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Immune markers in major depression and in antidepressive treatment

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3. The author designed the study, performed the clinical scales, carried out statistical analysis, and wrote the first draft of the manuscript.
4. The author participated in the study design, performed the clinical scales, carried out statistical analysis, and wrote the first draft of the manuscript.

ABBREVIATIONS

ACTH	–	Adrenocorticotropic hormone
anti-TPO	–	Anti thyroid peroxidise auto-antibodies
AVP	–	Arginine vasopressin
BDI	–	Beck Depression Inventory
BMI	–	Body mass index
CSF	–	Cerebrospinal fluid
CRH	–	Corticotropin releasing hormone
DA	–	Dopamine
DSM-IV	–	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
FE	–	MDD, first episode
FR	–	MDD, full remission
HAMD	–	Hamilton Depression Rating Scale
HC	–	Healthy controls
HPA axis	–	Hypothalamic-Pituitary-Adrenal axis
ICPE	–	International Consortium of Psychiatric Epidemiology
IDO	–	Indoleamine 2,3-dioxygenase
IFN-α	–	Interferon-α
IFN-γ	–	Interferon-γ
IL	–	Interleukin
IL-1	–	Interleukin-1
IL-1RA	–	Interleukin-1 receptor antagonist
IL-6	–	Interleukin-6
IL-8	–	Interleukin-8
IL-12	–	Interleukin-12
IRS	–	Inflammatory response system
LPS	–	Bacterial cell wall lipopolysaccharide
MADRS	–	Montgomery-Asberg's Depression Rating Scale
MDD	–	Major depressive disorder
M.I.N.I. 5.0.0	–	Mini International Neuropsychiatric Interview Version 5.0.0
NA	–	Noradrenalin
NIMH	–	National Institute of Mental Health
NK	–	Natural killer
NR	–	Non-responder
NS	–	Non significant
PGE2	–	Prostaglandin E2
R	–	Responder
RE	–	MDD, recurrent depressive episode
S	–	Stimulated
SD	–	Standard deviation
sIL-2R	–	Soluble interleukin-2 receptor

sIL-6R	–	Soluble interleukin-6 receptor
SSRI	–	Selective Serotonin re-uptake inhibitor
TCA	–	Tricyclic antidepressant
TDO	–	Tryptophan 2,3-dioxygenase
Tc cells	–	Cytotoxic T cells
Th cells	–	T-helper cells
TNF-α	–	Tumour necrosis factor α
US	–	Unstimulated
5-HT	–	Serotonin (5-hydroxytryptamine)

INTRODUCTION

Unipolar depression is a common, often chronic and episodic psychiatric disorder (Andrade et al., 2003; Weissman et al., 1996). Depression is a major health problem worldwide for two reasons: it is highly prevalent in the general population, and causes a significant loss of quality of life and social functioning in the affected individual. The prevalence rates vary widely across studies and different countries; for example, the one-month prevalence of depression is found from 2.2–20.7 % (Angst and Merikangas, 1997; Angst et al., 2002; Ialongo et al., 2004; Kessler et al., 1993; Regier, 1993; Regier et al., 1988). Prevalence rates have been found to be higher in women than in men. Further, depression contributes to a poorer outcome of co-morbid mental and somatic conditions.

The fact that mood disorders, including unipolar depression, have a great impact on distress of the affected individual and his or her family, lifetime disability, and suicide highlights the importance of etiologic research for their treatment and prevention (Merikangas et al., 2002). The available data consistently demonstrate an association of all mental disorders with considerable disability burden in terms of the number of work days lost and generally low treatment rates. Only 36.5% of cases with mood disorders had had any consultation with professional health care services; the finding suggests a considerable degree of unmet need (Wittchen and Jacobi 2005). In addition, there is evidence suggesting that biological mechanisms underlie a bidirectional link between mood disorders and many medical illnesses. Moreover, mood disorders may affect the course of medical illnesses (Evans et al., 2005).

Major depression is a complex disorder caused by genetic and environmental factors and interactions between them. There are many factors associated with depression: age, gender, living with a partner, ethnic background, education, immigrant status, urban-rural differences, life stress, childhood traumas, co-morbidity with other mental and physical disorders (Aluoja et al., 2004; Paykel et al., 2005; Smit et al., 2004). Despite some promising leads, no confirmed linkage in mood disorders has been established as yet. Impediments to gene findings include the lack of phenotypic validity, variation in ascertainment sources and methodology across studies, and genetic complexity (Merikangas et al., 2002).

A large body of evidence in recent years suggests that major depression is associated with activation of the inflammatory response system (IRS) (Connor and Leonard, 1998; Maes et al., 1995; Schiepers et al., 2005). Cytokines are small glycoproteines that function as signalling molecules between immune cells. When examining systemic activity of the immune system in depressed patients, differentiation into two major groups is justified, namely, pro-inflammatory and anti-inflammatory cytokines. An increased production of pro-inflammatory cytokines may play a crucial role in the immune and acute phase responses in depression (Van West 2005). There is evidence implying that

antidepressive treatment with various antidepressive agents has an immuno-modulative effect. However, the existing data are conflicting.

The purposes of this study were (i) to find associations between depression, depressive symptoms and soluble interleukine- 2 receptor (sIL-2R) and tumour necrosis factor-α. (TNFα) and anti thyroid peroxidise auto-antibodies (anti-TPO); (ii) to investigate the acute and chronic effects of selective serotonin re-uptake inhibitor, escitalopram, on serum levels of interleukin-8 (IL-8), sIL-2R and TNFα in patients with major depression; (iii) to clarify whether the addition of bupropion in escitalopram-resistant patients with major depression causes additional changes in the immune system; (iiii) to explore whether serum cytokine concentrations and/or anti-TPO positivity can predict treatment response to antidepressants.

REVIEW OF LITERATURE

1. Major depressive disorder (MDD)

Mood is defined by DSM-IV as a "pervasive and sustained emotion that colours the perception of the world. In contrast with affect, which refers to more fluctuating changes in emotional 'weather', mood refers to a more pervasive and sustained emotional *climate*" (DSM-IV). Mood is obviously modified by the events that occur in the real world. Negative events lower our mood and positive events tend to make us happier. On the one hand, mood affects all of our cognitions, judgements, and expectations. Through these continuous variations mood is influenced by the ever-changing flow of life events; mood determines our attitude towards life, and the basic characteristic of normal mood is that it is subject to change. Pathological mood is no longer influenced by changes in reality and remains steady and still, regardless of any occurrence. On the other hand, the state of mood continues to determine the cognition and the interpretation of reality (Faravelli et al., 2005). The lack of response to external stimuli is therefore the basis of the pathology of mood, rather than the intensity of the mood.

Four major clusters of symptoms and signs are recognized in the diagnosis of depression. They include mood (anhedonia, dysphoria, guilt, anxiety), cognition (attention deficit, difficulty to make decisions, lack of interest, thought content deals with sadness, hopelessness, lack of future, low self-esteem, death from boredom with life to actual self-harm), psychomotor (retardation, agitation), and neuro-vegetative (disturbed sleep, appetite, and sexuality) areas. The European psychopathological tradition indicates melancholy as the psychopathological nucleus of depression. It includes somatic symptoms and signs (worsening in the morning, early morning awakening, significant anorexia or weight loss), marked psychomotor retardation or agitation, guilty feelings and specific features of the mood (loss of interest or pleasure in all or almost all activities, lack of reactivity to usually pleasurable stimuli, peculiar characteristic of the depressed mood, which is qualitatively different from normal sadness). Atypical symptoms are opposites of melancholic ones. They include mood reactivity (i.e. mood brightens in response to actual or potential positive events) and inverse neuro-vegetative symptoms (increased appetite or significant weight gain, hypersomnia). Psychotic features considered by DSM-IV are delusions and hallucinations that might be mood-congruent or mood-incongruent.

1.1. Epidemiology of MDD

The one-month prevalence of depression is found between 2.2 and 20.7 % (Angst and Merikangas 1997; Angst et al., 2002; Ialongo et al., 2004; Kessler et al., 1993; Regier 1993; Regier et al., 1988). Life-time prevalence varies between 3% in Japan and 16.9% in the US according to the surveys of the International Consortium of Psychiatric Epidemiology (ICPE) (Andrade et al., 2003) and amounts to 16.2% in the National Co-morbidity Survey Replication (NCS-R) (Kessler et al., 2003). The high variation is due to different assessment instruments (e.g. semi-structured interviews or standardized interviews) and classification systems. Very low rates of major depression have been reported in studies conducted in Eastern Asian nations. Sociodemographic differences (e.g. discrepancies in the distribution of marital status) or cross-cultural variations (e.g. different social acceptability of the expression of emotions) could explain the discrepancies between the results. Prevalence rates have been consistently found to be 1.5–2.5 times higher in women than in men (Jacobi et al., 2005).

Depression is highly co-morbid with other mental disorders, especially anxiety. In most surveys, between one-third and half of respondents with a lifetime history of major depressive episode also had a history of at least one anxiety disorder (Andrade et al., 2003; Rush et al., 2005). Major depression and dysthymia frequently coexist; the disorder is sometimes referred to as ‘double depression’. The lifetime prevalence of double depression has been reported to range between 1.5% and 2.5% (Bland 1997). The median prevalence of current or lifetime alcohol problems in depression is 16% and 30%, respectively (Sullivan et al., 2005).

The median age of the onset of MDD is in the range 20 to 25 in most countries by the ICPE Survey. Consistent socio-demographic correlates included being female and unmarried (Andrade et al., 2003).

Family studies have shown that the risk of depression onset and severity is associated with family history of depression (Rohde et al., 2005). Sullivan et al. reported the relative risk for MDD subjects versus first-degree relatives of MDD patients 2.84 (Sullivan et al., 2000).

Suicide is one of the most serious aspects of major depression although there is evidence that suicide risk loci are independent of susceptibility loci of mood disorders (Zubenko et al., 2004). Suicide has been reported to occur in 10–15 % of patients previously hospitalized for major depressive disorder (Angst et al., 1999). In a study conducted by Murphy (1998), women were more likely to experience episodes of major depression but were 25% less likely than men to commit suicide.

1.2. Aetiology and pathogenesis of MDD

There is general agreement that the clinical syndrome of major depression must be associated with characteristic neurobiological changes in the brain. However, it is unclear to what extent specific syndromes correlate with particular neurobiological changes. In addition, there is some evidence that depression is associated with a distinct cellular and structural pathology.

1.2.1. Monoamine neurotransmitters

The monoamine hypothesis suggests that depressive disorder is due to an abnormality in the monoamine neurotransmitter system at one or more sites in the brain (Cowen 2005). Alterations were found both in receptors and in the concentrations or the turnover of the amines. Three monoamine transmitters have been implicated: serotonin (5-hydroxytryptamine; 5-HT), noradrenalin (NA), and dopamine (DA).

The synthesis of 5-HT in the brain depends on the availability of L-tryptophan. Plasma tryptophan levels are decreased in depressed patients, particularly in those with melancholic depression (Anderson et al., 1990). Recent development in brain imaging with selectively labelled ligands has allowed assessment of certain brain 5-HT receptor subtypes in vivo. There is evidence that unmedicated depressed patients differ from healthy volunteers in the density of 5-HT2a, 5-HT1a receptors in certain brain regions, and there are probably reductions in brain stem 5-HT transporter sites in depressed subjects, consistent with a decrease in the density of 5-HT cell bodies (Cowen 2005; Willeit et al., 2000). The efficacy of the serotonin re-uptake inhibitors (SSRI) suggests clinically the importance of serotonergic neurotransmission for the pathogenesis of depression.

The function of NA and DA has been studied less than that of 5-HT. However, considerable experimental evidence, neuroimaging techniques, and clinical evidence support the role of NA and DA (Elhwuegi 2004; Nutt et al., 2007). DA neurons play a key role in decreased incentive motivation, anhedonia, loss of interest and reward, the processes that are disrupted in depression, particularly in the case of melancholic states. NA is associated with loss of energy, fatigue, and low mood (Cowen 2005; Nutt et al., 2006). In addition, the reciprocal interactions between 5-HT, NA, and DA systems may account for the full picture of depression (Nutt et al., 2006; Trivedi et al., 2008).

