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# Distinct Cytokine Profiles in Patients with Oligoarticular Juvenile Idiopathic Arthritis after *in Vitro* Blockade of T Cells by Cyclosporine and Abatacept

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## Abstract

**Oligoarticular juvenile idiopathic arthritis (oJIA) is an antigen-driven and lymphocyte-mediated disorder affecting the adaptive immune system. Auto reactive T cells produce pro-inflammatory cytokines as IFN-$\gamma$ and IL-17. Failure of regulatory T cells leads to decreased production of anti-inflammatory IL-10 and results in the loss of immune tolerance. Therapeutic strategies suppress T cell dependent immune responses and consequently inhibit the process of inflammation. The aim of the study was to investigate the effect of T cell suppression on the cytokine network in oJIA patients. Therefore we examined the cytokine concentration after *in vitro* inhibition of T cells by cyclosporine and abatacept in patients with persistent oJIA and healthy control subjects. This single center cohort study consisted of oJIA affected children and control subjects. Cytokine profiles from cell culture supernatants were examined with multiplex fluorescent bead immunoassay by flow cytometry. High amounts of IL-17 were only observed in the collective of oJIA patients after T cell stimulation. Cyclosporine suppresses its concentration effectively. IL-2 and IFN-$\gamma$ are present in both groups. We found IL-6 and TNF-$\alpha$ in high concentrations after T cell activation. While TNF-$\alpha$ concentration is suppressed by both drugs, IL-6 concentration remains high in oJIA patients. Concentrations of IL-4 and IL-10 were not found to be influenced in status of activation or suppression. In conclusion, the results of the present study imply that IL-17 is the crucial T cell cytokine in oligoarticular JIA. Only cyclosporine could inhibit the secretion of IL-17 effectively. IL-2 and IFN-$\gamma$ are not specific for oligoarticular JIA. Both cytokines are found as well in healthy control subjects after T cell stimulation. Relevant pro-inflammatory macrophage cytokines in oli-**

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**goarticular JIA are TNF-$\alpha$ and IL-6. T cell suppression by cyclosporine and abatacept inhibits TNF-$\alpha$ but not IL-6 effectively. Production of anti-inflammatory cytokines is not influenced by T cell suppression.**

## Keywords

**Juvenile Idiopathic Arthritis, Pathogenesis, Cytokines, T Cell Inhibition**

## 1. Introduction

Juvenile idiopathic arthritis (JIA) refers to a group of chronic childhood arthropathies of unknown aetiology which represents the most common rheumatic condition in children. Seven subtypes of JIA are described by the latest (2001) International League of Associations for Rheumatology (ILAR) criteria. Persistent oligoarticular JIA is the most common form among Caucasians [1] [2]. Present data suggest an autoimmune pathogenesis involving both adaptive and innate immune responses. The association between susceptibility to oligoarticular JIA and HLA class II alleles implicates $CD4^+$ T cells in the pathogenesis of chronic arthritis [3]. This is supported by the fact that activated $CD4^+$ T cells clustered around dendritic cells are found in the synovia of the inflamed joint in oligoarticular JIA patients [4]. There exist two hypotheses for the development of autoimmune phenomena in oJIA: Massa *et al.* reported that T cell cross-recognition (molecular mimicry) of exogenous and self HLA-derived antigens generates an abnormal regulatory circuit which maintains and expands T cells, which may participate in autoimmune inflammation by generation of pro-inflammatory cytokines [5]. Another hypothesis is that auto-antigens derived from cartilage and other joint-related tissue, such as aggrecan, fibrillin and matrix-metalloproteinase 3 (MMP3), may be able to activate auto-reactive $CD4^+$ T cells, including Th1 and Th17 cells which are correlated with autoimmune symptoms of oligoarticular JIA [4] [6] [7]. Recent studies suggest that Th17 cells producing pro-inflammatory IL-17 are crucial for initiation and maintenance of autoimmune arthritis in oligoarticular JIA [8]. IL-17 receptors are widely expressed including epithelial cells, B and T lymphocytes, synovial fibroblasts, vascular endothelial cells and chondrocytes explaining its pleiotropic effects [9] [10]. In synovial fibroblasts, the cytokine IL-17 stimulates the secretion of MMPs, which trigger the destruction of cartilage tissue [11]-[13]. IL-17 synergizes with IL-1$\beta$ and TNF-$\alpha$, inducing pro-inflammatory cytokines and chemokines (e.g. IL-8) from monocytes resulting in neutrophil attraction to the inflamed joint [11]. Interestingly, IL-17 is able to maintain disease activity independent of TNF-$\alpha$ [14].

On the contrary, regulatory T (Treg) cells play a critical role in immune tolerance to auto-antigens by suppressing the function of effector T cells [15]. Activation-induced Treg cells produce anti-inflammatory cytokine IL-10 and are able to inhibit the secretion of pro-inflammatory cytokines IFN-$\gamma$ and IL-17 from effector Th1 and Th17 cells [16]. It has been supposed that Treg cells are the most important regulator of immune responses encouraged by the fact that their presence in the inflamed joint was associated with a limited and less severe course of arthritis in JIA [17]-[19].

In autoimmune arthritis a status of dysregulation is postulated in which the pro-inflammatory effect of Th1 and Th17 cells overcome the anti-inflammatory effect of Treg cells resulting in the loss of immune tolerance [20]. The dysfunctions of T cell tolerance may subsequently activate adaptive and innate immune responses including neutrophil granulocytes and macrophages leading to the production of pro-inflammatory cytokines IL-1$\beta$, TNF-$\alpha$ and IL-6. The pleiotropic effect of these cytokines leads to activation of macrophages, neutrophil granulocytes, endothelial cells and osteoclasts and proliferation of fibroblasts. This pathogenetic process results in chronic inflammation of the joint [21]. Consequently high levels of pro-inflammatory cytokines in serum and inflamed joint were found in patients with oligoarticular JIA [3].

