Serotonergic Dysfunction in Major Depressive Disorder: A role for pro-inflammatory cytokines

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List of Abbreviations

MDD	Major Depressive Disorder
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders IV
SSRi	Selective Serotonin Reuptake inhibitor
SNRi	Selective noradrenergic reuptake inhibitor
MAOi	monoamine oxidase inhibitor
MRI	Magnetic Resonance Imaging
SERT	Serotonin Transporter
IL	Interleukin
TNF-α	Tumor Necrosis Factor - alpha
Th	T-helper cell
5-HT	5-Hydroxytryptamine
CNS	Central Nervous System
TPH	Tryptophan Hydroxylase
5-HTP	5-Hydroxytryptophan
5-HIAA	5-Hydroxyindoleacetic acid
CSF	Cerebrospinal fluid
BDNF	Brain-Derived Neurotrophic Factor
VEGF	Vascular Endothelial Growth Factor
VGF	VGF nerve growth factor inducible
BPAP	benzofuranylpropylaminopentane
NGF	Nerve Growth Factor
Cdk	Cycline dependent kinase
BrdU	Bromodeoxyuridine
SERT	Serotonin Transporter
PFC	Prefrontal Cortex
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
BBB	Blood-Brain Barrier
IL-6R	Interleukin-6 Receptor
sIL-6R	Soluble Interleukin-6 Receptor
nf-κB	Nuclear factor-kappa B
NIK	nf-κB Inducing Kinase
TRAF6	TNF-receptor Associated Family 6
TRADD	TNFR-associated-death-domain protein
FADD	Fas-associated-death-domain protein
RIP-1	Receptor interacting protein-1
cIAP	cellular Inhibitor of Apoptosis Protein
JAK	Janus-protein tyrosine kinase
IRS	Insulin Receptor Substrate
IFNAR	Interferon alpha receptor
IFNGR	Interferon gamma receptor
ROS	Reactive oxygen Species

1. Introduction to depression

Major Depressive Disorder (MDD) is a mood-disorder characterized by lowered self-esteem, anhedonia, fatigue, appetite and sleep disturbances, a general thought of worthlessness, suicidal ideas and a general lack of interest in pleasure[1]. In order to be diagnosed with MDD a depressive episode, with at least 5 of these symptoms, should last for at least 2 weeks[1]. The estimated 12-month prevalence of MDD according to the fourth Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria[2] is 6,6%[1] and lifetime prevalence is 16,2%[1]. Because of the mood lowering nature of depression, patients often perform worse at work, retain from participating in social activities and interact less with friends and family members.

Depression is also a major societal burden financially. It was estimated to have cost America $83.1 billion[3] in 2000 and Europe an estimated €118 billion[4].These cost consist out of health-care costs but also lowered productivity, thus both direct and indirect costs to society. This makes depression one of the most burdening mental disorders on society[5].

Due to the high cost and prevalence of depression, therapies were needed. Perhaps the most known antidepressant prescribed today is fluoxetine or Prozac. Fluoxetine is a selective serotonin reuptake inhibitor (SSRi), thus an indirect activator of the serotonergic neurotransmitter system. Other therapies include selective noradrenergic reuptake inhibitors (SNRi) or monoamine oxidase inhibitors (MAOi), which inhibits MAO, an enzyme that metabolizes serotonin and noradrenaline, diminishing their effects.

This provides evidence for the importance of the monoaminergic brain systems, the immune system and the hippocampus in depression. Indeed, Magnetic Resonance Imaging (MRI) studies have shown decreased hippocampal volumes in depressed patients[6,7] which predicted duration of depression, not age of patients, in women[8]. Depressed patients were also shown to have lowered serotonin urinary excretion[9], reduced serotonin transporter (SERT) density[10], indicating a role of altered serotonin activity in depression.

