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# The impact of agonists and antagonists of TLR3 and TLR9 on concentrations of IL-6, IL10 and sIL-2R in culture supernatants of peripheral blood mononuclear cells derived from patients with systemic lupus erythematosus*

## Wpływ agonistów i antagonistów receptorów TLR3 i TLR9 na stężenie IL-6, IL-10 i sIL2R w nadsączu hodowlanym komórek mononuklearnych krwi obwodowej chorych na toczeń układowy rumieniowaty

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## Summary

Toll-like receptors (TLR), especially TLR3, 7 and 9, play an important role in the pathogenesis of systemic lupus erythematosus (SLE). In our study blood was collected from 16 patients with SLE and from 8 healthy volunteers. Concentrations of IL-6, IL-10 and sIL-2R were measured by ELISA in mononuclear cell culture supernatant after 24 hours of stimulation by agonists and antagonists of TLR3 and 9 (for TLR3-poli I/C, resveratrol and for TLR9-ODN2006, IRS 945). Stimulation of TLR9 by ODN2006 led to an increase of IL-6 concentration in cell culture supernatants from the cells of healthy volunteers compared with unstimulated cells from controls. Inhibition of TLR3 activation by resveratrol caused a significantly lower concentration of IL-10 in cell culture supernatants derived from both patients and healthy donors. Moreover, resveratrol significantly decreased the level of IL-10 and sIL-2R in culture supernatants of cells derived from patients with active disease compared to the inactive stage. A positive correlation was also found between IL-6 concentration following ODN2006 administration and disease activity. In conclusions, our results indicate that TLRs play a role in the modulation of the inflammatory response in SLE patients. This suppressive action on IL-10 synthesis demonstrated by resveratrol suggests that it may be useful in SLE therapy.

**Keywords:** **IL-6 • IL-10 • sIL-2R • ODN2006 • resveratrol • poli I/C, SLE, TLR**

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**Abbreviations:** **ACR** - American College of Rheumatology, **CD** - cluster of differentiation, **CpG DNA** - a cytosine triphosphate deoxynucleotide ("C") followed by a guanine triphosphate deoxynucleotide ("G"), **dsRNA** - double-stranded ribonucleic acid, **IFN** – interferon, **IL** – interleukin, **LPS** – lipopolysaccharide, **PBMC** - peripheral blood mononuclear cell, **poli I/C** - polyinosinic: polycytidylic acid, **SLAM** - Systemic Lupus Activity Measure, **SLE** - systemic lupus erythematosus, **SLEDAI** - SLE Disease Activity Index, **ssRNA** - single-stranded ribonucleic acid, **TIR** - Toll/IL-1R receptor domain, **TLR** - Toll-like receptor, **TNF** - tumour necrosis factor, **TRIF-TIR** - domain containing adapter inducing interferon beta.

## Introduction

Systemic lupus erythematosus (SLE) is a chronic and incurable autoimmunological disease whose pathogenesis is complex and not fully known. Immunological disturbances play a major role, together with the production of antigen-specific autoantibodies, which form deposits in important tissues and organs [12]. A considerable body of data indicates that Toll-like receptors (TLRs), especially TLR3, 7 and 9, play an important role in the pathogenesis of SLE. They recognize dsRNA, ssRNA and CpG-DNA nucleic acids derived from pathogens or from dead or destroyed host cells, which elicit the development of autoimmune diseases [6]. Patients with SLE demonstrate apoptotic disturbances and defects in the elimination of apoptotic bodies, which increase the expression of autoantigens [3]. Moreover, literature data suggests that abnormalities associated with TLRs may be connected with the development of autoimmune processes [14]. Mutations in the receptor genes and proteins, which are part of the signaling cascade, may contribute to forwarding through the immunological response of TLRs to the host antigens and development of autoimmune disease [14]. It was shown that TLR activation induces the synthesis of major cytokines which promote the loss of tolerance to host antigens in people prone to SLE development [11,13,24].