Therapeutic strategies block T cell dependent immune responses and consequently repress the process of autoimmunity. Pharmaceutics targeting T cells and used in JIA patients are cyclosporine and abatacept. Cyclosporine is part of the DMARD group (Disease Modifying Antirheumatic Drug) and its therapeutic effect is based on regulation of gene expression. It binds cytoplasmatic cyclophilin and the cyclosporine/cyclophilin-complex binds and inhibits transcription factor calcineurin. Activated calcineurin leads to intranuclear translocation of transcription factors resulting in gene expression of IL-2 and IFN-$\gamma$. Cyclosporine thus inhibits production of IL-2 and IFN-$\gamma$ and subsequent T cell activation [22] [23]. The use of cyclosporine in oligoarticular JIA

patients is approved for difficult to treat uveitis. For this purpose no controlled randomized studies have been realized so far [24]. Abatacept is a recombinantly produced, fully humanized, soluble fusion-protein. It consists of the extracellular domain of Cytotoxic T-lymphocyte-Associated Antigen-4 (CTLA-4) and a fragment of the Fc-part from human IgG1. CTLA-4 binds CD80/86 on antigen-presenting cells (APC) and inhibits the co-stimulatory signal needed for T cell activation [25]. In a controlled randomized study JIA-Patients resistant to therapy with methotrexate and TNF-$\alpha$-inhibitor showed benefits after being treated with abatacept [26].

Few studies exist to date on the effect of T cell suppression on the cytokine network of patients with oligoarticular JIA. We therefore established an *in-vitro* model to investigate the influence of pharmaceutics targeting T cells on the cytokine network with focus on the shift between pro- and anti-inflammatory cytokines. Aim of the study was to test for differences in cytokine expression in patients with oligoarticular JIA and healthy control subjects.

## 2. Methods

### 2.1. Patients and Samples

This single center cohort study consisted of oJIA affected children and control subjects and was conducted in the period 2009-2011. All JIA patients fit the International League of Associations for Rheumatology criteria for persistent oligoarthritis and provided written informed consent before enrolment. None of the patients was treated with immunosuppressive pharmaceutics used in our *in-vitro* model. Heparinized peripheral blood was collected from ten patients of our pediatric rheumatological outpatient clinic and from fifteen healthy adult volunteers. The study protocol was approved by the institutional ethics committee (#837.169.08).

### 2.2. Patient Demographics

Ten patients with persistent oligoarthritis were enrolled after consent. Comprehensive clinical information was collected at each patient visit, including history, physical examination, and clinical laboratory values [erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)]. Clinical status at each visit was graded according to a scoring system (Steinbrocker) to grade severity of arthritis. Patients were under no therapy, antiphlogistic (Naproxene) and/or immunosuppressive (Methotrexate) therapy dependent upon their disease status. Characteristics of the study subjects are shown in **Table 1**.

### 2.3. Isolation and Stimulation of PBMCs

Peripheral blood mononuclear cells were isolated from heparinized peripheral venous blood using Ficoll-Hypaque density gradient. The cells were suspended in culture medium [RPMI 1640 + 10% human AB-Serum + Penicillin (100 U/ml) + streptomycin (100 µg/ml)] and kept in a final concentration of $1 \times 10^6$ cells per ml in 96-well culture plates (VWR International, Germany; separation and culture media from PAA-Laboratories GmbH, Cölbe, Germany).

Polyclonal T cell stimulation for cytokine secretion was performed with phytohemagglutinin (PHA) from *Phaseolus vulgaris* (Sigma-Aldrich, Munich, Germany) in a final concentration of 10 µg/ml.

### 2.4. Stimulation and Blockade of T Cell Induced Cytokine Secretion

*In-vitro* T cell suppression was performed using cyclosporine from *Tolypocladium inflatum* (Sigma-Aldrich) in a final concentration of 150, 2 µg/l and abatacept (Bristol-Myers Squibb) in a final concentration of 10 µg/ml. To determine the basal level of cytokine secretion *in vitro* by PBMCs from JIA patients and healthy adults, PBMCs were cultured in absence of PHA according to the procedure described above.

Cell cultures were adjusted to a final volume of 200 µl/well and incubated at 37˚C in a humidified atmosphere containing 5% $CO_2$. After 24 hours 30 µl culture supernatants were collected and stored at −20˚C until used for the measurement of cytokine concentration. Final concentration and volume of each well are presented in **Table 2**.

### 2.5. Multiplex Fluorescent Bead Immunoassay (ELISA) for Determination of Cytokine Profiles

Two-colour flow cytometry was applied to cell culture supernatants of oJIA patients and healthy control subjects,

**Table 1.** Patient demographics.

| | |
|---|---|
| Number of samples analysed patients/controls | 10:15 |
| Age at enrollment [median (range) years] | 13.4 (0 - 26) |
| Age at onset [median (range) years] | 4.3 (0 - 12) |
| Disease duration [median (range) years] | 4.7 (1 - 11) |
| Disease activity (Steinbrocker) | |
| I [n] | 2 |
| II [n] | 7 |
| III [n] | 1 |
| ESR (mm/h) | 13 (3 - 58) |
| CRP (mg/l) | 3 (1 - 68) |
| Male/female | 4:6 |
| ANA positive | 5 |
| RF positive | 2 |
| HLA B27 positive | 1 |
| Patients on | |
| DMARDS (MTX) | 2 |
| NSAIDS | 8 |
| No therapy | 2 |
| Steroids | 0 |
| Biologicals | 0 |

**Table 2.** Final concentrations and volumes of *in-vitro* model.

| Suspension of cells | Culture medium | PHA | Pharmaceutical |
|---|---|---|---|
| 100 µl PBMC ($2 \times 10^6$/ml) | 100 µl | - | - |
| 100 µl PBMC ($2 \times 10^6$/ml) | 90 µl | 10 µl | - |
| 100 µl PBMC ($2 \times 10^6$/ml) | 80 µl | 10 µl | 10 µl cyclosporine |
| 100 µl PBMC ($2 \times 10^6$/ml) | 80 µl | 10 µl | 10 µl abatacept |

to investigate the concentrations of Interleukin (IL)-12p70, Interferon (IFN)-$\gamma$, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17A, IL-$\beta$, Tumor necrosis factor (TNF)-$\alpha$ and TNF-$\beta$. All cytokines were measured by commercial kits, Human Th1/Th2 11 plex Flow Cytomix Kit and Human IL-17A simplex Kit (BenderMedSystems GmbH, Vienna, Austria) according to the manufacturer's instructions for the use of tubes.