1.2.2. Hypothalamic-Pituitary-Adrenal axis (HPA)

The HPA axis consists of a feedback loop including the hypothalamus, pituitary, and adrenal glands. The axis receives important regulation from the hippocampus, amygdala, bed nucleus of the stria terminalis, and paraventricular nuclei. It is frequently stated that about half of the patients whose depressive disorder is at least moderately severe, and those with melancholic features,

hypersecrete cortisol. In addition, MDD is associated with early escape from dexamethasone-induced cortisol suppression and a blunted adrenocorticotropic hormone (ACTH) response to corticotropin releasing hormone (CRH) or dexamethasone/CRH challenge (Nemeroff and Vale 2005). The cause is not clearly established. Although researchers had initially thought that cortisol changes might simply be a marker of distress or depression, the view that it plays a provocative role in this regard has received increasing attention lately. Nevertheless, it has been suggested that the balance between glycocorticoid and mineralocorticoid receptors may be a pivotal factor in determining stress reactions and depressive outcomes. In addition, there is growing evidence that CRH hyperfunctioning in hypothalamic and extra-hypothalamic sites (locus coeruleus, amygdala, hippocampus, and nucleus accumbens), CRH1 receptor, and arginine vasopressin (AVP) are associated with depression (Anisman et al., 2008). The data indicate that in some patients stressful life experiences may interact with a predisposition to abnormal HPA axis regulation to produce sustained HPA overactivity (Cowen 2005).

There is some evidence that corticosteroids regulate the genomic expression and function of monoamine receptors in the brain, which could lead, for example, to a decrease in 5-HT neurotransmission (Cowen 2005).

1.2.3. Brain structure in depression

Computerized tomography (CT) and magnetic resonance imaging (MRI) have identified a number of abnormalities in MDD patients, particularly in those with more severe and chronic disorders. The most consistent findings include enlarged lateral ventricles, volume loss in frontal and temporal lobes, a decreased hippocampal volume, and a decreased volume of basal ganglia structures. Researchers have suggested that changes in the brain volume may represent long-term consequences of depression, perhaps associated with cortisol hypersecretion. However, there is also some evidence that changes are manifested early in the course of the illness and represent vulnerability factors (Frodl et al, 2002). In MDD, increased deep white matter hyperintensities are associated with the late onset of depression, greater illness severity and poorer treatment response, apathy, psychomotor slowness, and retardation (Chen et al., 2006; Steffens and Potter 2008; Taylor et al., 2003). A general report of imaging studies concludes that patients with structural abnormalities are less likely to respond to treatment (Cowen 2005).

1.3. Genetics of MDD

MDD is a complex disorder that does not result from either genetic or environmental influences alone but rather from both. Family, adoption, and twin studies help to delineate genetic and environmental effects in humans. Sullivan et al. have meta-analysed the available studies and found that in five family studies the odds ratio for proband versus first-degree relative status was 2.84. Statistical summation of five twin studies suggested that familial aggregation was due to additive genetic effects, with a minimal contribution of environmental effects common to siblings and substantial individual-specific environmental effects. The recurrence best predicts the familial aggregation of MDD (Sullivan et al., 2000)

The first genome-wide linkage survey identified nineteen statistically significant chromosomal regions for MDD [1p, 1q, 2q (2), 4q, 5q (2), 8p, 10p, 10q (3), 11pter, 11q, 15q, 18q, 19p, 19pericentric, Xq]; ten of those were highly-significant linkages (Zubenko et al., 2003). A year later the same team reported about chromosomal loci of genes that influence the risk of suicidal behaviour [2p12, 6q12, 8p22-p21 and Xq25-26.1] (Zubenco et al., 2004). Additionally, Zubenko et al. (2002) found 2q33-34 to be related to recurrent, early-onset MDD in women and Abkevich et al. (2003) showed that 12q22-12q23.2 was related to MDD in men. Some studies have focused on chromosome 15q (Holmans et al., 2004; Verma et al., 2008) and chromosome 10 (Neff et al., 2008).

In association studies in MDD, candidate genes regularly include members of the main neurotransmitter systems, such as monoamines, glutamate, and also pathways that influence several neuroendocrine systems, for example, HPA-axis (Lekman et al., 2008). Such candidate alleles are chosen on the basis of the current understanding of the biology of the disease. The simplest and common form of association studies is the case-control approach; unrelated affected individuals and unrelated healthy controls are genotyped to investigate whether the hypothesized susceptibility gene variant is overrepresented in the affected group. Several meta-analyses have been done in that area with conflicting results. Controversial findings have been published, for example, about the serotonin transporter linked promoter region, catehol-*O*-methyltransferase gene, promoter region of monoamine oxidase A gene, dopamine receptor 2, interleukin-1beta, dopamine β-hydroxylase, tryptophan hydroxylase, tyrosine hydroxylase genes, and brain-derived neurotrophic factor locus (Furlong et al., 1998a,b, 1999; Ho et al., 2000; Johansson et al., 2001; Kõks et al., 2006; Schumacher et al., 2005; Wood et al., 2002; Yu et al., 2003). That is why the NIMH support efforts to identify the most heritable, more homogenous subtypes and endophenotypes of mood disorders for genetic studies (Merikangas et al., 2002). Previous evidence from genetic association studies on cytokine genes in depression has been limited and inconsistent. No significant associations between polymorphisms from IL10 (Jun et al., 2002), IL6 (Hong et

al., 2005) and IL1-beta (Yu et al., 2003) genes and MDD have been found. On the other hand, the same IL1-beta polymorphisms have been found to be a risk factor for the appearance of depressive symptoms in patients with schizophrenia spectrum disorders (Rosa et al., 2004) and with Alzheimer's disease (McCulley et al., 2004). Furthermore, polymorphisms from monocyte chemoattractant protein-1 (MCP1) (Pae et al., 2004) and the tumour necrosis factor-alpha (TNF) (Jun et al., 2003) genes may have a potential role for susceptibility to MDD. Our recent study established an increased risk of MDD related to the IL20 and IL24 haplotype although none of the SNPs were individually associated with MDD (Traks et al., 2009). In another unpublished study we scanned a large number of single-nucleotide polymorphisms (SNPs) located on the chromosomal region 1q32, which contains four genes from IL10 family: IL10, IL19, IL20, and IL24, in groups of patients with major depressive disorder (MDD, n=312) and panic disorder (PD, n=210), and matched the findings with healthy controls (n=356). We found no significant associations between the SNPs of IL-10 family genes and MDD or PD.

One of the latest approaches in genetics is pharmacogenetics. Pharmacogenetic strategy studies how genetic variation could affect the response of patients to psychotropic drugs and their susceptibility to adverse drug reactions. These studies cold be useful to predict response rate to different antidepressants (rev by Rausch 2005; Serretti 2005; Tsao et al., 2006).

2. Immune system cells and cytokines

The primary function of the immune system is to protect the individual from bacterial and viral insults. The most important immune cells are monocytes, T- and B-lymphocytes, neutrophils, and natural killer cells. Monocytes (and also macrophages and dendritic cells) recognize microorganisms, take them up via phagocytosis, and degrade the microorganisms in small peptides that bind with endogenous major histocompatibility class II proteins. This complex is expressed at the cell membrane in such a way that T- and B-lymphocytes can recognize a foreign protein. Neutrofils are phagocytic cells at the site of the infection; natural killer cells destroy infected or malignant cells. Lymphocytes are cells of the acquired immune system; their actions are antigen-specific. B-lymphocytes recognize a membrane-bound antibody and then proliferate and differentiate into antibody-producing plasma cells. These antibodies opsonize the respective microbes, which facilitates phagocytosis by phagocytic cells. T-lymphocytes can be divided into two classes: T-helper cells (Th cells) and cytotoxic T cells (Tc cells). Activated Th-cells secrete certain molecules (cytokines) that regulate the activity of other immunecompetent cells; Tc cells mainly destroy cells infected with intracellular microorganisms (Aniaman et al., 2008; Van West et al., 2005).

Cytokines are small (15 to 44 kD) glycoproteines which function as signalling molecules between different immune cells. In addition to immune cells, they are produced by endothelial, epithelial, and neuronal cells.

2.1. Classification of cytokines

In the context of major depression, two major groups of cytokines are important: pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines are mainly produced by activated immune cells and stimulate others, so they enhance inflammatory reactions. Anti-inflammatory cells tend to inhibit activated cells. Monocytes and macrophages initially produce pro-inflammatory cytokines interleukin-1 (IL-1), tumour necrosis factor-α (TNFα), interleukin-6 (IL-6) and interleukin-12 (IL-12); after the initial activation anti-inflammatory cytokines or proteins IL-10 and IL-1 receptor antagonist (IL-1RA) are produced by these cells.

Th-1 cells produce interferon-γ (IFN γ), IL-2 and TNFα, tumour necrosis factor-β, IL-12, and IL-18. Th-2 cells produce IL-3, IL-4, IL-5, IL-10, IL-13, and IL-6. Th-1 cytokines stimulate cell-mediated immunity (mainly phagocytic cells); Th-2 cells promote humoral immunity (antibodies, allergic reactions) (Schwarz et al., 2001; Van West et al., 2005).

2.2. Cytokines and depression

Growing evidence suggests that, in addition to providing communication between immune cells, specific cytokines play a role in signalling the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioural changes. For example, pro-inflammatory cytokines, such as stressors, increased HPA axis functioning and influenced a range of monoaminergic and peptidergic extra-hypothalamic sites (rev by Anisman et al., 2008; Kronfol and Remick 2006). The pro-inflammatory cytokines TNF-α, IL-1 and IL-6 are primary HPA stimulating cytokines. IL-6 is a potent stimulator of CRH production, which leads to elevated HPA activity characterized by increased ACTH and cortisol levels (O'Brien et al., 2004). In addition, pro-inflammatory cytokines acutely stimulate 5-HT turnover and reduce the production of 5-HT by stimulating the enzyme indoleamine 2,3-dioxygenase (IDO), which converts tryptophan the precursor of 5-HT, into kynureine (Wichers and Maes 2002; Schiepers et al., 2005). IL-1, interferon- α (IFN-α), IFN-γ, and TNF-α have been shown to up-regulate the serotonin transporter, which may reduce extracellular 5-HT levels (Hayley et al., 2005; Miller and Raison 2006; Wichers and Maes 2002) and anti-inflammatory cytokine IL-4 was shown to induce a reduction of 5-HT uptake, so that the synaptic level of 5-HT synaptic level increases (Mössner et al., 2001). Some underlying mechanisms have been reported from animal

studies; for example, IL-1 and TNF-α act on serotonin transporter by activating P38 mitogen-activated protein (MAP) kinase (Miller and Raison 2006).

There is evidence that patients who receive cytokine immunotherapy frequently show depressive symptoms, which may be attenuated by antidepressant medication. This fact supports the causal role of cytokines in MDD (Capuron et al., 2004; rev Capuron and Miller 2004). However, despite the link with depression and immunotherapy, the question remains open whether the effects observed are a genuine manifestation of neurochemical changes underlying depression, or whether the symptoms are rather a reflection of general malaise or toxicity. Recent studies seem to have indicated that the neurovegetative and mood-related features introduced by IFN-α therapy are independent of one another, as paroxetine primarily affected the mood-related symptoms, with only minor effects on fatigue and anorexia (Raison et al., 2005).

Because cytokines are closely associated with central neurotransmitters and cytokine regulation is affected by stress, a number of studies have investigated the possible role of cytokines in major psychiatric disorder, including major depression. Maes et al. reported an increased plasma concentration of IL-1 (1993), IL-6 (1995 and 1997), sIL-2R, soluble IL-6 receptors (sIL-6R), and acute phase proteins (1995) in depressed patients. They concluded that there is an increase in pro-inflammatory cytokines in MDD patients. Unfortunately, these observations have not been consistently replicated. Table 1 provides a brief summary of controversial studies on cytokines in MDD patients versus healthy controls.