Studies show that the stimulation of TLRs by specific ligands causes the development of autoimmune diseases. *In vitro* and *in vivo* animal models have also shown that blockers of TLR inhibit the inflammatory response mediated by TLRs [1,2].

The aim of the present study was to determine the concentrations of IL-6, IL-10 and sIL-2R in supernatants from culture mononuclear cells derived from blood samples obtained from SLE patients after stimulation by agonists and antagonists of TLR 3 and TLR 9.

The choice of the cytokines was based on a different mechanism of their action on the inflammatory response, i.e. promotion (IL-6) and inhibition (IL-10) of the development of inflammation. Moreover, the soluble form of IL-2 receptor was used as the activation marker of the immune response.

The effect of the investigated TLR agonists and antagonists on the concentration of the cytokines may be indicative of a TLR-mediated immune response defect. This will not only contribute to a better understanding of the pathogenesis of inflammatory diseases, but also lead to eliminate this defect using appropriately designed drugs.

## Materials and methods

### Patient characteristics

The analysis was performed using blood taken from 16 patients with SLE under observation at the Outpatient Clinic and Department of Dermatology and Venereology, Medical University of Lodz, Poland and from eight healthy volunteers. The diagnosis of SLE was made if the patient fulfilled at least 4 ACR (American College of Rheumatology) criteria. The activity of the disease was also measured using SLAM (Systemic Lupus Activity Measure). In the analyzed group, nine patients were in the active phase of the disease while seven were in the inactive phase. The clinical characteristics of patients are presented in Table 1. The study was approved by the Bioethics Committee of the Medical University of Lodz, Poland, and was consistent with the Helsinki Declaration.

**Table 1.** Patient characteristics

| Parameter | Value | % |
|---|---|---|
| Number of patients | 16 | 100 |
| Number of women | 15 | 93.75 |
| Number of men | 1 | 6.25 |
| Age of the patients | 42 (29-62) | |
| Duration of the disease (years) | 8.5 (0.25-20) | |
| Activity of the disease | | |
| < 7 points | 7 | 43.7 |
| > 7 points | 9 | 56.25 |
| With immunosuppressive treatment | 10 | 62.5 |
| Without immunosuppressive treatment | 6 | 37.5 |

### Cell cultures

Peripheral blood mononuclear cells (PBMC) were derived from whole blood by density gradient centrifugation at a speed of 3600 rpm for 20 minutes using Ficoll-Histopaque-1077 reagent (PAA Laboratories, Austria). Cells in the interphase were collected, added to 5 ml of Hanks liquid (Biomed, Lublin, Poland) and then centrifuged at the speed of 3600 rpm for 10 minutes. The supernatant was poured out and 5 ml of RPMI 1640 culture medium (PAA Laboratories, Pasching, Austria) was added. The mixture was centrifuged at 1100 rpm for five minutes. The obtained mononuclear cells were suspended in RPMI-1640 culture medium with 10% thermally inactivated fetal calf serum, 1.5 mM L-glutamine, 100 U/ml penicillin and 100 µg/mL streptomycin (all reagents from PAA laboratories, Pasching, Austria). The PBMC suspension was then incubated for 24 hrs with the examined substances (TLR agonists and antagonists: ODN 2006 IRS 964, poli I/C, resveratrol) at a concentration of $1x10^6$ mononuclear cells/ml on 24-well culture plates (Nunc, Roskilde, Denmark), at a temperature of 37°C with 5% CO2 and 98% humidity. After two hours of incubation, the TLR antagonists were added to the cell culture with agonist. Cultures of PBMC without any examined substances were used as controls. The isolation and formation of the culture were made in aseptic conditions. After culturing, the cell suspension was poured into a centrifuge tube and centrifuged at a speed of 1100 rpm for five minutes. The supernatants were poured out and stored at a temperature of - 80°C for later assay of cytokine concentrations.