## 2.6. Statistical Analysis

Descriptive analysis was performed to compare data of healthy controls and oJIA patients. In order to evaluate the change of cytokine production, we formed the ratio of the values of stimulated cell cultures with biological and the values of unstimulated cell cultures. Mann-Whitney *U* test was used to compare data of healthy controls and oJIA patients. Only the values of $p < 0.05$ were considered to be statistically significant in all analyses. Statistical analysis was performed with commercial software (SPSS Statistics Software version 20.0; SPSS Inc.).

## 3. Results

We examined the presence of twelve cytokines in leukocyte culture supernatants of 10 oJIA patients as well as 15 healthy individuals by flow cytometry analysis (multiplex fluorescent bead immunoassay). In order to evaluate the change of cytokine production after PHA stimulation and cytokine inhibition by CSA and abatacept, the ratio of the cytokine median values of stimulated cell cultures with biologic and the cytokine median values of unstimulated cell cultures was formed (**Figure 1**, **Figure 2** and **Figure 3**). Cytokine concentrations (median values, minima and maxima) are shown in **Tables 3-5**. Error bars are shown in **Figure 4**, **Figure 5** and **Figure 6**.

### 3.1. Pro-Inflammatory T Cell Cytokines IL-2, IFN-$\gamma$ and IL-17A

We stated that PHA stimulated leukocytes of healthy individuals secrete more IL-2 respectively IFN-$\gamma$ than leukocytes of oJIA patients (**Figure 1(a)**, **Figure 1(b)**, **Figure 4(a)**, **Figure 4(b)**). In contrast, PHA induced IL-17A

**Table 3.** Median concentrations (pg/ml), minima and maxima of cytokines IL-2, IFN-gamma and IL-17A in control subjects (CS) and patients with persistent oligoarthritis (OA).

| | IL-2 | | IFN-gamma | | IL-17A | |
|---|---|---|---|---|---|---|
| | Control subjects | OA | Control subjects | OA | Control subjects | OA |
| Unstimulated | 0 (0 - 32.5) | 31.6 (0 - 61.3) | 0 (0 - 38.1) | 2.2 (0 - 48.4) | 0 (0 - 0) | 0 (0 - 0) |
| PHA stimulation | 314.09 (28.5 - 1999.2) | 151.1 (32.5 - 1400.3) | 1768.7 (0 - 3517.1) | 351.5 (33.3 - 1862.7) | 0 (0 - 341.3) | 176.4 (0 - 943.3) |
| PHA stimulation + CSA | 135.8 (33.0 - 711.7) | 77.7 (0 - 1159.2) | 1292.0 (4.8 - 3634.9) | 196.3 (8.64 - 1862.7) | 0 (0 - 2.0) | 0 (0 - 206.5) |
| PHA stimulation + abatacept | 121.4 (0 - 1514.8) | 140.3 (36.3 - 1535.7) | 643.9 (0 - 4255.4) | 362.0 (27.6 - 1594.7) | 0 (0 - 167.7) | 204.2 (0 - 768.3) |

**Table 4.** Median concentrations (pg/ml), minima and maxima of cytokines IL-1$\beta$, IL-6 and TNF-$\alpha$ in control subjects and patients with persistent oligoarthritis (OA).

| | IL-1$\beta$ | | IL-6 | | TNF-$\alpha$ | |
|---|---|---|---|---|---|---|
| | Control subjects | OA | Control subjects | OA | Control subjects | OA |
| Unstimulated | 0.95 (0 - 970.5) | 34.1 (0 - 10498.5) | 114.5 (0 - 4241.7) | 92 (0 - 13265.2) | 8.8 (0 - 281.3) | 9.8 (0 - 6832.3) |
| PHA stimulation | 4505.3 (151.4 - 10660.7) | 4829.9 (1255.1 - 12024) | 10175.3 (35.4 - 21752.7) | 14536.9 (8755.8 - 20223.8) | 1017.4 (134.5 - 4437.2) | 2374.9 (46.5 - 6696.4) |
| PHA stimulation + CSA | 3511.1 (96.4 - 10202.9) | 4009.4 (1046.2 - 12841) | 6028.3 (869.2 - 22894.9) | 15362 (9667.6 - 21730.5) | 1409.9 (25.7 - 4632.8) | 571.3 (48.4 - 7059.4) |
| PHA stimulation + abatacept | 3211.2 (31.7 - 12575.8) | 4313.9 (1217 - 10847.3) | 5762.1 (25.2 - 21752.7) | 14226.4 (7462.8 - 20223.8) | 963.8 (14.9 - 3545.7) | 1322 (50.4 - 3674) |

**Table 5.** Median concentrations (pg/ml), minima and maxima of cytokines IL-4 and IL-10 in control subjects and patients with persistent oligoarthritis (OA).

| | IL-4 | | IL-10 | |
|---|---|---|---|---|
| | Control subjects | OA | Control subjects | OA |
| Unstimulated | 0 (0 - 37) | 0 (0 - 44.2) | 0 (0 - 0) | 0 (0 - 346.8) |
| PHA stimulation | 0 (0 - 34.5) | 29.8 (0 - 67.4) | 1113.8 (14.5 - 4260.9) | 1182 (691 - 5004.3) |
| PHA stimulation + CSA | 0 (0 - 39.4) | 22.3 (0 - 39) | 884.1 (18.4 - 2667.1) | 866.2 (472.4 - 4301) |
| PHA stimulation + abatacept | 0 (0 - 40.6) | 29.9 (0 - 67.4) | 1019.8 (0 - 3143.9) | 1207.3 (664.9 - 5004) |