The immune system is also a candidate cause for depression: Depressed patients show an increase in the pro-inflammatory cytokines Interleukin-6 (IL-6)[11,12], Tumor Necrosis Factor - alpha(TNF-α)[12,13], IL-1β[14] and a decrease in anti-inflammatory cytokines IL-10[14] and IL-4[15]. Another immunological factor that has been implicated to play a role in depression is the T-helper cell 1(Th1) and T-helper cell 2(Th2) balance. An imbalance in Th1/Th2 cell levels in favor of Th1-cells results in heightened pro-inflammatory cytokine excretion. Research has indicated, via cytokine profiling, that such an imbalance is present in depressed patients[14,15].

Lipopolysaccharide is an endotoxin of bacteria that, when introduced in an organism, induces inflammatory responses and is used in research as such. Both Fluoxetine[16] and Imipramine[17], a serotonergic and noradrenergic reuptake inhibitor, protect the hippocampus from Lipopolysaccharide-induced apoptosis, indicating an effect on the immune response as well.

Thus, research has provided evidence for a role of the monoaminergic neurotransmitter systems and a role for the immune response in the pathophysiology of depression. This paper will discuss the possible link between the immune system and the serotonergic neurotransmitter system, focusing on its implications in hippocampal dysfunction in depression.

2. The serotonergic neurotransmitter system

2.1 Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT) is a neurotransmitter used by serotonergic neurons. It is a metabolite of tryptophan, an essential amino acid. In the central nervous system (CNS), tryptophan hydroxylase 2 (TPH2) metabolizes tryptophan into 5-Hydroxytryptophan (5-HTP) in contrary to the peripheral system, where TPH1 metabolizes tryptophan. The step from Tryptophan to 5-HTP is the rate-limiting step in 5-HT production, which is then decarboxylated into 5-HT by the enzyme aromatic amino acid decarboxylase[18]. This process is shown in figure 1. TPH2 is primarily expressed in the raphe nuclei, a group of serotonergic neurons[19]. 5-HT is released into the synaptic cleft between presynaptic and postsynaptic neurons where it exerts its effect on different 5-HT receptors. MAO metabolizes serotonin into 5-Hydroxyindolacetaldehy which is then metabolized into 5-Hydroxyindoleacetic acid (5HIAA)[18]. 5HIAA can serve as a biomarker for serotonin when measured in the cerebrospinal fluid (CSF). Research has shown that suicidal depressed patients show lowered 5-HIAA levels in the CSF[20].

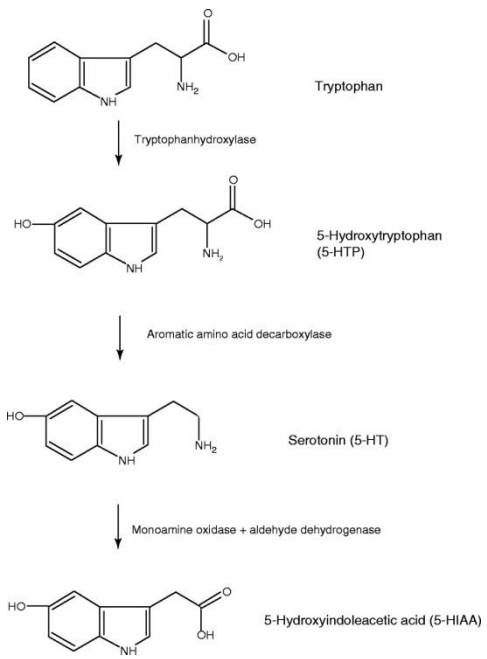

Figure 1. The serotonin metabolism: TPH2 metabolizes Tryptophan, creating 5-HTP, which is then decarboxylated into 5-HT by aromatic amino acid decarboxylase. 5-HT gets catabolized by MAO and aldehyde dehydrogenase into 5-HIAA[21].