### Examined substances

The synthetic oligodeoxynucleotide ODN2006 (5'-CGTCGTTTTGTCGTTTTGTCGTT-3', Genomed, Warsaw, Poland) with a phosphorotioester core resistant to nuclease was dissolved in RPMI-1640 culture medium and used at a concentration of 5 µg/mL. The synthetic oligodeoxynucleotide IRS 954 (5›-TGCTCCTGGA-GGGGTTGT-3› Genomed, Warsaw, Poland), also with a phosphorotioester core, was dissolved in RPMI-1640 culture medium and used at a concentration of 5 µg/mL. Resveratrol (Sigma-Aldrich, Poznan, Poland), synthetic 3,4›,5' - trihydoxytrans-stilbene, was dissolved in 96% ethanol according to the manufacturer's instructions, added to RPMI-1640 culture medium, and used in a concentration of 5 µg/mL. Poli I/C (Invivogen, San Diego, CA, USA) synthetic polyinosinic: polycytidylic acid, a dsRNA analogue, was dissolved in sterile, endotoxin free, water provided by the producer and used at a concentration of 5 µg/mL.

### Assessment of cytokine expression

The concentrations of IL-6, IL-10 and sIL-2R in culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) method using commercially available kits. The ELISA kits were purchases from R&D Systems Inc. ELISA (Biokom, Warsaw, Poland).

### Statistical analysis

Statistical analysis was performed using Statistica v. 9.1. The analyzed parameters were presented as arithmetic mean (x), standard deviation (SD) and median. Data distribution was assessed using the W-Shapiro Wilk test. Due to the fact that the distributions of the variables under consideration differ from the normal distribution, nonparametric tests were applied for further analyzes using the nonparametric U-MannWhitney test and Kruskal-Wallis test. Correlations between measured variables were assessed using Spearman's rank correlation coefficient (ρ). Differences of $p<0.05$ were considered statistically significant.

## Results

PBMCs from SLE patients and healthy controls were stimulated *in vitro* with ODN2006 and poli I/C alone or in presence of IRS954 or resveratrol for 24 h. We analyzed the significance of the differences between the level of cytokine production by the stimulated cells and non-stimulated cells derived from SLE patients vs healthy volunteers. The TLR9 activation by ODN2006 resulted in a higher IL-6 production by healthy volunteer cells in comparison with non-stimulated cells ($p=0.05$), but poli I/C did not have stimulatory effect (Fig. 1). PBMCs from patients with SLE stimulated with ODN2006 or poli I/C did not significantly increase IL-6 production. The inhibition of TLR9 and TLR3 activation was found to have no significant impact on IL-6 concentrations in cell culture supernatants from both SLE patients and healthy controls (Table 2 and Table 3). Moreover, we revealed a positive correlation between IL-6 concentration in cell culture supernatant after using ODN 2006 and the activity of the disease ($p<0.005$) (Table 4) (Fig. 2). With respect to IL-10,

**Table** 2. Mean and median values of IL-6, IL-10 and sIL-2R concentrations in cell culture supernatants

| Ligand TLR Patients | | IL6 | | IL-10 | | sIL-2R | |
|---|---|---|---|---|---|---|---|
| | | Volunteers | Patients | Volunteers | Patients | Volunteers | |
| ODN2006 | mean±SD | 327.13± 124.53 | 292.39± 110.22 * | 106.56± 228.69 | 37.18± 32.21 | 27.31± 33.22 | 11.87± 7.08 |
| | median | 374.40 | 323.35 | 25.5 | 27.0 | 11.5 | 8.5 |
| ODN 2006 + IRS 954 | mean±SD | 343.11± 140.56 | 228.44± 117.39 | 118.13± 249.31 | 40.08± 32.55 | 21.25± 25.71 | 12.38± 6.19 |
| | median | 386.0 | 220.4 | 26.6 | 38.4 | 11.5 | 9.0 |
| ODN 2006 + resveratrol | mean±SD | 221.89± 141.86 | 110.19± 107.94 | 31.37± 57.39 | 47.28± 50.89 | 10.69± 10.33 | 10.50± 7.98 |
| | median | 242.35 | 70.35 | 7.05 | 32.8 | 7.0 | 7.5 |
| poli I/C | mean±SD | 300.19± 65.05 | 182.75± 145.39 | 104.44± 193.36 | 93.95± 101.13 | 16.56± 21.91 | 11.63± 11.10 |
| | median | 365.15 | 133.25 | 22.0 | 61.3 | 7.5 | 7.5 |
| poli I/C + IRS 954 | mean±SD | 282.75± 64.63 | 180.19± 167.84 | 115.94± 258.22 | 38.40± 35.59 | 18.5± 24.93 | 10.38± 9.74 |
| | median | 356.95 | 128.10 | 17.7 | 33.0 | 8.5 | 5.5 |
| poli I/C + resveratrol | mean±SD | 228.70± 158.02 | 68.65± 62.74 | 21.38± 31.44 ** | 23.30± 34.27 *** | 12.69± 15.22 | 8.75± 7.94 |
| | median | 364.15 | 43.8 | 12.0 | 5.7 | 6.0 | 6.5 |
| medium non-stimulated cells | mean±SD | 283.11± 55.50 | 144.54± 143.35 * | 92.69± 184.61 ** | 37.15± 43.62 *** | 15.56± 22.82 | 13.38± 7.98 |
| | median | 364.15 | 89.80 | 27.0 | 19.5 | 7.5 | 7.0 |