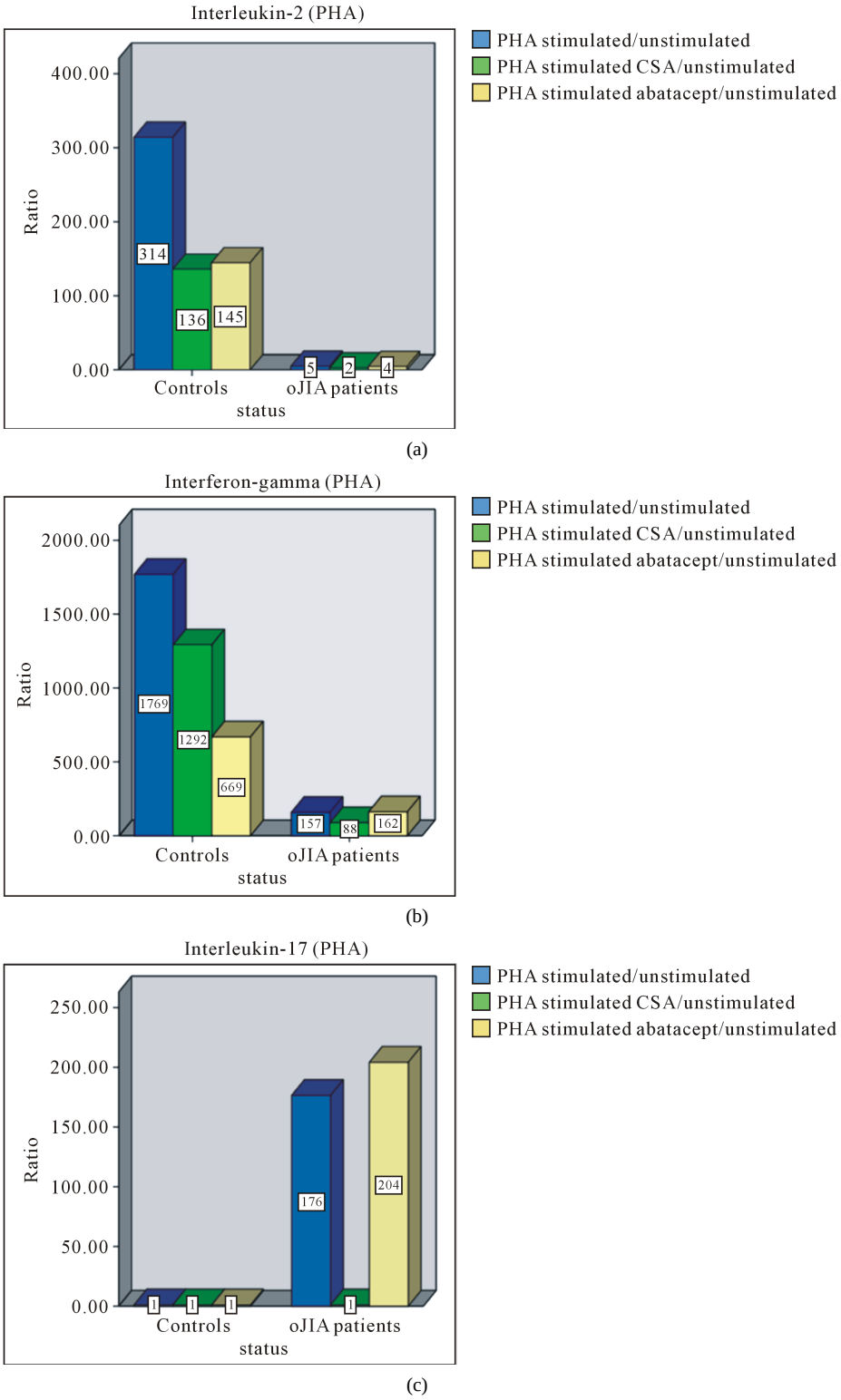



**Figure 1.** Representative bar diagrams showing the ratio of cytokine values of (a) IL-2, (b) IFN-γ and (c) IL-17A in healthy controls (left) and oJIA patients (right). Blue filled bars: ratio of the values of PHA stimulated cell cultures and the values of unstimulated cell cultures. Green filled bars: ratio of the values of PHA stimulated cell cultures and the values of stimulated cell cultures with CSA. Yellow filled bars: ratio of the values of PHA stimulated cell cultures and the values of stimulated cell cultures with abatacept.