The association between cytokine levels and scores of depression rating scales is also unclear. Leo et al. (2006) reported IL-1 β, IL-6, and TNF-α level to be related to HAMD scores. By contrast, Kagaya et al. (2001) and Tuglu et al. (2003) did not found any correlation between TNF-α concentration and HAMD scores; the latter authors used additionally the BDI scale, but no correlation was still found. There is evidence that cytokine activity may be related to chronicity of illness, neurovegetative features, or some other aspects (such as typical or atypical, melancholic or non-melancholic, sleep pattern) or symptoms of MDD (Anisman and Merali 2002).

There is evidence implying that antidepressive treatment with various antidepressive agents has an immunomodulative effect. In vitro studies suggest that when human monocytes are incubated with different classes of antidepressants together with bacterial cell wall lipopolysaccharide (LPS), which stimulate the release of pro-inflammatory cytokines, the synthesis and release of IL-1, IL-6, and TNF-α is markedly inhibited (Xia et al., 1996). Additionally, Kubera et al. (2000a) showed that an increased concentration of serotonin after administration of SSRI antidepressants is associated with an increased release of anti-inflammatory cytokine IL-10 and a decreased synthesis of INF-γ.

The effects of antidepressants on the activity of the immune system have been studied in animal studies. The enhanced lymphocyte proliferation and an increased production of IL-1 and IL-2 in rats under chronic mild stress is

reversed following chronic treatment with imipramine (Kubera 1996) while the production of IL-10, an anti-inflammatory cytokine, increases after desipramin treatment (Kubera 2001b). A decreased production of TNF-α after the administration of different antidepressants has been reported several times in animal studies (Brustolim et al., 2006; Obuchowicz et al., 2005, Reynolds et al., 2005; Roumestan et al., 2006). However, data about treatment effects on immune system activity are controversial. Table 2 summarizes the findings of antidepressant effects in HC, and Table 3 does the same in MDD patients.

Table 1. Cytokine levels in different studies in MDD patients vs healthy controls

Authors	IL-1α	IL-1β	IL-2	IL-4	IL-6	IL-7	IL-8	IL-10	IL-12	IL-15	IFN-γ	TNF-α	TGF-β1	sIL-2R	sIL-6R	Il-1Ra
Maes et al., 1993	↑															
Weizman et al., 1994		↓	↓													
Maes et al., 1995					↑									↑	↑	
Maes et al., 1997					↑										≈	↑
Frommberger et al., 1997					↑											
Song et al., 1998					↑		↑									↑
Kubera et al., 2000b					≈↑			≈↑								≈↑
Lanquillon et al., 2000					≈							↑				
Kagaya et al., 2001		≈			≈							≈		≈		
Mikova et al., 2001					≈									≈		
Rief et al., 2001					≈										≈	↑
Rothermundt et al., 2001		≈														
Nunes et al., 2002		≈			↓							≈		≈↓		
Tuglu et al., 2003												↑				
Bauer et al., 2003												≈		≈		
Brambilla et al., 2004		≈										≈				
Kubera et al., 2004					↑							≈				
Schlatter et al., 2004			↑	≈												
Basterzi et al., 2005					≈											
Myint et al., 2005				≈							≈		≈↓			

Table 1. Continued

Authors	IL-1α	IL-1β	IL-2	IL-4	IL-6	IL-7	IL-8	IL-10	IL-12	IL-15	IFN-γ	TNF-α	TGF-β1	sIL-2R	sIL-6R	Il-1Ra
Lee and Kim, 2006									↑				≈			
Leo et al., 2006		↑			↑							↑				
Pike and Irwin, 2006					↑									≈		
Kim et al., 2007			↓	↓	↑						↓	↑	↑			
Marques-Deak et al., 2007		≈			≈						≈					
O'Brien et al., 2007					↑		≈	≈				↑			≈	
Sutcigil et al., 2007			↑	≈					↑			↑	≈			
Simon et al., 2008	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑					

↑ – Higher levels in MDD patients than in healthy controls
↓ – Lower levels in MDD patients than in healthy controls
≈ – No statistical difference between MDD patients and healthy controls
≈↑ – A slight increase, statistically not significant
≈↓ – A slight decrease, statistically not significant

Table 2. Effect of antidepressants on different markers of the immune system in healthy volunteers

Authors	Study group	Antidepressant	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12	INF-γ	TNF-α	TGF-β	IL-1Ra
Xia et al., 1996	Healthy volunteers	TCA	↓			↓				↓	↓		
Maes et al., 1999	Healthy volunteers	Clomipramine Sertraline Trazodone						↑		↓			
Lin et al., 2000	Healthy voluteers	Moclobemide				≈	↓	↑		≈	↓		≈
Szuster-Ciesielska et al., 2003	Healthy volunteers	Imipramine, Mianserin Lithium		≈(US) ↓(S) ↑	≈(US) ↓(S) ↓			↑ ↑	≈(US) ↓(S) ≈	↑(US) ↓(S) ↑		≈(US) ↑(S) ↑	
Maes et al., 2005	Healthy volunteers	fluoxetine						≈		↓	↓		
Diamond et al., 2006	Healthy volunteers	Desipramine Clomipramine Fluoxetine, Reboxetine	↑ ↓ ≈					≈↑ ≈↑ ≈↑		↓ ↓ ↓	≈ ≈ ≈		

↑ – increased after treatment
↓ – decreased after treatment
≈ – no difference between the baseline and at the end of treatment
≈↑ – tend to increase after treatment
≈↓ – tend to decrease after treatment
S – Stimulated production
US – Unstimulated production

Table 3. Effect of antidepressants on different markers of the immune system in patients with MDD

Authors	Study group	Antidepressant	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12	INF-γ	TNF-α	TGF-β	sIL-2R	sIL-6R	IL-1Ra
Weizman et al., 1994	MDD patients	Clommipramine 4 weeks	↑	≈											
Maes et al., 1995	MDD patients	Fluoxetine TCA				≈							≈	≈	
Frommberger et al., 1997	MDD patients	?				↓									
Maes et al., 1997	MDD patients	Trazodone Trazodone + pindolol Trazodone + fluoxetine 5 weeks				≈								↓	≈
Kubera et al., 2000b	MDD patients	? 6 weeks				≈		≈							≈
Lanquillon et al., 2000	MDD patients	Amitiiptyline 6 weeks				↑(R) ↓(NR)					↓(R) ≈(NR)				
Kagaya et al., 2001	MDD patients	Mainly clomipramine, combinations with others 1month	≈			≈					↑		≈		

Table 1. Continued

Authors	Study group	Antidepressant	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12	INF-γ	TNF-α	TGF-β	sIL-2R	sIL-6R	IL-1Ra
Kubera et al., 2001a	MDD, treatment-resistant patients in vitro	Imipramine, Venlafaxine Fluoxetine						↑ ↑		≈ ↓					
Mikova et al., 2001	MDD patients	Different 8 weeks				≈	≈				≈		≈		
Tuglu et al., 2003	MDD patients	Sertraline, Citalopram, Fluoxetin, Fluvoxamine Paroxetine									↓				
Kubera et al., 2004	MDD, treatment-resistant patients in vitro	Imipramine Venlafaxine Fluoxetine				↑					≈				
Tucker et al., 2004	Chronic posttraumatic stress disorder	Double-blind (citalopram, sertraline, placebo)	↓										↑		

Table 1. Continued

Authors	Study group	Antidepressant	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12	INF-γ	TNF-α	TGF-β	sIL-2R	sIL-6R	IL-1Ra
Basterzi et al., 2005	MDD patients	SSRI 6 weeks				↓									
Myint et al., 2005	MDD patients	different			↓					≈		↑			
Himmerich et al., 2006	MDD patients	Different groups, also combinations, Li allowed; 8 weeks									≈				
Lee and Kim, 2006	MDD patients	Different 6 weeks							≈↓			↑			
Leo et al., 2006	Drug-naïve first episode MDD patients	Sertralin Citalopram 6 weeks	↓			↓					↓				
Narita et al., 2006	MDD patients	Fluvoxamine Paroxetine Milnacipran Longer than 6 months									↓				

Table 1. Continued

Authors	Study group	Antidepressant	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12	INF-γ	TNF-α	TGF-β	sIL-2R	sIL-6R	IL-1Ra
Kim et al., 2007	MDD patients	Different classes 6 weeks in vitro		↓	≈	↓				≈	≈	≈			
Sutcigil et al., 2007	MDD patients	Sertraline 8 weeks			↑				↓			↑			

↑ – increased after treatment
↓ – decreased after treatment
≈ – no difference between baseline and at the end of treatment
≈↑ – tend to increase after treatment
≈↓ – tend to decrease after treatment
S – Stimulated production
US – Unstimulated production

3. Thyroid function in major depression

There is evidence that abnormalities in thyroid function are more common in patients with mood disorders than in healthy subjects (Bauer et al., 2008; Joffe and Marriott 2000). Subclinical hypothyroidism is found to be related to an increased risk of elderly depression (Chueire et al., 2007). Thyroid hormones, particularly T3, are known to accelerate the clinical response to antidepressant therapy in MDD (Aronson et al., 1996; Abraham et al., 2006; Agid and Lerer 2003). Additionally, there is evidence that depressive patients with subclinical hypothyroidism respond worse to antidepressant intervention (Duval et al., 1996; Joffe and Levitt 1992). Gitlin et al. (2004) found that low values of the thyroid-stimulating hormone (TSH) correlated with greater improvement of depressive symptoms during treatment with SSRIs.

3.1. Anti thyroid peroxidise auto-antibodies (anti-TPO)

The association between thyroid autoimmunity and mood and anxiety disorders was found in several studies (Carta et al., 2004; Pop et al., 1998), but there have also been some negative findings in that field (Chueire et al., 2007; Engum et al., 2005; Horning et al., 1999). Only a few studies have focused on the impact of anti-TPO positivity to the treatment effect of MDD, and this data is also controversial. Haggerty et al. (1997) reported that the presence of antithyroid antibodies predicts a poor response to antidepressant treatment. However, Fountoulakis et al. (2004) failed to demonstrate such an association.

AIMS OF THE STUDY

The general aim of the study was to explore a possible involvement of IL-8, TNF-α, and sIL-2R in the pathogenesis of MDD. Based on this, the specific aims of the study were as follows:

1. To compare TNF-α and sIL-2R serum levels between patients with major depression and healthy controls.

2. To find correlations between the levels of cytokines and the severity of depression measured by HAM-D in patients with major depression.

3. To find correlations between the levels of cytokines and single symptoms of depression according to HAMD items.

4. To examine effects of escitalopram treatment on the levels of IL-8, TNF-α, and sIL-2R in patients with major depression.

5. To find out whether bupropion augmentation changes the production of IL-8, TNF-α, and sIL-2R in escitalopram-resistant patients with major depression.

6. To detect possible associations between IL-8, TNF-α, and sIL-2R serum concentrations and treatment response in patients with major depression.

7. To find out whether anti-TPO positivity or thyroid hormones have an impact on efficacy of escitalopram treatment in patients with major depression.

MATERIALS AND METHODS

1. Ethical considerations

The Ethics Review Committee on Human Research of the University of Tartu approved the study protocols and the informed consent forms of the subjects. All participants signed the written informed consent.

2. Characteristics of study participants and study design

All the subjects who participated in this study – patients and healthy controls – were Caucasians living in Estonia. MDD patients were recruited at the Psychiatric Clinic of the Tartu University Hospital. The age of all subjects was between 15 and 65 years. The diagnosis according to DSM-IV criteria was verified using M.I.N.I. 5.0.0 and substantiated by psychiatric history and medical records. To assess the severity of depressiveness, HAMD and BDI scales were used; MADRS was used additionally in the treatment phase. All healthy subjects were interviewed using the M.I.N.I. 5.0.0, and only those without a personal or family (defined as first-degree relatives) history of psychiatric disorders and not taking medications were included in the study. There were no significant age and gender differences between the patients and the healthy volunteers.