*p=0.05; **p<0.03; ***p<0.05

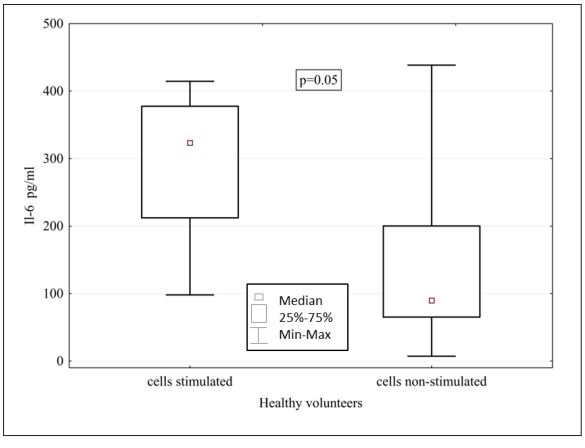


**Fig. 1.** Median of IL-6 concentrations in culture supernatants of cells obtained from healthy volunteers after stimulation with ODN 2006 and in non-stimulated cultures (in medium alone). Number of patients in particular group is posted in the Table 1. Significance levels are indicated for Mann-Whitney test

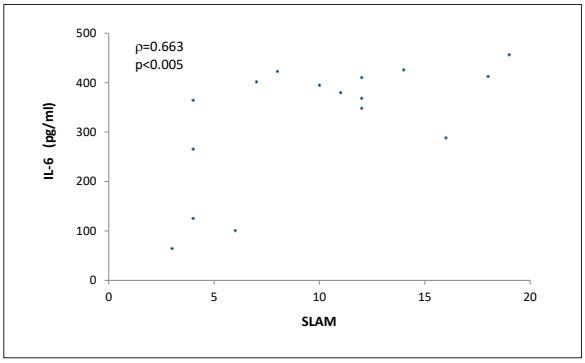


**Fig. 2.** Correlation between concentration of IL-6 in supernatants from culture of SLE patient cells after ODN2006 stimulation and activity of the disease according to the SLAM scoring system. Number of patients in each particular group is posted in the Table 1

stimulation with both ODN2006 and poli I/C had no significant effect on SLE patients and controls. The inhibition induced by resveratrol was associated with lower concen-