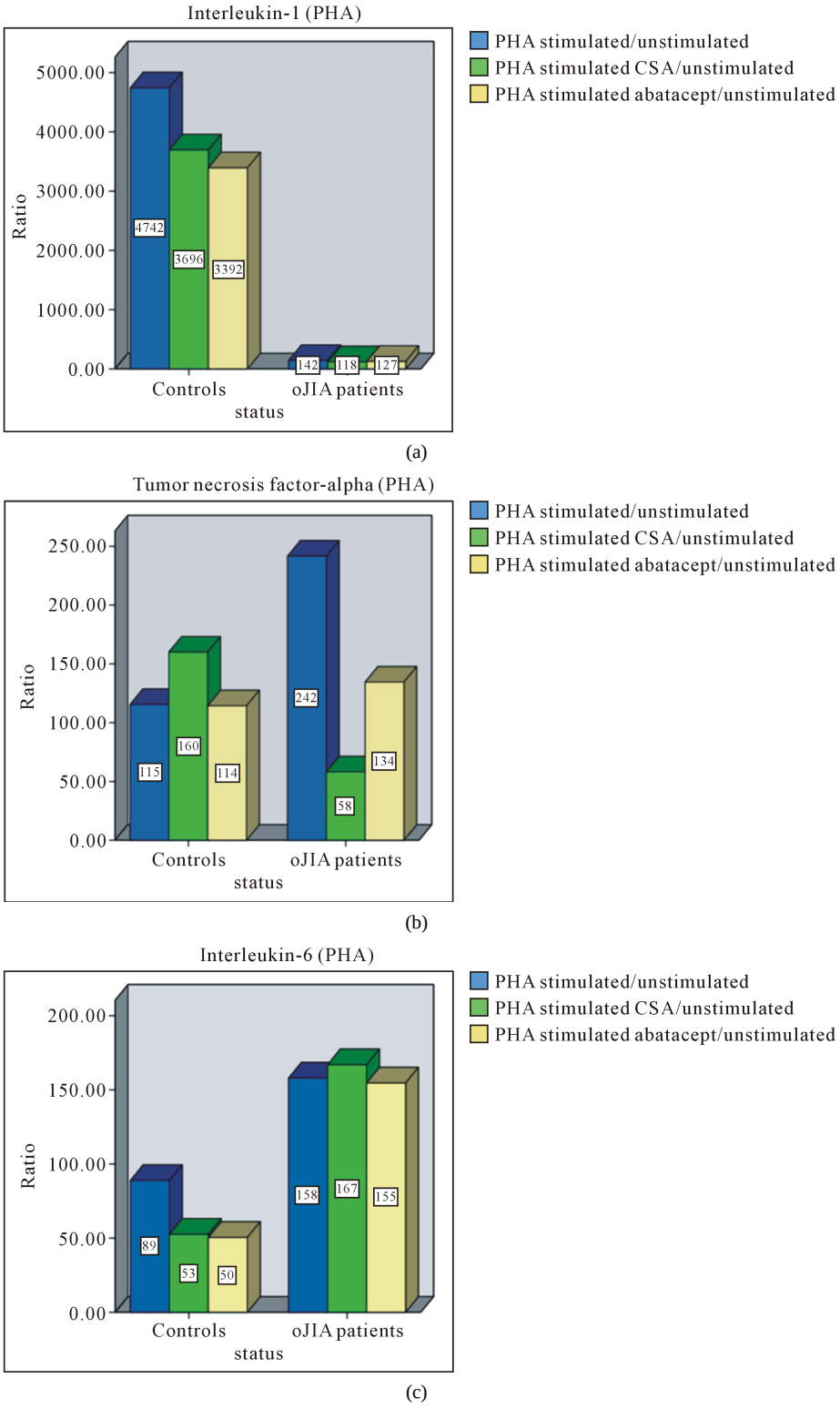



**Figure 2.** Representative bar diagrams showing the ratio of cytokine values of (a) IL-1, (b) TNF-$\alpha$ and (c) IL-6 in healthy controls (left) and oJIA patients (right). Blue filled bars: ratio of the values of PHA stimulated cell cultures and the values of unstimulated cell cultures. Green filled bars: ratio of the values of PHA stimulated cell cultures and the values of stimulated cell cultures with CSA. Yellow filled bars: ratio of the values of PHA stimulated cell cultures and the values of stimulated cell cultures with abatacept.

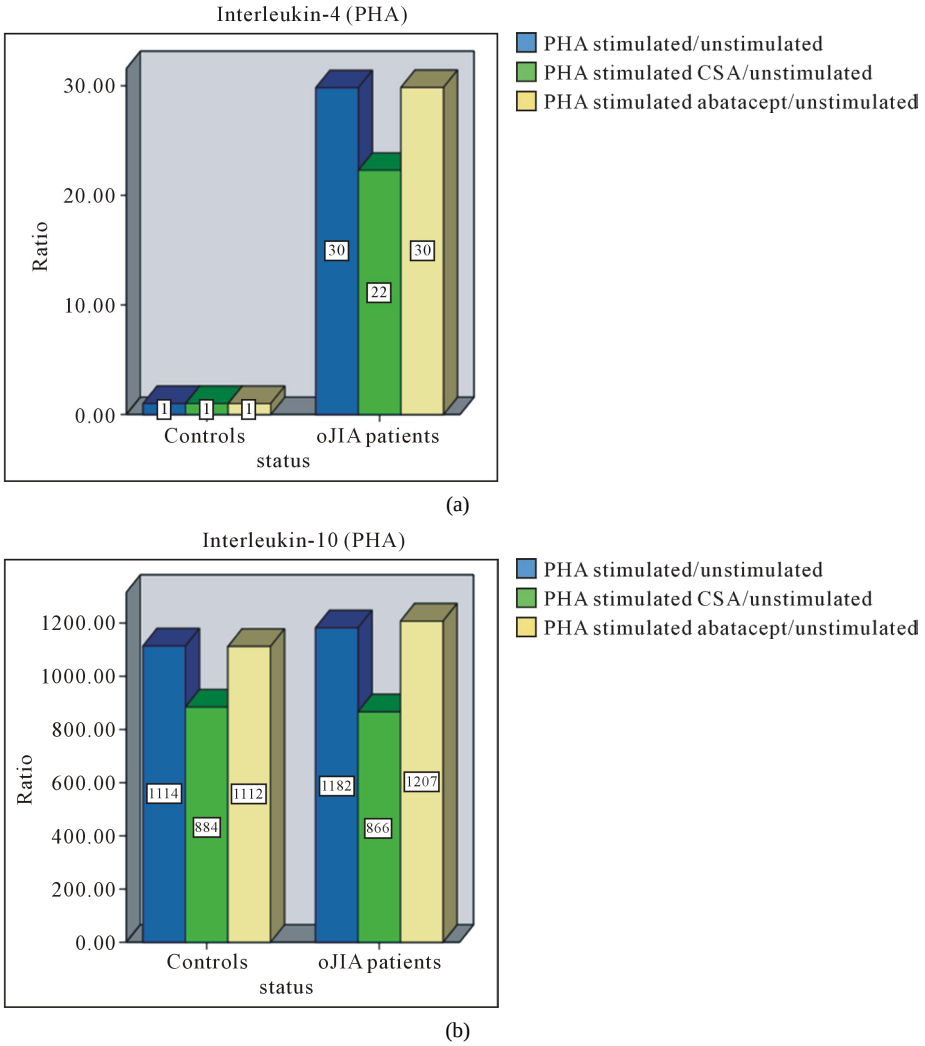
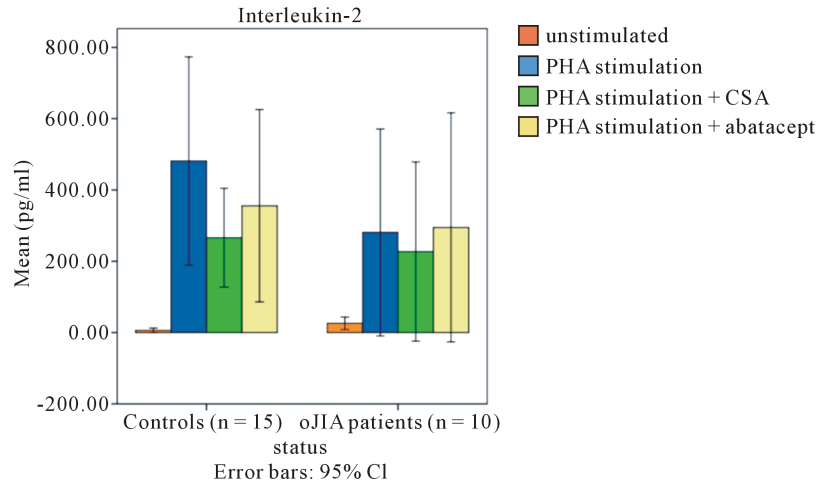



**Figure 3.** Representative bar diagrams showing the ratio of cytokine values of (a) IL-4 and (b) IL-10 in healthy controls (left) and oJIA patients (right). Blue filled bars: ratio of the values of PHA stimulated cell cultures and the values of unstimulated cell cultures. Green filled bars: ratio of the values of PHA stimulated cell cultures and the values of stimulated cell cultures with CSA. Yellow filled bars: ratio of the values of PHA stimulated cell cultures and the values of stimulated cell cultures with abatacept.