129 MDD patients were selected for the treatment phase with escitalopram. These subjects were required to have a MADRS score at least 23 or higher and the wash-out period from previous antidepressive drugs had to be at least two weeks, if the subject had received treatment for current depressive episode. No other regular medication, including anti-inflammatory drugs, was allowed during the study, except for hormonal contraceptives and zolpidem or zopiclon for insomnia. All patients started treatment with 10 mg escitalopram per day for the first 4 weeks. The patients showing at least a 50% decline in the MADRS total score at week 4 continued taking 10 mg of escitalopram until the end of the study. The dose of escitalopram was increased and kept at 20 mg in patients who demonstrated less than a 50% decrease in the MADRS score at week 4 or who showed exacerbation of depressive symptoms during any of the following visits. At the end of week 12 the patients were defined as responders (R) if the decrease in the MADRS total score was at least 50% and as remitters if the score was less than 12. The patients who did not meet these criteria were defined as non-responders (NR). As almost all responders fulfilled the criteria of remission on the MADRS, the analyses were made only between the groups of R and NR. Bupropion 150–300 mg per day was added to escitalopram in those NRs who agreed to continue the study (n=28) for additional 6 weeks. At

the end of the augmentation period the patients were again defined as R if the decrease in the MADRS total score was at least 50% during this period or as NR if the MADRS total score decreased less than 50%. All the patients were visited every two weeks; blood samples for cytokines were taken at week 0, week 4, week 12, and week 18; for antibodies blood was collected only once, during the baseline visit. Table 4 shows the characteristics of the study participants.

Table 4. Characteristics of study participants

	Total number of participants	Sex (male/female)	Age (year) Mean ± SD	Age range (years)
MDD patients	247	73/174	33.5 ± 12.8	17–63
MDD patients in the first treatment phase	100	35/65	32.1 ± 11.9	19–63
MDD patients in the augmentation phase	28	11/17	31.2 ± 9.5	19–48
Healthy controls	94	36/58	32.5 ± 13.3	15–66

3. Laboratory analyses

Initial cytokine selection was made on the basis of the literature: IL-1β, IL-6, IL-8, IL-10, TNF-α, and sIL-2R. Unfortunately, it was not possible to measure IL-1β, IL-6, and IL-10 in our patients. The blood was collected between 9.00 and 11.30 a.m. for all the study groups. After complete clot formation the samples were centrifuged, and the serum was divided. The probes were collected and analysed in one batch by means of the IMMULITE system using solid-phase, enzyme labelled, and chemiluminescent sequential immunometric assay. The intra-assay coefficient of variation for sIL-2R was 3.7%, 3.8% for IL-8, and 3.6% for TNF-α; the inter-assay coefficients were 8.1%, 7.4%, and 6.5%, respectively.

Anti-TPO testing was performed for 129 patients using the ImmunoCAP 100 system (Phadia, Uppsala, Sweden). TSH, total T3, freeT3, and freeT4 were assessed by means of the chemiluminescence method, using the IMMULITE200 analyser. The reference values are 0.4–4.0 mU/L for TSH; 1.3–2.8 nmol/L for total T3, 2.7–6.5 pmol/L for free T3, and 10.3–25.0 pmol/L for free T4. The coefficients of variance for these hormones were less than 10% for freeT3, 9% for free T4, 12.5% for TSH, and 15% for T3. The anti-TPO test values over 100 IU/ml were taken as positives.

4. Statistical analysis

The analyses were performed using the software package Statistica 7.0 (Tulsa, OK, USA). As cytokine levels did not follow the Gaussian distribution, logarithms were used to normalize the data. The significance level of the tests for declaring a probability value as significant was set at 0.05. Different statistical tests were used in different studies and are described in the publications.

RESULTS

1. Differences in cytokine levels between MDD patients and healthy controls

The levels of sIL-2R and TNF-α were compared between 75 currently depressed subjects, 17 patients in full remission and 55 healthy controls in Study I (demographic data in Table 5). First, sIL-2R and TNF-α were compared in the 4 study groups: MDD with the recurrent depressive episode (RE), MDD with the first episode (FE), MDD in full remission (FR) and healthy controls (HC). The results showed a significant difference in the level of sIL-2R between the groups (Table 5). The levels of sIL-2R were significantly lower in FR than in RE and HC. There was a trend towards a lower level of sIL-2R in FR compared to FE. Previous use of antidepressants did not influence these results.

No group differences were found in the levels of TNF-α between 4 groups (Table 5), but a comparison of the currently euthymic subjects (HC and FR) and depressed subjects (FE and RE groups) showed lower levels of TNF-α in the currently depressed subjects. Additionally, the subjects with previous antidepressive treatment had significantly lower levels of TNF-α and differed significantly from drug-naïve patients and HC. There was no difference between HC and drug naïve patients.

When only drug-naïve patients, drug naïve remissions, and HC were included in the analysis, REs were associated with increased levels of sIL-2R by comparison with FE, FR, and HC. There was no difference in the levels of TNF-α between the groups.

HAM-D scores were significantly and positively associated with TNF-α but not with sIL-2R levels in the currently depressed patients. BDI scores were not related to the levels of TNF-α and sIL-2R. Both biomarkers did not correlate with the number of depressive episodes, with the duration of the current episode, smoking habits, or melancholic features. Additionally, it appeared that sIL-2R levels were related to two HAM-D items: decreased activity (the 7^{th} item of HAM-D) and agitation (the 9^{th} item of HAM-D). TNFα levels were associated with decreased activity and suicidality (the 3^{rd} item of HAM-D). IL-2R and TNFα levels were not related to any BDI items.

Table 5. The demographic data, the mean scores of HAM-D and BDI and the concentrations of interleukin-2 receptor (IL-2R) and tumour necrosis factor alpha (TNFα) in the Study I groups with statistical comparisons.

	FE	RE	FR	HC	p-value
Male/female	4/8	14/49	7/10	23/32	*NS
Age (±SD)	32.50 (14.28)	37.24 (12.35)	35.76 (14.54)	32.75 (14.10)	**NS
BMI (±SD)	25.38 (4.02)	22.22 (3.24)	22.99 (2.90)	24.15 (4.47)	**NS
HAMD (±SD)	24.27 (4.54)	24.14 (3.11)	3.19 (3.54)	1.08 (1.22)	***<0.001
BDI (±SD)	30.40 (7.88)	30.08 (8.86)	4.85 (3.98)	4.32 (4.81)	***<0.001
Melancholic symptoms Yes/No	8/4	56/7			*=0.050
IL-2R (kU/l) Mean (±SD)	431.75 (111.24)	506.90 (174.06)	354.94 (142.78)	453.55 (136.10)	***<0.001
TNFα (ng/l) Mean (±SD)	5.48 (1.72)	6.46 (3.16)	7.72 (3.74)	7.29 (3.56)	***NS

*Fisher's exact test
**Kruskal-Wallis' test
*** Group – effect of ANOVA
post hoc for HAMD: FE/RE (p=0.889), FR/HC (p=0.008), FE/HC (p=0.000), FE/FR (p=0.000), RE/HC (p=0.000), RE/FR (p=0.000)
post hoc for BDI: FE/RE (p=0.894), FR/HC (p=0.812), FE/HC (p=0.000), FE/FR (p=0.000), RE/HC (p=0.000), RE/FR (p=0.000)
post hoc for IL-2R: FE/RE (p>0.05), FR/HC (p=0.004), FE/HC (p>0.05), FE/FR (p=0.080), RE/HC (p>0.05), RE/FR (p=0.0001)

2. Escitalopram treatment effects on IL-8, TNF-α, and sIL-2R levels in MDD patients

The treatment effects of escitalopram on IL-8, TNF-α, and sIL-2R levels in MDD patients were assessed in Study II. In this study, the study group consisted of 100 patients (35 males and 65 females) and 45 HC (19 males and 26 females). The demographic and clinical data of the study cohort are presented in Table 6.

There were no significant differences in age or sex distribution between the R and NR or between the patient groups and HC. The NR had more previous depressive episodes, earlier age of disease onset, and was more melancholic and less drug-naive than R. At baseline, the severity of depression on MADRS did not significantly differ between the R and NR groups.

There was a statistically significant effect of group x time interaction but no group effect in sIL-2R measurements during the study (weeks 0, 4, and 12). There were different patterns of sIL-2R changes for R and NR – in the NR group sIL-2R decreased significantly between weeks 4 and 12 and in the R group between weeks 0 and 4. No significant effects of escitalopram treatment could be reported for either IL-8 or the TNF-α level. By week 12 there were no differences in cytokine levels between the 3 study groups (Table 7).

Table 6. Demographic and clinical data and the baseline measurements of the cytokines of the study II cohort: healthy volunteers, responders and non-responders to treatment with escitalopram

Variables	Healthy volunteers	Responders	Non-responders	p-value
Number of patients	45	74	26	
Gender (male/female)	19/26	29/45	6/20	** NS
Age	32.9. (14.1)	31.5 (±12.2)	34.2 (±11.0)	****NS
Number of episodes		4.60 (±5.08)	7.25 (±6.63)	*<**0.01**
Age of onset of the first episode		23.62 (±10.47)	19.67 (±10.32)	*=**0.050**
Duration of current episode (months)		10.8 (±14.3)	14.7 (±16.8)	*NS
Melancholic symptoms (with/without)		50/24	23/3	**<**0.05**
Drug-naive/previously treated		44/32	9/15	**<**0.05**
MADRS before the treatment		28.5 (±5.9)	29.5 (±4.4)	*NS
MADRS at the end of the treatment		4.5 (±5.1)	22.8 (±6.3)	*<**0.001**
Baseline sIL-2R (kU/l)	471.17 (±136.57)	524.56 (±175.33)	499.18 (±138.90)	***NS
Baseline IL-8 (ng/l)	7.74 (±1.95)	6.31 (±1.95)	6.64 (±1.99)	***NS
Baseline TNF-α (ng/l)	6.42 (±1.94)	5.70 (±1.55)	6.38 (±2.02)	*** **<0.05**

*t-test
**Chi-squire test
***group-effect of ANCOVA with age as covariance and gender as second factor
****t-test with Bonferroni correction

Table 7. Measurements of the cytokines in escitalopram treatment-week 0, 4 and 12 in responder and non-responder groups

Time	sIL-2R (kU/l)		IL-8 (ng/l)		TNF-α (ng/l)	
	Responders	Non-responders	Responders	Non-responders	Responders	Non-responders
Week 0	524.56 (±175.33)	499.18 (±138.90)	6.31 (±1.95)	6.64 (±1.99)	5.70 (±1.55)	6.38 (±2.02)
Week 4	493.99 (±167.42)	518.18 (±154.08)	6.46 (±2.04)	7.66 (±2.67)	5.91 (±1.90)	6.79 (±2.24)
Week 12	515.42 (±208.65)	451.00 (±109.66)	6.93 (±2.32)	7.09 (±2.64)	6.27 (±1.94)	6.40 (±2.38)
P*	<0.05		NS		NS	

*time × group effect of RM design ANOVAs

3. Bupropion augmentation effects on IL-8, TNF-α, and sIL-2R levels in escitalopram-resistant MDD patients

The MDD patients who did not respond to 20 mg escitalopram treatment had a possibility to continue the study in the augmentation phase. Twenty-eight patients were selected for Study III. The HC group was the same as in Study II. The demographic and clinical assessment data of patients are presented in Table 8. There were no significant differences in age, sex, or body mass index (BMI) between R, NR, and HC. The NR scored significantly higher on MADRS both before and after the treatment.

There were no group or group x time interaction effects in the augmentation phase. However, there was a significant time effect for IL-8 as the levels of IL-8 increased during 6 weeks of treatment (Table 8). No correlations were noticed between cytokine levels and the severity of depression on MADRS total scores at any time of measurement.