**Table 3.** Mean and median values of IL-6, IL-10 and sIL-2R concentrations in cell culture supernatants

| Ligand TLR Active patients | | IL6 | | IL-10 | | sIL-2R | |
|---|---|---|---|---|---|---|---|
| | | Inactive patients | Active patients | Inactive patients | Active patients | Inactive patients | |
| ODN2006 | mean±SD | 387.47 ± 49.02 | 249.54 ± 151.93 | 20.87 ± 15.03 | 216.74 ± 324.45 | 21.22 ± 28.73 | 35.14 ± 39.13 |
| | median | 395.2 | 265.90 | 13.80 | 39.00 | 9.00 | 15.00 |
| ODN 2006 + IRS 954 | mean±SD | 380.53 ± 114.81 | 295.00 ± 164.38 | 24.58 ± 15.14 | 238.40 ± 353.66 | 10.67 ± 7.07 | 34.86 ± 34.66 |
| | median | 418.70 | 316.50 | 24.40 | 63.00 | 9.00 | 18.00 |
| ODN 2006 + resveratrol | mean±SD | 248.38 ± 105.01 | 187.83 ± 182.22 | 10.79 ± 15.91 | 57.83 ± 80.28 | 5.44 ± 3.28 | 17.43 ± 12.57 |
| | median | 243.40 | 92.60 | 3.60 | 27.00 | 6.00 | 14.00 |
| poli I/C | mean±SD | 372.54 ± 100.12 | 207.16 ± 191.80 | 40.86 ± 51.47 | 186.20 ± 275.82 | 13.33 ± 22.18 | 20.71 ± 22.55 |
| | median | 398.70 | 91.80 | 21.40 | 45.00 | 7.00 | 10.00 |
| poli I/C + IRS 954 | mean±SD | 339.60 ± 129.11 | 209.66 ± 185.62 | 16.47 ± 10.12 | 243.83 ± 364.19 | 6.66 ± 4.25 | 33.86 ± 32.26 |
| | median | 367.2 | 124.90 | 12.00 | 51.00 | 8.00 | 27.00 |
| poli I/C + resveratrol | mean±SD | 267.44 ± 147.45 | 178.89 ± 168.19 | 7.93 ± 7.20 */** | 38.66 ± 42.22* | 5.00 ± 2.29*** | 22.57 ± 19.22*** |
| | median | 254.50 | 91.10 | 4.40 | 27.00 | 5.00 | 19.00 |
| medium non-stimulated cells | mean±SD | 348.47± 91.73 | 199.09± 185.97 | 21.60 ± 14.14 ** | 184.09± 260.02 | 6.44 ± 3.68 | 27.27 ± 31.61 |
| | median | 366.90 | 147.30 | 18.40 | 36.00 | 7.00 | 13.00 |

*p=0.05; ** p<0.02; ***p<0.05

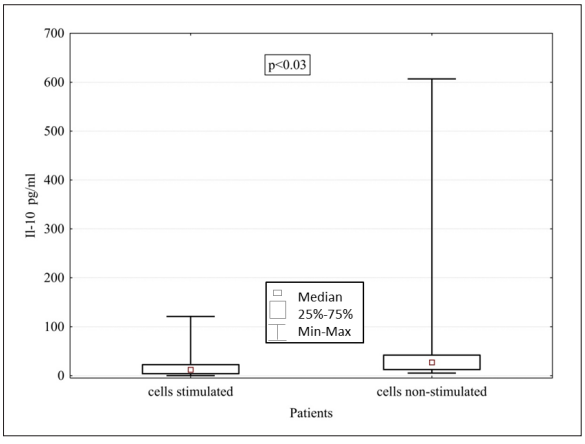


**Fig. 3.** Median concentration of IL-10 in supernatants from culture of cells obtained from patients after stimulation with ODN 2006 and in non-stimulated cultures (in medium alone). Number of patients in each particular group is posted in the Table 1. Significance levels are indicated for Mann-Whitney test

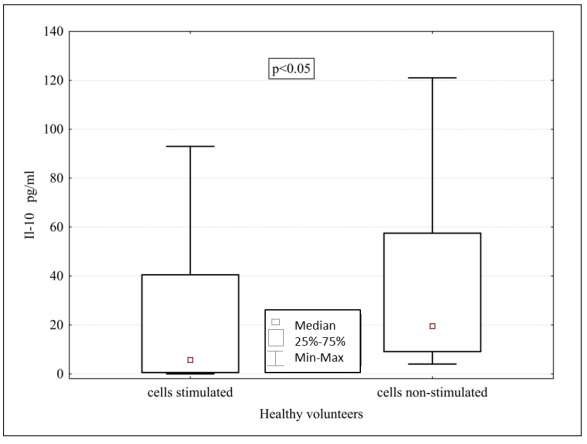


**Fig. 4.** Median concentrations of IL-10 in supernatants from culture of healthy volunteer cells stimulated with poli I/C and resveratrol versus non-stimulated cells (in medium alone). Number of patients in each particular group is posted in the Table 1. Significance levels are indicated for Mann-Whitney test

trations of IL-10 in culture supernatant from both type of patients and healthy volunteers in comparison with non-stimulated cells (p<0.05) (Table 2, Fig. 2, 3 and 4).