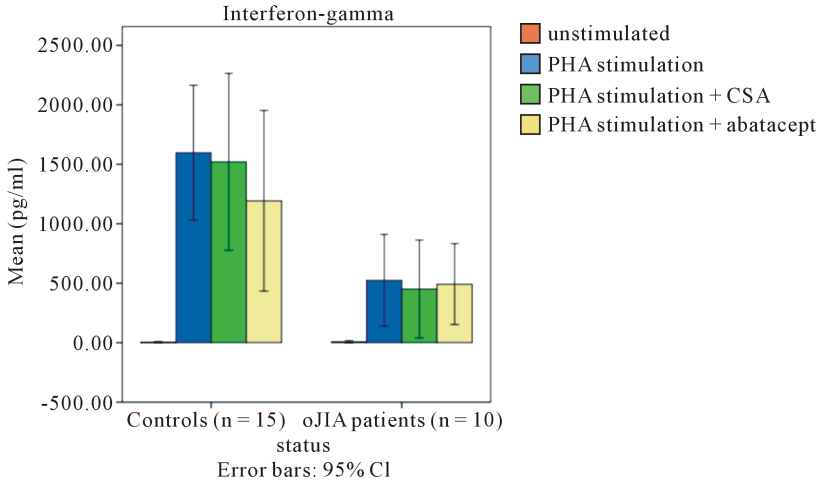
production was generally much higher in oJIA patients (**Figure 1(c)**, **Figure 4(c)**). By the use of CSA an inhibition of IL-2 and IFN-$\gamma$ was attained in healthy subjects and oJIA patients equally. Although abatacept shows an inhibitory effect on IL-2 and IFN-$\gamma$ in the control group it still seems to reinforce IFN-$\gamma$ secretion in oJIA patients (**Figure 1(b)**, **Figure 4(b)**). Furthermore, an almost complete suppression of IL-17A was attained in oJIA patients and healthy individuals equally by the use of CSA (**Figure 1(c)**, **Figure 4(c)**). Interestingly, abatacept did not alter IL-17A levels.

### 3.2. Pro-Inflammatory Macrophage Cytokines IL-1, IL-6 and TNF-$\alpha$

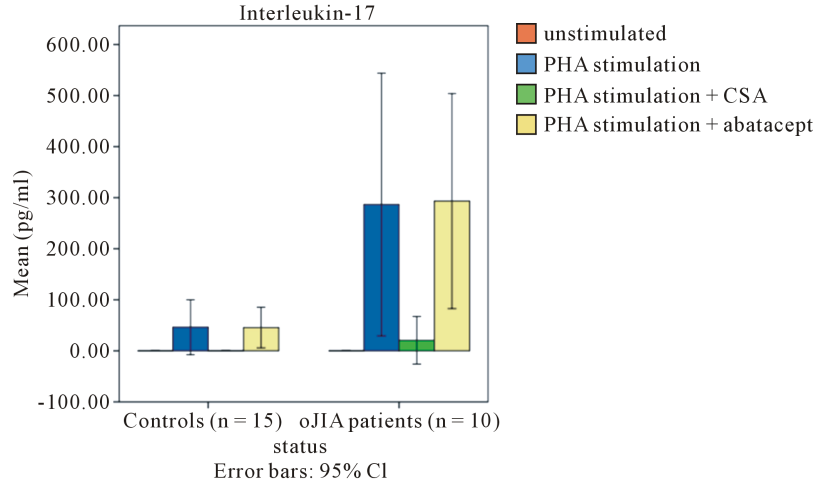
As expected, we found before and after PHA stimulation higher amounts of IL-6 and TNF-$\alpha$ in leukocyte cultures of oJIA patients than in leukocyte cultures of the control group (**Figure 2(b)**, **Figure 2(c)**, **Figure 5(b)**, **Figure 5(c)**). In addition, PHA stimulated leukocytes of healthy individuals secrete slightly more IL-1 than leukocytes of oJIA patients (**Figure 2(a)**, **Figure 5(a)**). We discovered that CSA and abatacept suppress secretion of IL-1 and TNF-$\alpha$ in control subjects and oJIA patients while IL-6 concentration remains high in oJIA patients (**Figure 2** and **Figure 5**).




(a)

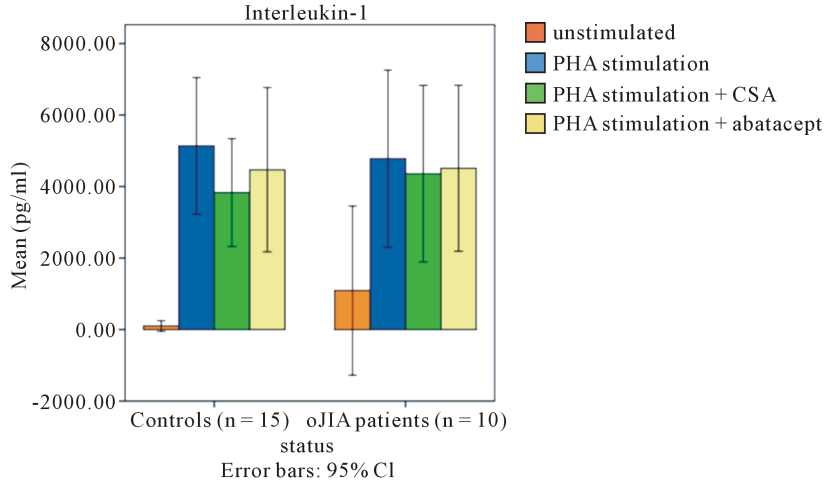



(b)