Table 8. Demographic and clinical characteristics and concentrations of sIL-2R, IL-8 and TNF-α in responders (R) and non-responders (NR) in bupropion augmentation

Variables	Responders	Non-responders	p-value
Number of patients	18	10	
Gender (male/female)	5/13	6/4	NS
Age	31.7 (±9.8)	30.4 (±9.4)	NS
BMI	23.9 (±3.7)	24.9 (±3.2)	NS
Number of episodes	7.3 (±8.0)	6.7 (±9.7)	NS
Age of onset of the first episode	18.3 (±6.7)	18.6 (±8.8)	NS
Duration of current episode (months)	13.9 (±16.8)	14.9 (±15.1)	NS
Melancholic symptoms (Yes/No)	15/3	8/2	NS
MADRS before the combined treatment	19.8 (±4.3)	25.7 (±6.9)	**=0.010**
MADRS at the end of the treatment	7.3 (±4.6)	24.6 (±5.5)	**<0.001**
sIL-2R kU/l (±SD) week 0	525.47 (265.11)	493.11 (143.56)	a) NS b) NS
sIL-2R kU/l (±SD) week 6	563.78 (216.47)	534.80 (151.47)	c) NS
IL-8 ng/l (±SD) week 0	6.54 (2.13)	7.35 (3.36)	a) NS b) <**0.05**
IL-8 ng/l (±SD) week 6	6.94 (2.34)	7.64 (2.29)	c) NS
TNF-α ng/l (±SD) week 0	6.55 (2.89)	6.37 (2.47)	a) NS b) NS
TNF-α ng/l (±SD) week 6	7.04 (2.85)	7.21 (2.41)	c) NS

a) RM ANOVA Group effect
b) RM ANOVA Time effect
c) RM ANOVA Time x group effect

4. Associations between IL-8, TNF-α, and sIL-2R baseline serum concentrations and treatment response in MDD patients

In the treatment phase I (Study II) the comparison of baseline cytokine levels between R, NR, and HC demonstrated a statistically significant between-group difference for TNF-α but not for other cytokines (Table 6). R showed a lower baseline TNF-α level in comparison with NR or HC, whereas the two latter groups did not differ from each other. However, there was a significant gender effect – NR males had a higher level of TNF-α than R males, NR females, or R females.

In the augmentation phase, the baseline levels of IL-8, TNF-α, and sIL-2R did not significantly differ between the R, NR and HC groups (Table 6).

5. Thyroid function and treatment response

Anti-TPO positivity was found in eight depressive and two healthy females without a statistically significant difference between these groups. As anti-TPO was not seen in either of the male groups, all further statistical analyses were carried out only in females. There were no significant differences in the levels of thyroid hormones (particularly, total T3, free T3, freeT4, and TSH) between female responders and non-responders; however the latter group showed a tendency for a higher prevalence of anti-TPO than the responders. Eleven patients had elevated total T3 and/or free T3 and/or free T4 levels, and one of them had anti-TPO.

Table 9. Demographic and clinical data of female responders and non-responders.

Variable	Responders (n=60)	Non-responders (n=30)	P (Mann-Whitney) P* (Chi-square) P** (Fisher exact test)
Age (years ± SD)	35.2 ± 13.1	32.7 ± 10.5	NS
Anti-TPO (pos/neg)	3/57 (5.3 %)	5/25 (20.0 %)	***NS
MADRS baseline (± SD)	28.1 ± 4.7	29.1 ± 5.3	NS
MADRS endpoint (± SD)	3.8 ± 3.7	23.0 ± 6.4	**<0.001**
HAMD baseline (± SD)	20.2 ± 4.0	21.4 ± 5.0	NS
HAMD endpoint (± SD)	3.8 ± 3.2	16.8 ± 5.6	**<0.001**
Duration of current episode (months ± SD)	13.1 ± 17.8	12.1 ± 15.2	NS
Age of onset of the first episode (years ± SD)	26.8 ± 12.3	18.6 ± 8.9	**<0.005**
Number of previous episodes (± SD)	4.2 ± 5.0	7.7 ± 8.1	**<0.05**
Comorbid anxiety	23/60	17/30	*NS
Comorbid melancholia	45/60	25/30	*NS

DISCUSSION

I. TNF-α

There were no significant differences in the TNF-α levels between main groups: RE, FE, FR, and HC. Study I showed lower TNF-α serum levels in the currently depressed than euthymic subjects. Further analysis revealed that the lower levels of TNF-α were associated with previous antidepressive treatment and were not found in drug-naïve patients. Narita et al. (2006) reported that the levels of TNF-α were significantly lower in remitted MDD patients receiving maintenance antidepressive treatment for longer than 6 months in comparison with the healthy controls. Unfortunately, it is not clear how long this immunosuppressive effect could last after the discontinuation of depression treatment. The lower than normal levels of TNF-α were also observed in young patients with dysthymia, but not in those with MDD (Brambilla et al., 2004).

TNF-α is a multifunctional cytokine which participates in the pathogenesis of various diseases, including autoimmune, inflammatory, neurodegenerative diseases, diabetes, septic shock, and congestive heart failure (Tayal and Kalra 2008), and it has been associated with psychiatric disorders, including MDD (Table 1).

Although the general opinion is that MDD is associated with higher levels of pro-inflammatory cytokines, especially IL-6, IL-1β, and TNF-α, not all human studies, including study I for TNF-α, have reported an increase in pro-inflammatory cytokines in depressed patients versus healthy controls (Table 1). Like in HPA axis activity, which could be hyper- or hypoactive depending on subtype of depression (Antonijevic 2006; De Beaurepaire 2002), it may be that some subgroups have opposite reactions in cytokine profiles, e.g. a decrease in pro-inflammatory cytokine production. Our study suggests this hypothesis with the finding of lower levels of TNF-α in currently depressed patients. Lower TNF-α level could be a state marker, as in remission phase there were no differences between healthy and affected subjects. This is in agreement with a study by Kagaya et al. (2001), showing that after pharmacotherapy TNF-α levels of depressed patients increased. This hypothesis needs to be further tested in different subgroups of patients with MDD.

There is also a finding of midlife women reporting higher levels of depressive symptoms associated with a decreased in vitro production of IL-1β, IL-6, and TNF-α compared with their less-depressed counterparts (Cyranowski et al., 2007). Similarly, TNF-α level was significantly lower in healthy students with high anxiety scores during psychological stress (Chandrashekara et al., 2007). These reports suggest the relationship between decreased synthesis of pro-inflammatory cytokines and symptoms of depression and anxiety. Like in major depression, the data are also controversial in healthy controls. Maes et al. (1997a) found that, in students, examination stress significantly increased the stimulated production of TNF-α. Higher BDI scores were associated with

greater expression of TNF-α (Suarez et al., 2004). However, Marsland et al. (2007) did not find significant associations between TNF-α and any psycho-social parameters in middle-aged community volunteers.

The possible reasons for conflicting findings could be explained by different study cohorts, subtypes of depressive disorders, and different cytokine measurement techniques. Depression is often (approximately 50%) associated with HPA axis hyperactivity (Cowen 2005), and deregulation of the feedback mechanism appears to occur in depressive disorders (Schiepers et al., 2005). Pro-inflammatory cytokines are potent activators of the HPA axis and play a critical role in activating the HPA axis in MDD. These cytokines counteract the negative feedback action of corticosteroids on the HPA axis (Myint and Kim 2003; Schiepers et al., 2005). There is a hypothesis that cytokines could induce corticosteroid receptor resistance in the hypothalamus and the pituitary gland (Pace et al., 2007) – a higher level of pro-inflammatory cytokines implies a stronger resistance of corticosteroid receptors.

2. TNF-α in the escitalopram-treatment phase

The lower levels of TNF-α in Study II were associated with a better treatment response. The R group of patients had lower levels of TNF-α than NR or HC. There was no difference between NR and HC. Bauer et al. (2003) have previously reported that patients with treatment-resistant depression did not differ from HC in their baseline levels of TNF-α and sIL-2R. There is also evidence that elevated HPA axis activity in acute depression suppresses TNF-α activity, while in remission, when HPA axis activity is normalized, the TNF-α system seems to gain influence on the HPA system (Himmerich et al., 2006).

The difference between responders and non-responders has been assessed in several studies. After a six-week treatment period with amitriptyline, TNF-α levels normalized only in responders (Lanquillon et al., 2000). However, the pre-treatment levels of TNF-α in this study were increased in both, responders and non-responders as compared to healthy controls.

TNF-α activates serotonin transporters, providing a mechanism by which cytokines can modulate serotonergic signalling and influence emotional cognitive processing (Miller and Raison 2006; Zhu et al., 2006). Additionally, there is evidence that antidepressants have an effect on the production of pro-inflammatory cytokines, including TNF-α (Diamond et al., 2006). Table 2 contains results of different studies of the treatment effect on TNF-α. Heiser et al. (2008) found that the incubation of the platelets in vitro MDD patients with cortisol and dexamethazone at baseline resulted in an apparent increase in the secretion of TNF-α in the R group compared with HC while the values of the NR group did not differ from the data of the HC group in this respect. These data underscore the likelihood that this type of glycocorticoid actions may be present under special conditions despite the commonly assumed immuno-

supressive effects of the steroids. Therefore, it could well be possible that such a mechanism of glycocorticoids is at least partly responsible for the increased levels of this cytokine in MDD patients (Heiser et al., 2008; Lanquillon et al., 2000, Mikova et al., 2001). Heiser et al. (2008) believe that glycocorticoids may be involved in the psychopathology of MDD and the TNF-α system, supported by the correlation between these parameters in the responder subgroup of their study. They also found that the dynamics of the glycocorticoid receptor system is related to psychopathological normalization in the other system. In that study responders to antidepressive therapy showed more dynamic changes with the time of treatment under both basal and stimulated immune conditions. It suggests indeed that the dynamics of neuroendocrine-immune interactive system are related to a positive therapy response.

3. TNF-α in the augmentation phase with bupropion

Previous studies have reported that bupropion may lower the TNF-α level in various physical illnesses including Crohn's disease (Kast 2003). In this study bupropion was added to escitalopram in treatment-resistant patients, and the effect was not detectable. To our knowledge there were no previous studies on the effects of antidepressant augmentation on cytokines production. Hernandez et al. (2008) studied MDD patients during antidepressive treatment with SSRIs for 52 weeks and measured several cytokines at weeks 0, 5, 20, 36, and 52. They found that the changes in certain cytokine levels were not linear. The production of some cytokines changed only at the beginning of treatment (interferon- α), but IL-4, for example, increased from the baseline to week 20 and started to decrease after that. Unfortunately, TNF-α, IL-8 and sIL-2R were not assessed in their study. In this study, at the end of the augmentation phase the treatment had lasted for 18 weeks and the reactions of the immune system might be much faster at the beginning of the treatment. Among the limitations was the relatively small study group in the particular treatment phase.

4. IL-8

There were no differences in levels of IL-8 between the groups in our study. IL-8 is a chemokine produced by monocytes, macrophages, fibroblasts, keratinocytes, and endothelian cells after stimulation by IL-1 and TNF-α (Janeway et al., 2005). It includes several functions of human polymorphonuclear leukocytes, such as chemotaxis, release of granule components and respiratory burst (Mikova et al., 2001). There are only a few studies on IL-8 in major depression and even in animal studies this marker has been rarely measured. Simon and Song found elevated levels of IL-8 in major depression (Simon et al., 2008; Song et al., 1998). As in our study, Mikova et al. (2001) could not repeat that

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finding. There is evidence that in the case of bipolar depression the levels of IL-8 are higher than in HC (O'Brien et al., 2006). Also, some papers have reported higher IL-8 levels in schizophrenia (Maes et al., 2002) or in negative symptoms of schizophrenia (Zhang et al., 2002). However, Song et al. (2007) reported lower levels of IL-8 in post-traumatic stress disorder compared with HC.

There is a study showing higher IL-8 concentrations in patients with fibromyalgia (Wang et al., 2009). Notably, pain is common in patients with major depression. Severity and duration of chronic pain was reported as directly proportional to severity of depression (Evans et al., 2005; Fishbain 2002). Some theories support the immunological link between pain and depression (Campbell et al., 2003). It is possible that IL-8 levels are related to pain symptoms of depression, but this hypothesis need to be tested.

