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# Regulatory T cells increased while IL-1β decreased during antidepressant therapy

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ABSTRACT

*Background:* Regulatory T cells (Tregs, $CD4^+CD25^{hi}$) are specialized in steering the immune response and cytokine release to maintain tolerance to self-antigens. As cytokines such as interleukin (IL)-1β, IL-6 and interferon (IFN)-α have been shown to be involved in the pathophysiology of depression and cytokine levels have been shown to change during successful antidepressant treatment, we tested the involvement $CD4^+CD25^{hi}$ Tregs in these immunological processes during antidepressant therapy.
*Methods:* 16 patients suffering from a depressive episode were included into the study and treated with antidepressants according to their doctor's choice. Blood samples were collected during the first week after admission and after 6 weeks of treatment. Therein, we determined plasma levels of IL-1β, and measured IL-1β, IL-6 and IFN-α levels in the stimulated blood by performing a whole blood assay. We distinguished lymphocytes and identified $CD4^+CD25^{hi}$ Tregs by multiparameter flow cytometry. The psychopathological status was assessed using the Hamilton Depression Rating Scale (HAMD-21).
*Results:* HAMD-21 score, IL-1β serum levels as well as LPS-stimulated IL-1β and IL-6 production had decreased significantly at the end of treatment. In contrast, the amount of $CD4^+CD25^{hi}$ cells increased significantly from 2.74% ± 0.88 (mean value ± standard deviation) to 3.54% ± 1.21; $p = 0.007$. No significant changes in virus-induced IFN-α production was observed.
*Conclusions:* The increase in $CD4^+CD25^{hi}$ Tregs during antidepressant therapy may be the reason for the decrease in cytokine production and the recovery from depression.


## 1. Introduction

In recent years, tremendous progress has been made in understanding a subpopulation of T cells: the T regulatory cells (Tregs). Tregs are specialized in suppressing the immune response so that tolerance to self-antigens can be maintained. They belong to a group of T cells expressing specific cell surface molecules denoted as cluster of differentiation (CD) 4 and CD25 which is abbreviated as $CD4^+CD25^+$. Due to their high expression of CD25, they are currently named $CD4^+CD25^{hi}$. The immunomodulating effects of Tregs are mediated by membrane molecules and the modulation of cytokine expression (Liu and Leung, 2006).

A lack or defect of Tregs has been shown to be involved in a variety of autoimmune diseases and previous studies clearly show that reduced cell number or impaired function of Tregs is correlated with increased autoimmunity (Loser and Beissert, 2007). In turn, Treg count increases in patients responding to pro-inflammatory cytokine antagonist therapy (Boissier et al., 2009). Evidence is emerging that a number of autoimmune diseases are also associated with high levels of interleukin (IL)-1β or genetic predisposition to IL-1β overproduction (Timms et al., 2004; Pascual et al., 2005; Maeda et al., 2005).

Pro-inflammatory cytokines such as interleukin IL-1β are not only involved in the pathogenesis of inflammatory diseases. They are also involved in the pathophysiology of depression (Piletz et al., 2009; Konsman et al., 2008). Moreover, the so-called "cytokine hypothesis of depression" implies that an excessive secretion of certain cytokines represents a key factor in the propensity to precipitate depression in susceptible individuals (Maes, 2008). In accordance to this, elevated pro-inflammatory cytokine plasma levels such as IL-1β and IL-6 have repeatedly been found in patients suffering from affective disorders (Himmerich et al., 2008; Seidel et al., 1995, 1999; Kubera et al., 2001; Brambilla et al., 2004; Simon et al., 2008; Diniz et al., 2010; Howren et al., 2009). Interferon (IFN)-α is a cytokine released in response to viral infection

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which induces cellular release of IL-1β and IL-6, into systemic circulation (Dantzer et al., 2008). In combination with ribavirin, IFN-α is the primary treatment for patients with chronic hepatitis C virus (HCV) infection. But while efficacious, a substantial portion of patients develops major depression during treatment (Capuron and Miller, 2004). Several studies have demonstrated elevations in peripheral levels of IL-6 among patients undergoing IFN-α therapy (Wichers et al., 2007). Therefore it is meaningful to measure IFN-α production while investigating changes in IL-1β and IL-6 production during antidepressant therapy.

However, the changes of $CD4^+CD25^{hi}$ Tregs during antidepressant therapy have never been tested so far. We sought to investigate these changes during antidepressant therapy in patients with different degrees of depressive episodes and additionally determined *in vivo* concentrations of IL-1β and the *in vitro* production of IL-1β, IL-6 and IFN-α. Recently, Tregs have been shown to be decreased in the peripheral blood of patients suffering from major depression (Li et al., 2009). Therefore, we hypothesize that antidepressant therapy may increase Tregs and correct this immunological feature of depressed patients.

## 2. Methods

### 2.1. Subjects

$N = 16$ (5 males, 11 females) consecutive referrals to the department of psychiatry and psychotherapy of the Medical School of the RWTH Aachen University suffering from a depressive episode were included into the study. Mean age was 42 ± 8.2 years (mean ± SD years). Initially, 19 patients were included, but 2 patients refused standardized psychopathological examination and 1 patient was excluded because of not being depressed according to the initial study examination.