IL-10 levels in supernatants harvested from cultures of active SLE patient cells were also found to be lower than those obtained after the stimulation of inac-

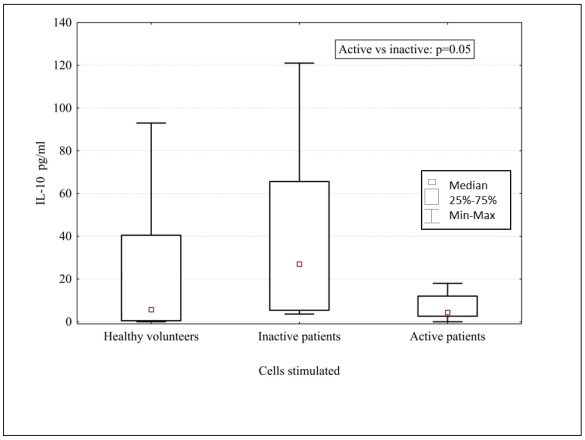


**Fig. 5.** Median IL-10 concentration in culture supernatants after cell stimulation with poli I/C and resveratrol. Cells were obtained from inactive and active phase of SLE as well as from healthy volunteers. Number of patients in each particular group is posted in the Table 1. Significance levels are indicated for Mann-Whitney test

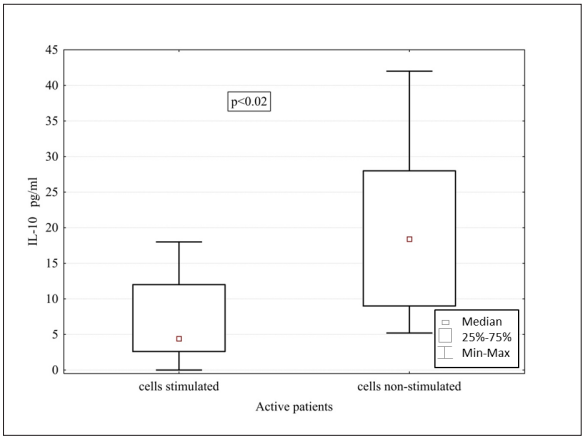


**Fig. 6.** Median IL-10 concentration after stimulation with poli I/C and resveratrol the cells from active phase of SLE and compared to non-stimulated cells (in medium alone). Number of patients in each particular group is posted in the Table 1. Significance levels are indicated for Mann-Whitney test

tive SLE patients (p=0.05) (Fig. 5). Moreover, statistically significant lower IL-10 production (p<0.02) was observed in the case of patients in the active phase of SLE than in unstimulated cells following the addition of resveratrol to the culture with poli I/C (Fig. 6 and Table 3). Simulation by ODN2006 or poli I/C did not reveal a correlation between the level of IL-10 production and the activity of the disease (Table 4). PBMCs stimulated with ODN2006 or poli I/C did not show changes in the levels of sIL2R level in all studied groups. Similar findings were obtained using antagonist of TLR9 and TLR3 (Table 2). However, resveratrol decreased poli I/C-induced sIL2R in culture supernatant from active phase patients compared to inactive phase patients (p=0.05) (Table 3, Fig. 7). Stimulation of TLR3 and TLR9 did not reveal a positive correlation between sIL-2R concentration in culture supernatants and the activity of the disease (Table 4).