There is no knowledge of the exact functions of IL-8 in the brain and its relationships with other cytokines and neurotransmitters. Thus, the results are rather difficult to interpret. There are two in vitro studies in healthy men and women showing a greater stimulated production of IL-8 correlating with higher BDI scores (Suarez et al., 2003, 2004), but this finding has not been replicated in depressed patients. Marsland et al. (2007) found among a middle-aged community volunteers a positive relationship between stimulated production of IL-8 and symptoms of depression and an inverse relationship between stimulated IL-8 production and perceived social support. Taken together, there are no affirmative data for a relationship between IL-8 and depression and broader assessments, including other immune markers, are necessary.

5. IL-8 in the escitalopram – treatment phase

The second study observed no changes in IL-8 production in MDD patients treated with escitalopram. There were only two previous studies evaluating associations of IL-8 with treatment outcomes in depression. Mikova et al. (2001) treated 14 patients with different antidepressants and did not find any effect on IL-8 levels. However, due to small sample size these findings remained inconclusive. O'Brien et al. (2007) assessed SSRI-resistant patients in comparison to HC. They measured cytokines once, upon determining treatment-resistant status. The higher production of pro-inflammatory cytokines IL-6 and TNF-α was detected in currently euthymic patients with previous SSRI-resistant depression with no differences in IL-8 levels.

6. IL-8 in the augmentation phase with bupropion

Production of IL-8 during 12 weeks of escitalopram treatment did not change significantly, but started to increase during bupropion augmentation. This may be explained by differences in the effects of various antidepressants on IL-8

synthesis. For example, Kast et al. (2003) found that bupropion decreased and mirtazapine increased the production of TNF-α. Lin et al. (2000) reported that moclobemide suppressed unstimulated production of IL-8 and the TNF- α effect in HC in vitro. However, these data can not be equally transferred to in vivo findings of MDD patients. There is also a possibility that the changes in IL-8 production need more time to become statistically significant. Only Hernandez et al. (2008) have evaluated levels of different cytokines repeatedly during 52 weeks of antidepressive treatment. Unfortunately, IL-8 was not measured in this study. More studies on the effects of augmentation with another antidepressant on immune markers' production are necessary to confirm the trends seen in our study.

7. sIL-2R

Few studies have focused on cytokine receptors in MDD, and the results are even more inconsistent than in cytokines (Table 1; rev by Kronfol and Remick 2000). Cells that express a functional receptor for a cytokine will respond to the presence of that cytokine. Cytokine receptors can also be found in soluble form. Usually, a soluble receptor for a specific cytokine can inhibit the biological activity of the cytokine by inhibiting the binding of the cytokine to its membrane-anchored receptor (TNF-α). However, on rare occasions sIL-R and the specific cytokine complex add to the activity of this cytokine (IL-6) (Kronfol and Remick 2000). There is evidence that sIL-2R appears to regulate the immune function by binding IL-2, and thereby neutralizes its cellular effects (Bien and Balcerska 2008; Levine et al., 2001).

IL-2 is the growth factor of T-cells, which is involved mainly in the proliferation and differentiation of T-cells but also activates B-cells and natural killer (NK) cells (Tayal and Kalra 2008). sIL-2R is produced by T-cells similarly to IL-2; thus, it is a marker of T-cell activity (Janeway et al., 2005). IL-2 is also identified in various brain regions, including hippocampus, neostriatum, and the cortex. IL-2 is produced by cells in the central nervous system including astrocytes and microglial cells (Levine et al., 2001). Elevated levels of sIL-2R have been observed in a variety of autoimmune and inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, myasthenia gravis, sarcoidosis, celiac disease and Crohn's disease, among others (Bien and Balcerska 2007). In major depression, serum IL-2 is often immeasurable because of low concentration (Maes et al., 1995; Klabusay et al., 2006), and usually sIL-2R is measured for assessing T-cell activity. There is an opinion that increased sIL-2R is an adaptation mechanism to increased IL-2 (Levine et al., 2001). Nevertheless, there are currently insufficient data to confirm this hypothesis. On the contrary, there is evidence that IL-2 accumulation in the serum of patients with unipolar and bipolar depression is strongly correlated with a decrease in

sIL-2 serum levels (Androsova et al., 2001). An inverse relationship between IL-2 and sIL-2R levels has also been reported in studies in healthy individuals. Increased IL-2 and decreased sIL-2R levels, in addition to decreased IL-2R mRNA, were observed during examination periods in medical students compared with levels measured during low-stress periods (Mittwosh-Jaffe et al., 1995). Moreover, decreased IL-2 levels have been associated with negative emotions (Nakano et al., 1998) and economic stress (Anisman et al, 1999). sIL-2R levels were not measured in these studies.

Findings in patients with depression and healthy individuals have been, and in most studies only IL-2 levels, but not sIL-R, were measured. Some studies demonstrated lower levels of IL-2 in patients with depression compared with healthy controls (Hernandez et al., 2008; Kim et al, 2007; Pavon et al., 2006; Mendlovic et al., 1999). One of these studies included only inpatients with MDD (Pavon et al, 2006), while in the other studies, outpatients were included. In one study, no differences were observed between patients with MDD and healthy controls (Schlatter et al., 2004), although the cohort in this study was small, with 9 patients with depression and 9 healthy individuals. In contrast, higher IL-2 levels were observed in patients with depression compared with in healthy subjects in three separate studies (Simon et al., 2008; Sutcigil et al., 2007; Kim et al., 2008).

In this study decreased sIL-2R serum levels were found in MDD patients in full remission compared with MDD patients with a recurrent episode (trends for the first episode) and healthy controls. Maes et al. (1995) reported that sIL-2R concentrations appear to correlate with IL-2 secretion, and higher levels of sIL-2R may suggest an up-regulated production of IL-2. If so, then MDD patients in the remission phase have lower T-cell activation than other groups in the cohort of this study, The finding may suggest secondary adaptive changes in the immune system activity in the remission phase of MDD. The limitation of this study is a relatively small and heterogeneous FR group. The criteria for the duration of the remission have to be at least 2 months, but no further questions were asked to this effect. Therefore, one can not draw any conclusions about the time period when such changes appear.

On the other hand, Nunes et al. (2002) reported reduced levels of sIL-2R in moderate and severe depression, Kim et al. (2008) reported decreased IL-2 in suicidal MDD patients, Levine et al. (1999) reported lower cerebrospinal fluid (CRF) sIL-2R in MDD compared with healthy controls, and they failed to provide a good explanation to this effect. If lower levels of sIL-2R are related to MDD, it could well be that the finding of this study about lower levels of sIL-2R in the remission phase predicts future MDD episodes in those patients.

Increased serum levels of sIL-2R were observed in patients with MDD compared with healthy individuals in two previous papers (Maes et al., 1995; Seidel et al., 1995).

IL-2 is hypothesized to activate dopaminergic pathways (Levine et al., 2001; Müller and Ackenheil 1998). Study I found that sIL-2R concentrations

associated with decreased activity and agitation items of the HAM-D scale, which are symptoms that the dopamine system is involved (Nutt et al., 2007).

8. sIL-2R in the escitalopram- treatment phase

There are many possible mechanisms of the SSRI effect on the activity of the immune system. Chronic antidepressant treatment re-establishes the feedback mechanisms of glycocorticoid receptors, and the activity of the HPA axis normalizes (Holsboer 2000). In addition, there is evidence that both noradrenalin and serotonin act as immuno-modulators. Thus, a functional increase in the activities of the neurotransmitter systems by effective antidepressants could contribute to the normalization of the immune function that occurs in depressed patients following effective treatment (Leonard 2001).

In study II, changes in sIL-2R levels followed different patterns in responders and non-responders to escitalopram. While an increase in sIL-2R is an adaptive immunosuppressive mechanism, the R group reacts immuno-suppressively with an increased production of sIL-2R during the first four weeks, and production decreases after week 4. In the NR group the secretion of sIL-2R decreases between the baseline and week 4 and slightly increases after week 4. These findings, in combination with the results of Hernandez et al. (2008), suggest that the timepoint at which sIL-2R is measured is an important factor, and whether the concentration increases or decreases may depend on the duration of treatment.

Some other studies did not detect any changes in sIL-2R levels during antidepressive treatment when R and NR were analysed together. Subchronic use (~3 months') of fluoxetine or tricyclic antidepressants was not associated with changes in sIL-2R levels (measures at baseline and at treatment endpoint) in patients with major depression (Maes et al., 1995). In addition, treatment of patients with depression with clomipramine for 8 weeks did not have an effect on sIL-2R concentrations (Kagaya et al., 2001), and similar results were obtained using antidepressants from different classes, such as clomipramine (n=8), paroxetine (n=4), mianserin (n=1) and amitriptyline (n=1) (Mikova et al., 2001). Nor is there any difference between serum sIL-2R concentrations at baseline and week12 when R and NR were put together in this study. At the time of publication, Mikova et al. (2001) exhibited significantly higher serum IL-2R values in non-responders than in responders after treatment. However, the patient group that was assessed in this study was small (n=14), treatment was not standardized and, moreover, it included antidepressants from different classes.

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9. sIL-2R in the augmentation phase with bupropion

In the augmentation phase the secretion of sIL-2R did not change; nor were there any differences between R and NR groups. It seems that differences in immune system reactions occur at the beginning of the treatment. Again, the timepoint of assessment that was discussed in the preceding section may play a role here. As this study is the first to explore the effect of bupropion augmentation on cytokine profiles, further studies are needed to clarify the reactions of the immune system in augmentation or treatment changes.

10. Anti-TPO and thyroid hormones

Thyroid autoimmunity is one of the aspects of immune system activation. The study IV found a somewhat higher frequency of baseline anti-TPO positive cases in non-responders to escitalopram monotherapy as compared with responders, suggesting that anti-TPO positivity may predict the treatment response to antidepressant medication. A larger study group may be needed to have significant statistical power. There was no relationship between thyroid hormones and treatment response.

Notably, anti-TPO positivity has often been associated with bipolar depression (Haggerty et al., 1997, Kupka et al., 2001) and also with unipolar depression (Carta et al., 2004). There is evidence that atypical depression is related to higher thyroid microsomal antibodies (synonymous with anti-TPO). In addition, the presence of anti-TPO during early pregnancy was associated with the development of postpartum depression (Kuijpens et al., 2001). There are several negative findings in unipolar depression (Engum et al., 2005; Fountoulakis et al., 2004; Haggerty et al., 1997, Horning et al., 1999). One hypothesis might be that anti-TPO positivity predicts bipolar disorder for depressed patients without previous manias. Undiagnosed bipolar disorder may be a possible reason for poorer improvement with antidepressants (Berk and Dodd 2005).

The association between presence of anti-TPO and immune system is demonstrated in bipolar patients, while the level of anti-TPO was negatively correlated with the serum level of sIL-2R (Padmos et al., 2004). The previous finding of study II is that the responders showed a lower baseline TNF-α level in comparison with non-responders or healthy subjects, whereas the two latter groups did not differ from each other. So, both findings suggest that immune system activation is involved in the treatment response in major depression.

Alteration of thyroid function has also been reported in depressed patients. Compared with controls, patients with depression showed lower basal serum evening TSH (Duval et al., 1999). However, this has not been a consistent finding (Frye et al., 1999; Fountoulakis et al., 2004; Pop et al., 1998). Gitlin et al. (2004) have demonstrated that low baseline TSH correlated strongly with

greater improvement in depressive symptoms assessed by change in HAM-D scores. Unfortunately, assessment of thyroid hormones was included to our study protocol later and we could assess hormones only once in patients with depression but not in healthy individuals. Previously, Sagud et al. (2002) demonstarted that 4-week treatment with sertraline increased plasma T3 levels in depressed patients. The baseline levels of thyroid hormones (TSH, T3 and T4) of patients with depression did not differ from the values in healthy controls in their study. These results are similar to the findings in study IV.

