of soluble Fas in the sera of controls and patients, t-test (f).