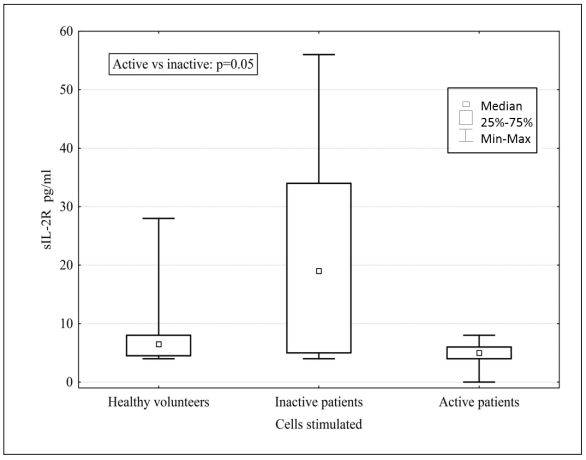


**Fig. 7.** Median concentration of sIL-2R in supernatant from cells stimulated with poli I/C and resveratrol. Cells were obtained from inactive and active phase of SLE as well as from healthy volunteers. Number of patients in each particular group is posted in the Table 1. Significance levels are indicated for Mann-Whitney test

**Table 4.** Correlation between IL-6, IL-10 and sIL-2R concentrations in cell culture supernatants following ODN 2006 or poli I/C administration and activity of the disease

| | | IL-6 | IL-10 | sIL-2R |
|---|---|---|---|---|
| Activity and stimulation by TLR9 agonist | p | 0.005145 | 0.307785 | 0.978279 |
| | R Spearmana | 0.662733 | -0.272194 | -0.007407 |
| | t(N-2) | 3.311349 | -1,05842 | -0.027717 |
| Activity and stimulation by TLR3 agonist | p | 0.071920 | 0.330108 | 0,602048 |
| | R Spearmana | 0.461547 | -0.260360 | -0.141159 |
| | t(N-2) | 1.946700 | -1.00897 | -0.533511 |

## Discussion

Our results show that mononuclear cells from SLE patients responded differently to stimulation by the examined ligands than those from healthy volunteers. The stimulation with ODN2006 induced a TLR9 stimulation in healthy volunteers associated with a higher concentration of IL-6 in the supernatants, whereas cells of SLE patients demonstrated that they are not sensitive to the compound. While our observations are consistent with the results obtained by other authors in this regard, they also confirm that PBMC from SLE patients have a weak response to activation. Our experimental model confirms that previous *in vitro* exposure of murine macrophages from the RAW264.7 cells line to CpG DNA caused the inhibition of cytokine release following re-stimulation by TLR9 ligand [29].

A positive correlation was also found between IL-6 concentration in cell culture supernatant following ODN2006 use and disease activity. This observation confirms previously published data that aggravation of the disease is correlated with the concentration of IL-6 [5,19,20]. No changes in IL-6 concentration were observed after the stimulation of PBMC by poli I/C, neither in patients nor healthy volunteers. In contrast, Wong et al. report that adding poli I/C to culture of PBMC from patients and healthy volunteers is associated with a higher concentration of IL-6 [26]. However, it is important to note that the group of SLE patients examined in their study was in the inactive phase of the disease according to SLEDAI (mean 3.13), and most of the group had been receiving immunosuppressive treatment (93.8% of patients used prednisone), which make them similar to the healthy population. The patients from our research remained in the active phase of the disease according to SLAM (mean 11.2), and 54.3% were receiving immunosuppressive treatment.

No changes in IL-10 concentration were observed following ODN2006 use in neither the patients nor in healthy volunteers. However, this observation stands in contrast with the results of previous studies regarding the *in vitro* impact of this synthetic TLR9 ligand on IL-10 concentration. Unlike our observations on the control group described in the present study, Wong et al. found that following ODN2006 stimulation, IL-10 concentration in the supernatant was elevated in healthy volunteers; however, the IL-10 concentration in patient cells remained similar to that of the control group, which is consistent with our results [26].