11. General discussion and future perspectives

We previously stated that about half of the patients whose depressive disorder is at least moderately severe, and those with melancholic features, have HPA-axis hyperactivity. However, not all melancholic patients have this kind of feature. Despite efforts over many years it is still not known how to clinically differentiate these patients from others or what the exact endophenotype of these depressive patients is. In addition, HPA hypofunction was found in a subgroup of depressed patients with hypersomnia, hyperphagia and lethargy or fatigue, commonly referred to as atypical depression (Antonijevic 2006; Levitan et al., 2002). Similarly, there is evidence that some of the depressive patients have changes in immune system activity, but the coexisting endophenotype is still unknown. There is some evidence that different subtypes of depression have different immune patterns, e.g. in melancholic versus non-melancholic depression (Kaestner et al., 2005; Rothermundt et al., 2001), but not all studies confirmed this relationship (Marques-Deak et al., 2007). Unfortunately, these studies investigated different cytokines making direct comparisons not feasible. This formed the basis for our evaluation of the relationships between single depressive symptoms and immune system markers as with study I. The available data are rather inconsistent and controversial, and do not allow clear conclusions to be drawn regarding the involvement of cytokines in depression. Most of the previous studies were limited by small or heterogeneous samples, whereas additional bias might be caused by concomitant use of antidepressive medications during the measurement of cytokines. Moreover, only a few of the previous studies have addressed the association between cytokines and specific symptoms of depression or the different stages of clinical course. Therefore, future studies need very homogenous cohorts; enough patients in each study group, including remission group. In addition, different studies assess different cytokines with different methods. It would be highly useful to assess the concentration of several cytokines and their receptors in one study so as to evaluate the co-effects. Moreover, there is evidence that cytokines are influenced by several factors, such as biological rhythms, circadian rhythms, smoking habits, previous food intake, and physical activity (Gokhale et al., 2007; Haack et al., 2004; Majde and Krueger 2005; Reichenberg et al., 2002).

There is no much knowledge about the influence of factors such as climate, race, gender and seasons. As concentrations of some (TNF-receptor), but not all (IL-2 receptor), immune markers change during the day, repeated measurements would be helpful (Haack et al., 2004). Measurement of certain cytokines, including TNF-α and sIL-2R, may predict response to treatment, but the association is likely to depend on the specific action of the antidepressant used. Clinical trials with standardized treatment with different classes of antidepressant should help to determine whether immune system reactions are associated with distinctive neuropharmacological profiles of antidepressant medication. Furthermore, as demonstrated by Hernandez et al. (2008), longer treatment periods with regular clinical and biomarker assessments are necessary to make further progress in this field. A framework of the effects of immune system in the pathogenesis of depression is presented in Figure 1.

Our studies were limited to measuring only three immune markers: TNF-α, sIL-2R and IL-8. In future studies more complex laboratory analyses would be needed. Using these three markers we could see that being drug-naive or not, could strongly influence results for an unknown time-period. More to the point, previous antidepressive treatment lowers the levels of TNF-α. There was no immune activation in depressed patients in our Estonian study cohort. However, lower levels of baseline TNF-α predicted better treatment response to escitalopram. Different patterns of change in the sIL-2R levels occurred in responders and non-responders which affirms the need for repeated measurements during a longer treatment period.

The weak result that anti-TPO positivity could negatively influence the response to escitalopram needs to be repeated with a larger study cohort because of the low prevalence rate. Thyroid hormones are known to be related to mood symptoms. However, repeated assessment during the treatment with different antidepressants would be better for ascertaining the associations between thyroid function and depressive states.

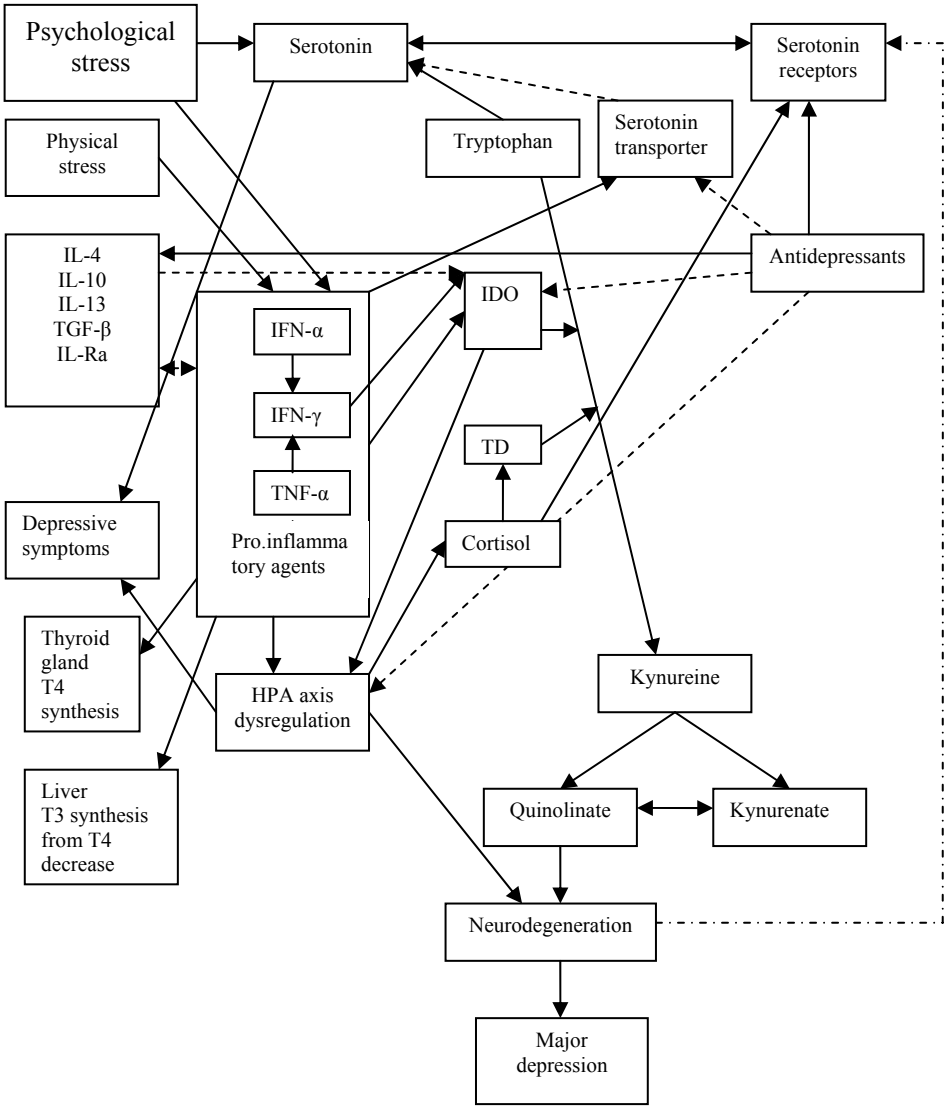

Figure 1. Immune system's effects in the pathogenesis of depression.

CONCLUSIONS

1. The levels of TNF-α were lower in currently depressed subjects compared with euthymic subjects in the study cohort. MDD patients with previous antidepressive treatment had significantly lower levels of TNF-α than drug-naïve patients and HC.

2. There was a positive correlation between TNF-α (but not sIL-2R) and the HAM-D total score in currently depressed subjects.

3. The levels of TNF-α were positively related to HAM-D items of decreased activity and agitation. The levels of sIL-2R were positively associated with HAM-D items of decreased activity and suicidality.

4. There were different patterns of changes in the levels of sIL-2R in responders and non-responders to escitalopram treatment: The concentrations of sIL-2R decreased later in non-responders than in responders. Treatment with escitalopram had no significant effect on the levels of IL-8 and TNF-α.

5. Augmentation of escitalopram treatment with bupropion caused a significant increase in IL-8 serum concentrations during 6 weeks of augmentation therapy. There was no effect on the levels of sIL-2R and TNF-α.

6. The lower baseline TNF-α level was found in the responder group in the escitalopram treatment phase. More specifically, male non-responders had higher levels of TNF-α than male responders or female responders and non-responders.

7. There was a trend for higher frequency of baseline anti-TPO positive cases in female non-responders to escitalopram monotherapy as compared with responders. There were no significant differences in the levels of thyroid hormones (particularly, total T3, free T3, freeT4, and TSH) between female responders and non-responders.

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SUMMARY IN ESTONIAN

Immunoloogilised muutused unipolaarse depressiooni korral ja antidepressiivse ravi käigus

Sissejuhatus

Unipolaarne depressioon on ühiskonnas sage häire, mis põhjustab olulist elukvaliteedi ja sotsiaalse funktsioneerimise langust. Depressioonil on märkimisväärset mõju ka kaasnevate psüühika- ja kehaliste häirete kulule ja ravitulemustele.

Depressioon on polüfaktoriaalne haigus, mille tekkes osalevad koosmõjuna nii geneetilised kui ka keskkonnategurid. Oluliseks on osutunud vanus, sugu, partnerlus, etniline kuuluvus, haridus, immigrandistaatus, linna-maapiirkonna erinevused, eluolukorrad, lapsepõlve traumad, kaasnevad haigused. Depressiooni patogeneesis on teadaolevalt roll monoamiinidel: serotoniinil, noradrenaliinil ja dopamiinil. Samuti on leitud, et umbes pooltel depressioonihaigetest on häirunud HPA-telje talitlus, esinevad häired glükokortikoid- ja mineralokortikoidretseptorite süsteemis. On alust arvata, et HPA-telje üleaktiivsus stressolukordade tagajärjel tekib neil isikuil, kellel on predispositsioon HPA-telje regulatsioonihäirele. Aju visualiseerimisuuringutel on püsivamateks leidudeks aju külgvatsakeste laienemine, otsmiku- ja temporaalsagarate, samuti hipokampuse ja mõnede basaaltuumade mahu vähenemine. Ühe hüpoteesi kohaselt võiks see olla kroonilise hüperkortisoleemia tulemus, kuid on ka arvamus, et muutused esinevad juba haiguse varases staadiumis, olles depressiooni teket ennustavaks ilminguks. Tulemused geneetiliste tegurite osas on seni veel lõplikult kindlaks tegemata, ilmselt nii depressiooni fenotüübiliste erinevuste kui ka geneetilise keerukuse tõttu. On leitud mitmeid kromosoomipiirkondi, mis seonduvad depressiooniga, näiteks 1p, 1q, 2q, 4q, 5q, 8p, 10p, 11pter, 11q, 15q, 18q, 19p, Xq. Samas on eraldi piirkonnad, mida seostatakse suitsidaalse käitumise ja depressiooni varase algusega. Geneetilised assotsiatsiooniuuringud keskenduvad geenidele, mida seostatakse põhiliste neuromediaatoritega või teiste patogeneesimehanismidega, näiteks HPA-teljega. Üks perspektiivseid suundi on depressiooni farmakogeneetika, mis uurib geneetilise variatsiooni mõju ravivastusele.

Viimaste aastate jooksul on enam tähelepanu hakatud pöörama ka immuunsüsteemi osatähtsusele depressiooni korral. Immuunsüsteemi primaarne roll on organismi kaitsmine viiruste, bakterite ja kasvajarakkude eest. Erinevad immuunrakud suhtlevad omavahel tsütokiinide abil. Laias laastus võib tsütokiinid jaotada kaheks: põletikutsütokiinideks (näiteks interleukiin-1, interleukiin-6, tuumor-nekroosfaktor alfa (TNF-α) ning põletikuvastasteks tsütokiinideks (interleukiin-10, interleukiin-1 retseptori antagonist). Lisaks sellele, et tsütokiinid toimivad virgatsainetena immuunsüsteemi rakkude vahel, on neil roll ka ajus, kus nad osalevad neurokeemiliste, neuroendokriinsete,

neuroimmuunsete ja käitumuslike muutuste tekkes. On leitud näiteks, et põletikutsütokiinid tõstavad HPA-telje aktiivsust, mõjutavad monoamiinide ja peptiidide taset hüpotaalamuse välistes piirkondades. Samuti on tõendeid, et põletikutsütokiinid aktiveerivad ensüüm indoleamiin-2,3-dioksügenaasi (IDO), mis konverteerib trüptofaanist kinureiini, mitte vajaliku serotoniini; samuti on leitud, et tõuseb serotoniin-transporteri aktiivsus, mis omakorda vähendab ekstratsellulaarse serotoniini taset.