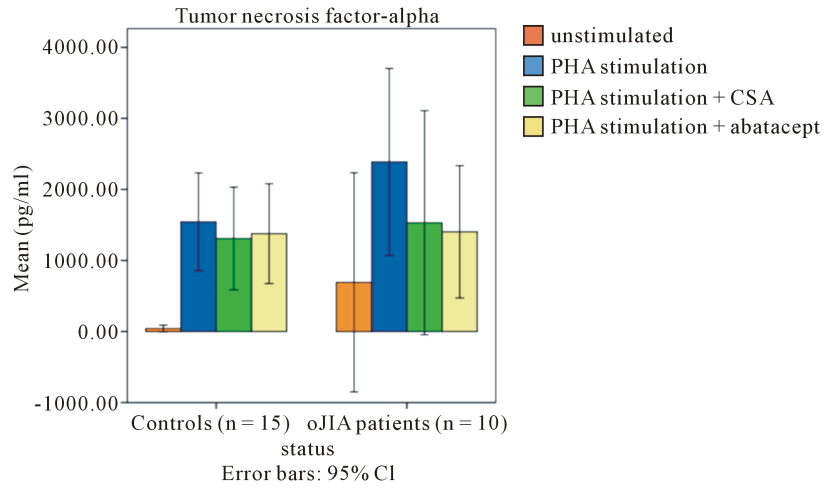



(c)

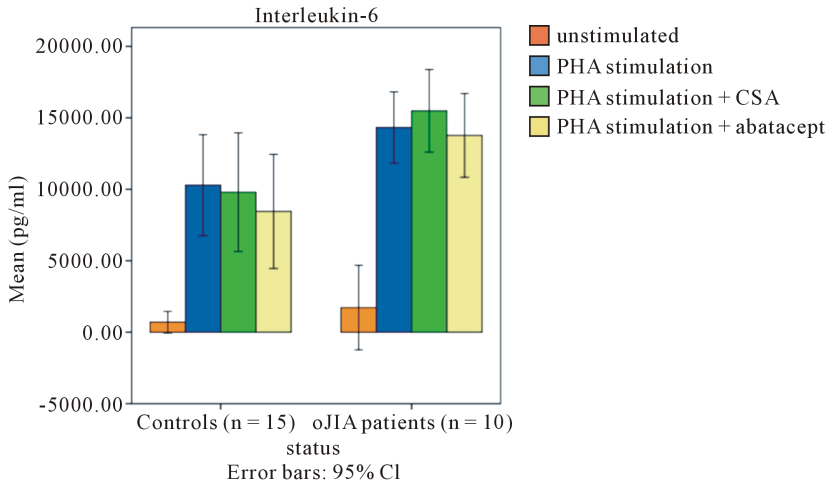
**Figure 4.** Representative error bar diagrams showing the mean concentrations of (a) IL-2, (b) IFN-$\gamma$ and (c) IL-17A in healthy controls (left) and oJIA patients (right). Orange filled bars: unstimulated cell cultures. Blue filled bars: PHA stimulated cell cultures. Green filled bars: PHA stimulated cell cultures with CSA. Yellow filled bars: PHA stimulated cell cultures with abatacept.




(a)




(b)




(c)

**Figure 5.** Representative error bar diagrams showing the mean concentrations of (a) IL-1, (b) TNF-$\alpha$ and (c) IL-6 in healthy controls (left) and oJIA patients (right). Orange filled bars: unstimulated cell cultures. Blue filled bars: PHA stimulated cell cultures. Green filled bars: PHA stimulated cell cultures with CSA. Yellow filled bars: PHA stimulated cell cultures with abatacept.

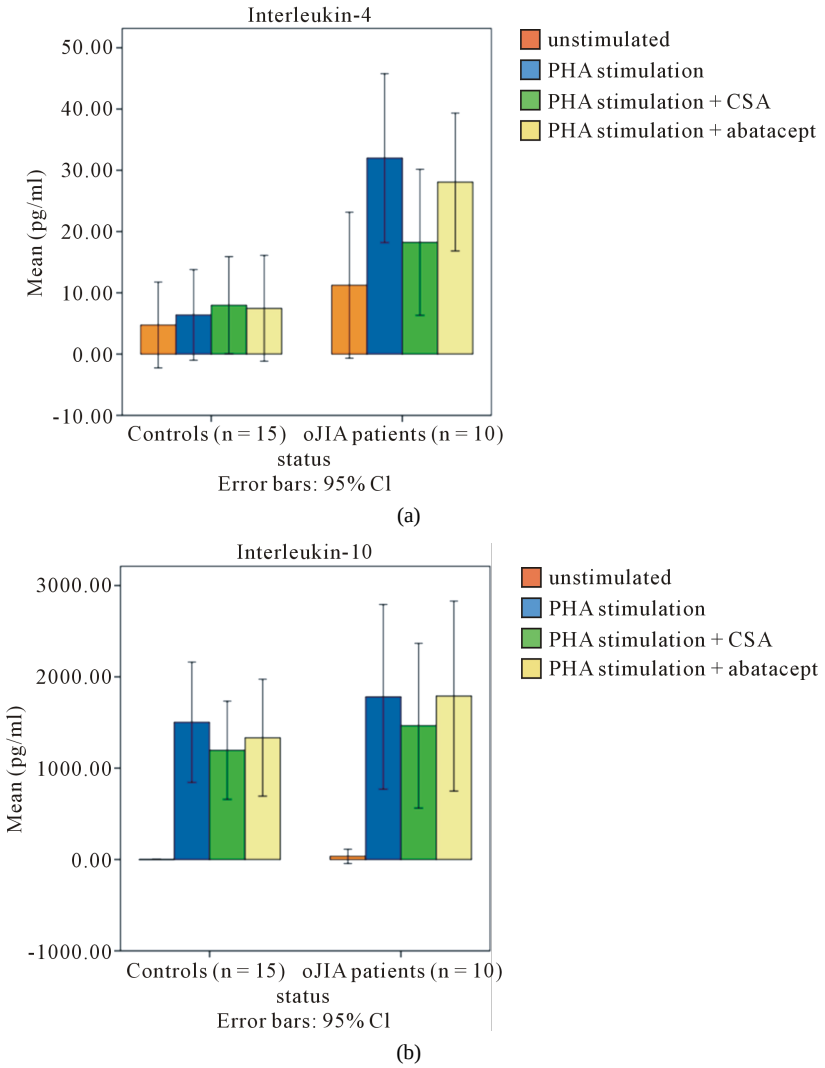



**Figure 6.** Representative error bar diagrams showing the mean concentrations of (a) IL-4 and (b) IL-10 in healthy controls (left) and oJIA patients (right). Orange filled bars: unstimulated cell cultures. Blue filled bars: PHA stimulated cell cultures. Green filled bars: PHA stimulated cell cultures with CSA. Yellow filled bars: PHA stimulated cell cultures with abatacept.