An assessment of PBMC response revealed higher concentrations of sIL-2R in the cell culture supernatant following TLR9 activation, especially in the active phase of SLE. TLR3 stimulation caused higher concentrations of sIL-2R only in the active phase. Moreover, TLR9 stimulation resulted in an increase of sIL-2R concentration more than three times higher than the control values, which were only twice those at baseline. This stronger response of cells from patients with active SLE to stimulation by TLR3 and TLR9 ligands may be connected with greater cell reactivity following aggravation by the disease and the involvement of the receptors of lymphocyte activation [23]. Wong et al. report the increase of sIL-2R serum concentration in SLE patients undergoing particularly of fungal or tuberculosis infection [27]. Hence, TLR activation by pathogens causes sIL-2R production. Moreover, it has been observed that sIL-2R concentration is reduced by the administration of antimalarial drugs, which are known to be inhibitors of endosomal TLR activation [25]. A correlation has been noted between sIL-2R concentration and the activity of lupus nephritis [9]. Activation of TLR3 and TLR9 has been shown to cause aggravation of lupus nephritis in animal models [16,17]. Dejic et al. report that the sIL-2R concentration (marker of lymphocyte activation) correlates with the grade of renal inflammation in SLE patients [4]. These findings imply that TLR3 and TLR9 activation and sIL-2R level may be mutually dependent in the development of the renal inflammatory process during the active phase of SLE.

Previous studies on animal experimental models have shown that inhibition of TLR activation using synthetic substances, such as ODN, cause the suppression of clinical symptoms of SLE and normalization of laboratory parameters [2,18,32]. The present study also examines the *in vitro* efficacy of resveratrol, a TLR3 antagonist, and IRS 954, a TLR9 antagonist. It was found that resveratrol administration lowers the concentrations of examined cytokines independently of previous stimulation by TLR3 and TLR9 ligands in both SLE patients and healthy volunteers. However, statistically significant differences were observed only in the case of IL-10 after poli I/C stimulation, which was then inhibited by resveratrol. This selective action of resveratrol on TLR3 has also been confirmed by other authors [30].

In addition, our results indicate that resveratrol also causes the inhibition of IL-10 synthesis. A literature search revealed only one congress report with no precise information about the influence of resveratrol on cytokine synthesis [22]. However, Xuzhu et al. report that the use of resveratrol in mice with collagen-induced arthritis resulted in the total inhibition of IL-17 production and lower concentrations of IFN-γ, TNFα, IL-1 and IL-6, but no influence on IL-2 or IL-10 concentrations [28]. The impact of resveratrol on cytokine concentrations depends on the dose. Reduction of IL-10 concentration may inhibit disease development, which is believed to be associated with its immune system stimulation [10]. Moreover, IL-10 induces the expression of IL-2R, which sensitizes B lymphocytes to the action of IL-2, and increases the differentiation rate of the cells [8]. Literature data has also shown that in SLE patients, the presence of higher amounts of immunological complexes and IFN-α disturb the function of IL-10 by diminishing its ability to inhibit the inflammatory process [31], which reduces the anti-inflammatory activities of this cytokine. Previous studies also found a correlation between higher concentrations of IL-10 and greater SLE activity [15]. However, Ishida et al. found IL-10 to have a negative impact on SLE activity [7].

Our findings indicate that following culture with resveratrol and poli I/C, cells obtained from patients in the active phase of the disease excreted significantly lower amounts of IL-10 and sIL-2R than those in the inactive phase. The addition of resveratrol reduced IL-10 concentrations in the cell culture supernatant, which indicates that resveratrol may also have an influence on cytokine synthesis mechanisms other than those dependent on TLR/TRIF. In addition, resveratrol diminishes the expression of costimulatory molecules on macrophages (CD80) and T lymphocytes (CD28), which impacts on the activation of those cells [21]. Resveratrol acts antago-

nistically to ligands stimulating TLR by blocking the increased expression of costimulatory molecules.

In conclusion, our findings suggest the involvement of resveratrol in the modulation of the inflammatory response. Although resveratrol was not found to have a selective influence on cells activated by TLR ligands, it was found to have anti-inflammatory activity. This beneficial effect of resveratrol may be of importance for development of future SLE therapies.

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The authors have no potential conflicts of interest to declare.