Patsientidel, kes saavad tsütokiin-immuunteraapiat, tekivad kõrvaltoimena sageli depressiooni sümptomid, mis antidepressantide abil taanduvad. Depressioonihaigetel on korduvalt näidatud kõrgemaid põletikutsütokiinide tasemeid kui tervetel kontrollisikutel, samas on erinevad uuringud olnud vastuoluliste tulemustega. Uurimise all on olnud seosed tsütokiinide ja depressiooni raskusastme, melanhooliasümptomite esinemise või unemustritega. Lahknevaid tulemusi on saadud erinevate antidepressantide kasutamisega. Üldine seisukoht on, et antidepressantidel on leitud depressiooni korral immunomoduleeriv toime. Kuigi enam rõhutatakse põletikutsütokiinide taseme langust ja põletikuvastaste tsütokiinide taseme tõusu antidepressantravi foonil, on ka siin tulemused vastuolulised, sõltudes kasutatavast ravimist, uuringu kestvusest ning mitmetest muudest teguritest.

Ka kilpnäärme talitlushäireid on seostatud meeleoluhäiretega, rõhuasetusega subkliinilisele alatalitlusele, mille korral on leitud ka halvemat ravivastust antidepressantravile. Oluliselt vähem on uuringuid, mis keskenduvad kilpnäärme vastaste antikehade rollile meeleoluhäirete korral. Vaid üks uuring toob välja, et kilpnäärme vastaste antikehade esinemine on seotud halvema ravile reageerimisega depressioonihaigetel.

Uurimuse põhieesmärgid

Uurimuse põhieesmärgiks oli selgitada välja võimalik interleukiin-8 (IL-8), TNF-α ja lahustuva interleukiin-2 retseptori (sIL-2R) haaratus unipolaarse depressiooni patogeneesis ning tuvastada nende põletikutsütokiinide taseme muutused antidepressiivse ravi käigus.

Uurimuse täpsemad eesmärgid olid järgnevad:

1. Võrrelda TNF-α ja sIL-2R seerumkontsentratsioone depressioonihaigetel ja tervetel vabatahtlikel.
2. Leida võimalike seoseid uuritavate tsütokiinide seerumkontsentratsioone ja depressiooni raskuse vahe, mida määrati HAM-D skaalaga.
3. Leida võimalikke seoseis uuritavate tsütokiinide ja depressiooni üksiksümptomite vahel.
4. Leida estsitalopraamravi mõju IL-8, TNF-α ja sIL-2R tasemetele depressioonihaigetel.
5. Selgitada välja, kas bupropioonaugmentatsioon põhjustab muutusi IL-8, TNF-α ja sIL-2R produktsioonis estsitalopraamresistentsetel depressioonihaigetel.

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6. Leida võimalikud seosed IL-8, TNF-α ja sIL-2R tasemete ja antidepressantravile reageerimise vahel depressioonihaigetel.
7. Selgitada välja, kas kilpnäärme peroksidaasi vastased antikehad või kilpnäärmehormoonid (täpsemalt TSH, T3, T4) mõjutavad patsientide estsitalopraamravile reageerimist.

Materjal ja meetodid

Kõik uuringusse kaasatud isikud olid Eesti elanikud. Depressioonihaiged vanusepiirides 15–65 aastat selekteeriti Tartu Ülikooli Kliinikumi Psühhiaatriakliinikusse pöördunud patsientide seast. Depressiooni diagnoos määrati DSM-IV kriteeriumite järgi, kasutades diagnostilist struktureeritud intervjuud M.I.N.I.5.0.0 ja psühhiaatrilist intervjuud. Raskusastme hindamiseks olid kasutusel Hamiltoni depressiooni skaala (HAMD), Becki depressiooni hindamise küsimustik (BDI) ning ravifaasis osalenutel ka Montgomeri-Asbergeri depressiooniskaala (MADRS). Tervetel isikutel välistasime depressiooni-episoodid minevikus, samuti esimese astme sugulastel. Raskemad kehaliselt haigused olid uurimuses osalemise välistavateks teguriteks.

Kokku osales uurimuses 247 depressioonihaiget ning 94 tervet kontrollisikut. Neist 166 depressioonihaiget osales ravifaasis, mille käigus uuritavad said antidepressantravi estsitalopraamiga. Esimese 4 nädala jooksul oli kõigil osalenutel raviannus 10 mg, neil patsientidel, kel selle aja jooksul depressiooni-skoor MADRS skaalal langes vähem kui 50%, oli edasine raviannus 20 mg. Raviannust tõstsime ka neil patsientidel, kel hilisemas uuringufaasis tekkis tagasilöök. Kokku kestis esimene raviperiood 12 nädalat. Raviperioodi lõpus analüüsisime eraldi paranenuid (MADRS skoor alla 12) ja mitte ravile reageerinud patsiente (skoori langus alla 50%). Kuna patsientide arv, kes ravile reageerisid skoori alanemisega enam kui 50%, kuid kelle lõppskoor oli siiski üle 12 punkti, oli väga väike, analüüsisime neid ühiselt esimeses rühmas. Ravi teise faasi kaasasime 28 estsitalopraamravile mitte reageerinud isikut, kes said järgneva 6 nädala jooksul lisaks 20 mg estsitalopraamile 150–300 mg bupropiooni (noradernaliini ja dopamiini tranporteri blokaator). Sarnaselt esimese faasiga, analüüsisime raviperioodi lõpus eraldi ravile reageerinud ja mittereageerinud patsiente.

Tsütokiinide määramisel kasutati IMMULITE süsteemi, kilpnäärmehormoonid määrati kemiluminestsentsmeetodil ja anti-TPO määramisel kasutati ImmunoCAP süsteemi.

Peamised tulemused

Esimeses uurimuses võrreldi TNF-α ja sIL-2R tasemeid käesolevalt esmase ja korduva depressiooniga patsientidel, remissioonis patsientidel ning tervetel kontrollisikutel. Uurimuses selgus, et sIL-2R tase seerumis oli remissioonis patsientidel oluliselt madalam võrreldes nii tervete kontrollisikute kui ka korduvate depressioonihaigetega võrreldes. Esmaste depressioonihaigete suhtes

täheldati samasuunalist tendentsi. Samuti selgus, et eelnev antidepressantravi kasutamine tulemust ei mõjutanud. TNF-α tase nende gruppide vahel ei erinenud, kuid käesolevalt depressiivsete haigetel oli TNF-α tase madalam kui eutüümsetel isikutel (remissioonis haiged koos tervete kontrollisikutega). Ilmnes, et eelnevalt antidepressantidega ravitud isikutel oli TNF-α tase madalam kui ravinaiivsetel haigetel ja tervetel kontrollisikutel. Ainult ravinaiivseid isikuid analüüsides leidsime, et korduva depressiooni korral oli sIL-2R tase kõrgem kui ülejäänud gruppides. Depressioonihaigetel korreleerusid HAMD skoorid positiivselt TNF-α tasemega. Seoseid depressioonepisoodi kestvusega, eelnevate episoodide arvuga, suitsetamisharjumuste, melanhoolsete sümptomite ja määratud tsütokiinide vahel esile ei tulnud. Üksiksümptomitest seostusid TNF-α väärtustega alanenud aktiivsustase ning agiteeritus; sIL-2R väärtustega alanenud aktiivsustase ning suitsidaalsus.

Teine uurimus keskendus estsitalopraamravi mõjule tsütokiinide tasemele. Selgus, et ravile reageerinud patsientidel alanes sIL-2R seerumkontsentratsioon ravi esimese 4 nädala jooksul, samas raviresistentsetel patsientidel sIL-2R tase esialgu kergelt tõusis, alanedes alles 4 ja 12 ravinädala vahel. IL-8 ja TNF-α osas kahe grupi vahel erinevusi ei täheldatud. Sama uuring näitas, et ravile reageerinud patsientidel oli ravieelne TNF-α tase madalam kui raviresistentsetel haigetel ja tervetel kontrollisikutel.

Bupropioonaugmentatsiooni faasis ei esinenud olulisi grupi- ja ajagrupi seoseid, kuid ilmnes ajaefekt IL-8 osas: 6 nädala jooksul tõusis IL-8 tase kogu grupi lõikes. Augmentatsioonifaasi ravitulemust ei õnnestunud ühegi tsütokiini taseme abil ette ennustada.

Kuigi anti-TPO esines vaid kaheksal naispatsiendil ja kahel tervel naisel, tuli uurimuses esile tendents, et estsitalopraamile raviresistentsetel naistel on enam anti-TPO positiivsust kui ravile reageerinud naistel. Olulisi seoseid kilpnäärmehormoonide, depressiooni parameetrite ja ravitulemuste vahel esile ei tulnud.

Järeldused

1. Depressiivsetel patsientidel oli TNF-α tase madalam kui eutüümsetel isikutel. Depressioonihaigete eelnev antidepressantravi oli seotud madalamate TNF-α väärtustega võrreldes ravinaiivsete haigete ja tervete kontrollisikutega.
2. Depressioonihaigetel korreleerus TNF-α tase positiivselt HAMD skooridega. sIL-2R taseme ja depressiooni raskusastmega seoseid esile ei tulnud.
3. TNF-α väärtused korreleerusid alanenud aktiivsuse ja agiteeritusega ja sIL-2R väärtused alanenud aktiivsustaseme ning suitsidaalsusega.
4. Immuunsüsteemi vastus, mõõdetuna sIL-2R produktsioonis, erines estsitalopraamravi resistentsete ja ravile reageerinud patsientide vahel. sIL-2R väärtused näitasid raviresistentsetel isikutel langustendentsi ajaliselt hiljem. IL-8 ja TNF-α osas erinevusi esile ei tulnud.

5. Estsitalopraamravi augmenteerimine bupropiooniga põhjustas olulise IL-8 produktsiooni tõusu 6 nädalase perioodi vältel. Ravi ei mõjutanud TNF-α ja sIL-2R taset.
6. Estsitalopraamravile reageerinud patsientidel esinesid madalamad TNF-α ravieelsed väärtused võrreldes raviresistentsete haigete ja tervete kontroll-isikutega. Oluline oli siin ka sooefekt: meestel, kes ravile ei reageerinud, esinesid kõrgemad TNF-α ravieelsed väärtused võrreldes ravile reageerinud meestega, samuti nii raviresistentsete kui ka ravile reageerinud naistega.
7. Raviresistentsete naiste hulgas oli enam anti-TPO positiivseid isikuid kui ravile reageerinud naiste hulgas. TSH, T3 ja T4 ravieelne tase ei seostunud raviefekti ega ka depressiooni parameetritega.

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My research has primarily focused on the changes in the immune system in mood disorders. I have participated in a number of studies on the genetics of mental disorders and in research into sleep habits in medical students.

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Täiendus

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Teadustöö

Teadustöö on keskendunud peamiselt immuunsüsteemi iseärasustele meeleoluhäirete korral. Olen osalenud ka mõnes psühhiaatrilise geneetika valdkonna uurimuses, samuti meditsiinitudengite uneharjumusi käsitlevas töös.

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List of Publications

1. **Eller T**, Aluoja A, Maron E, Vasar V. Soluble interleukin 2 receptor and tumour necrosis factor in depressed patients in Estonia. Medicina, 2009 (accepted).
2. **Eller T**, Aluoja A, Vasar V, Veldi M. Symptoms of anxiety and depression in Estonian medical students with sleep problems. Depress Anxiety. 2006; 23 (4): 250–6.
3. **Eller T**, Metsküla K, Talja I, Maron E, Uibo R, Vasar V. Thyroid autoimmunity and treatment response to escitalopram in major depression (submitted to Nord J Psychiatry, 2008).
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