### 3.3. Anti-Inflammatory T Cell Cytokines IL-4 and IL-10

We observed that PHA stimulated leukocytes of oJIA patients secrete more IL-4 and IL-10 than healthy individuals. Neither CSA nor abatacept seem to influence the secretion of these cytokines significantly.

## 4. Discussion

Autoreactive T cells producing pro-inflammatory cytokines are in the focus of pathogenic concepts of oJIA. We studied the effect of *in vitro* T cell suppression on the cytokine network in oJIA patients and healthy controls and found relevant differences.

### 4.1. T Cell Cytokines

After polyclonal T cell stimulation by PHA we found several differences in concentrations of T cell cytokines: Both groups showed an increase of IL-2 and IFN-$\gamma$. Interestingly, in the group of healthy control subjects the increase was distinctly higher. After T cell suppression by cyclosporine and abatacept we observed a markedly decrease of IL-2 an IFN-$\gamma$ in healthy control subjects, while in the group of oJIA-patients we found no relevant

changes in concentration. These results support the hypothesis that there is no specific role for both IL-2 and IFN-$\gamma$ in the pathogenesis of oJIA. After PHA-stimulation we found an increase of IL-17 in the oJIA-group, while in healthy control subjects no relevant concentration of this cytokine was observed. This supports the hypothesis of association between IL-17/$CD4^+$Th17 cells and autoimmune mechanisms in the pathogenesis of oJIA postulated by many studies [3] [8]. After blocking T cell function via cyclosporine IL-17 concentration is undetectable low. This reflects an inhibition of pro-inflammatory and autoreactive $CD4^+$Th17 cells that are considered to be crucial in JIA-pathogenesis. Former *in vitro* studies on cyclosporine and T cell cytokine expression correlate with our findings: The fact that cyclosporine suppresses concentration of IL-17 was proven for Behçet's disease, psoriasis and Vogt-Koyanagi-Harad-syndrome [27]-[29]. To our knowledge we are the first group reporting this finding for persistent oligoarticular JIA. For abatacept we found no relevant changes in concentration of IL-17.

### 4.2. Pro-Inflammatory Cytokines

After PHA stimulation we found relevant increase in concentration of IL-1$\beta$ in both groups. After T cell blockade by cyclosporine and abatacept a decrease in concentration of IL-1$\beta$ was found for healthy control subjects. Concentration of IL-6 after T cell stimulation was markedly higher in oJIA-patients compared to healthy control subjects suggesting a major role in the inflammatory process of chronic arthritis. Regarding the effect of T cell suppression by cyclosporine and abatacept it can be postulated that in healthy control subjects a decrease is achieved while patients still show high concentrations of IL-6. In our opinion, the unchanged IL-6 concentration is an indication that cyclosporine and abatacept can indeed attenuate the autoimmune component of inflammation, but have no effect on the secondary occurring auto-inflammation. In patients with polyarticular JIA (pJIA) IL-6 is increased in the serum and in synovial fluid and cytokine concentrations are positively correlated with the severity of joint involvement and C-reactive protein levels [30]. The same applies for the likely function of IL-6 in the pathogenesis of oligoarthritis. However, this should be verified in further studies. Up to 30% of pJIA patients still show signs of active disease although these patients may respond to methotrexate or biologics approved for pJIA [31] [32]. The study of Brunner *et al.* allowed the conclusions that treatment with IL-6 inhibitor tocilizumab provides safe, sustained and clinically meaningful improvement for patients with pJIA [33].

TNF-$\alpha$ is regarded as a crucial cytokine in JIA-pathogenesis [20] [34] [35]. Consistent with this hypothesis we found a distinct increase in concentration after PHA stimulation which was considerably higher in the group of patients compared to healthy control subjects. Both immunosuppressive drugs induced a decrease in concentration of TNF-$\alpha$ in the patient group. These results consolidate the important role for IL-6 and TNF-$\alpha$ in the pathogenesis of oligoarticular JIA that is supported by clinical studies: In a prospective study elevated serum levels of IL-6 and TNF-$\alpha$ were found in oJIA patients [35].

### 4.3. Anti-Inflammatory Cytokines

After PHA stimulation and T cell suppression there were no relevant differences in concentrations of IL-4 in both groups suggesting no important pathogenic role of this cytokine. After PHA stimulation concentrations of IL-10 markedly increased in both groups. T cell suppression led to no differences in concentration of this anti-inflammatory cytokine.

Although pathogenic concepts of oligoarticular JIA postulate a critical role of IL-10 producing Treg cells in immune tolerance to auto-antigenes T cell suppression seems not to focus on this pathway [15]. It can be asserted that both cyclosporine and abatacept suppress pro-inflammatory (T) cell activation and do not directly activate anti-inflammatory cells.

## 5. Conclusion

In conclusion, the results of the present study imply that IL-17 is the crucial T cell cytokine in oligoarticular JIA. Just cyclosporine blocks secretion of IL-17 effectively. IL-2 and IFN-$\gamma$ are not specific for oligoarticular JIA. Both cytokines are found as well in healthy control subjects after T cell stimulation. Relevant pro-inflammatory cytokines in oligoarticular JIA are TNF-$\alpha$ and IL-6. T cell suppression by cyclosporine and abatacept inhibts TNF-$\alpha$ but not IL-6 effectively. Production of anti-inflammatory cytokines is not influenced by T cell suppression.

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