After a complete description of the study, all patients gave written informed consent to participate in the investigation, which had been approved by an independent ethics committee (Ethics Committee of the Medical Faculty, RWTH Aachen University, Germany; EK002/08). All patients included in the study suffered from a depressive episode at the time of admission to the hospital, the day clinic or the outpatients unit. Individual diagnosis according to ICD-10 criteria varied within the range of affective spectrum disorders (F31, F32, F33). Substance dependence and severe medical conditions that might be related to depressive symptoms such as endocrine disorders or dementia precluded study enrolment. Physical examination, medical history and baseline laboratory investigations did not reveal any acute or chronic inflammation or infection. Autoimmune, cardiac, pulmonary, endocrine and haematological diseases were also ruled out. The study followed a naturalistic design, i.e. patients were treated with different kinds of antidepressant drugs according to their doctor's choice. The individual dose was adjusted according to clinical judgment and plasma levels.

### 2.2. Procedure

Blood samples were collected during the first week after admission and after 6 weeks of treatment. Patients' psychopathology was assessed using the Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960) as well as the Beck Depression Inventory (BDI) (Beck et al., 1961) on the same day. HAMD-21 at week 1 was 16.87 ± 6.16; range: 8–28.

Within the blood, we determined plasma levels of IL-1β. Additionally, a whole blood assay was performed in the blood samples as described previously (Kirchner et al., 1982; Seidel et al., 1996). Blood was cultured in a whole blood assay within 1–2 h after blood collection. Test tubes (5 ml, Greiner, Nürtingen, Germany) were filled with 900 μl RPMI 1640 medium supplemented with L-glutamine (2 mM) and penicillin/streptomycin (100 U/100 μg/ml) (Cambrex, Verviers, Belgium). Subsequently, 100 μl of blood was introduced into each tube. For induction of IL-1β and IL-6 production we used endotoxin (LPS, Sigma–Aldrich, Munich, Germany; 250 ng/ml) and for induction of IFN-α production we used Newcastle Disease Virus (NDV, kindly provided by Prof. R. Zawatzky, German Cancer Research Centre, Heidelberg; 80 haemagglutinating units (HAU) per ml). All tubes were covered and samples were incubated in an atmosphere of 5% CO2 and 37 °C for 24 h. Cell-free supernatants were harvested after incubation and stored at −70 °C. For quantification of IL-1β, IL-6 and IFN-α, antibody pairs (BD Bioscience) were used to analyze supernatants. Results were measured with a Magellan-ELISA plate reader (Tecan, Crailsheim, Germany). The detection limits of IL-1β, IL-6 and IFN-α were at 3.9 pg/ml, 4.6 pg/ml and 7.9 pg/ml respectively.

We used a multiparameter flow cytometry (FACS Calibur, BD Bioscience, Heidelberg, Germany) to distinguish lymphocytes and to identify $CD4^+CD25^+$ cells and $CD4+CD25^{hi}$ Tregs. The expression was detected by the monoclonal antibodies anti-CD4-FITC and anti-CD25PE (BD Bioscience). The different cell fractions could be distinguished via two-dimensional plots of forward and side scatter. Tregs were identified by gating on $CD4^+CD25^{hi}$. For details and for an example of multiparameter flow cytometry, see Fig. 1.

### 2.3. Statistics

Lillefors test of normality (Dallal and Wilkinson, 1986) indicated no significant deviation from the normality assumption for $CD4^+CD25^+$ and $CD4^+CD25^{hi}$ cells at the beginning and end of treatment (all $p > 0.35$). To test for differences in $CD4^+CD25^+$ and $CD4^+CD25^{hi}$ as well as to control for overall alpha-level, a multivariate repeated measures ANOVA with the within-factor beginning vs. end of treatment and two outcome measures ($CD4^+CD25^+$ and $CD4^+CD25^{hi}$) was computed. Only in the case of a significant overall multivariate F-test, univariate analyses followed. In addition, to explore potential determinants of the observed changes, correlations and *t*-tests for difference scores and percentages were computed with gender, age, severity of illness, magnitude of improvement or specific medications as exploratory variables. For these latter analyses, an uncorrected *p*-value of less than 0.05 was considered significant. Differences between beginning and end of treatment in stimulated IL-1, IL-6 and IFN-α levels and lymphocyte fraction were tested with the non-parameteric Wilcoxon-test because of non-normality in some parameters. All analyses were undertaken with SPSS 16.0 and R (R Development Core Team, 2008).

## 3. Results

At the end of treatment, HAMD-21 had improved from an initial mean score of 16.87 ± 6.16 to 7.69 ± 6.04. In 15 of the 16, HAMD-21 scores were lower at the end of treatment, while in one subject HAMD-21 scores had increased from 15 to 16. On average, scores were reduced by 9.2 ± 6.0 points or 55% ± 8.

At the beginning of the treatment, IL-1β levels in serum were below the detection limit in 10 of the 16 patients, while at the end of treatment all 16 patients had undetectable levels of IL-1β; this difference in frequencies was statistically significant (Fisher's exact test: $p = 0.018$). In the 6 patients that had measurable levels at baseline, these varied between 7.12 pg/ml and 60.87 pg/ml (mean: 31.29). In all six patients, IL-1β levels were no longer detectable at the end of treatment. The six patients with detectable IL-1β levels at baseline did not differ from the 11 other patients in depression