2.2 Raphe Nuclei

The raphe nuclei are a group of serotonergic neurons located in the brainstem which project mostly to the forebrain and the limbic structures. More specifically the raphe nuclei innervate the frontal cortex, amygdala, hippocampus, entorhinal cortex and anterior hypothalamus of the limbic system[22]. The raphe nuclei consist out of three areas: The caudal linear, dorsal and medial raphe nuclei[23]. Both the dorsal and medial raphe nuclei innervate the hippocampus in rats[24]. As stated before, the neurons located in the raphe nuclei primarily express TPH2, thus limiting the rate of serotonergic activity[19].

2.3 Neuroplasticity

The serotonergic neurotransmitter system is among the first systems to innervate the mammalian forebrain[25,26] indicating a role for serotonin in the early development of the mammalian brain. Indeed, delaying serotonergic fiber ingrowth has negative effects on the dendritic elongation and spine appearance[27] and synaptogenesis[28]. Research utilizing glial cell-cultures also indicates differentiating effects of serotonin on its non-neuronal targets[29]. Thus, serotonin plays a large role in neuronal and non-neuronal development. Serotonin also shows similar developmental effects in the mature brain: Removal of serotonin decreases MAP-2 and synaptophysin in rats[30], markers for dendrites and synapses. Removal of serotonin also decreases S-100β levels[31], a glial trophic factor excreted by astrocytes which is important for neuronal branching patterns in the mature brain[32]. Due to the large serotonergic innervation in the forebrain and the limbic system and the direct effects on neuronal maturation, this indicates that serotonin and the lack thereof play a large role in the structural and functional characteristics of the brain. However, S-100β is not the only trophic factor increased by serotonin. Fluoxetine, a SSRi, treatment also increases Brain-Derived Neurotrophic Factor (BDNF), Vascular Endothelial Growth Factor (VEGF) and VGF nerve growth factor inducible (VGF) in mice[33], and serotonergic activity enhancer benzofuranylpropylaminopentane (BPAP) increases Nerve Growth Factor (NGF) and BDNF[34]. These neurotrophic factors are significantly reduced in depression (BDNF[35,36], NGF[36]). Findings also indicate that serotonin deficiency has a direct effect on adult hippocampal neurogenesis in the dentate gyrus: Serotonin deficient rats show decreased bromodeoxyuridine (BrdU) labeled cells, a marker for cell-division and thus neurogenesis, thus indicating lowered neurogenesis[37]. Antidepressants (imipramine, fluoxetine and desipramine) also stimulate hippocampal neurogenesis, possibly via inhibiting cycline dependent kinase (Cdk) inhibitor p21 (p21), an inhibitor of cellular proliferation[38], however this may be independent of serotonin.

2.4 5-HT1a Autoreceptor

Tryptophan is metabolized into Serotonin by TPH1/2 enzymes, moved to the synaptic membrane via vesicles and released into the synaptic cleft. Serotonin Transporter (SERT), the main SSRI target, reuptakes serotonin after which it is metabolized by MAO. While in the synaptic cleft serotonin may bind to several G-coupled protein receptors: The 5-HTRs. The 5-HT receptor system is among the most complex systems: The 5-HT1a receptor is expressed both post- and presynapticly, as shown in Figure 2, with the presynaptic autoreceptor inhibiting firing rates of neurons. Indeed, 5-HT1a agonists have shown to decrease neuronal activity in the dorsal raphe nuclei[39], hippocampus[40] and prefrontal cortex (PFC)[41], showing antagonistic effects with 5-HT2 receptors: 5-HT2 receptors excite whilst 5-HT1a autoreceptors inhibit serotonergic activity.
5-HT1a receptors are negatively coupled too adenylyl cyclase in contrary to other 5-HT receptors[42]. Autoreceptors inhibit neuronal activity via inhibiting the cAMP-dependent activating pathway (shown in Figure 3). 5-HT1a receptors also further inhibit neuronal activity by hyperpolarizing cells

directly. 5-HT1a agonists activate Ca^{2+}-gated K^{+}-channels[43].

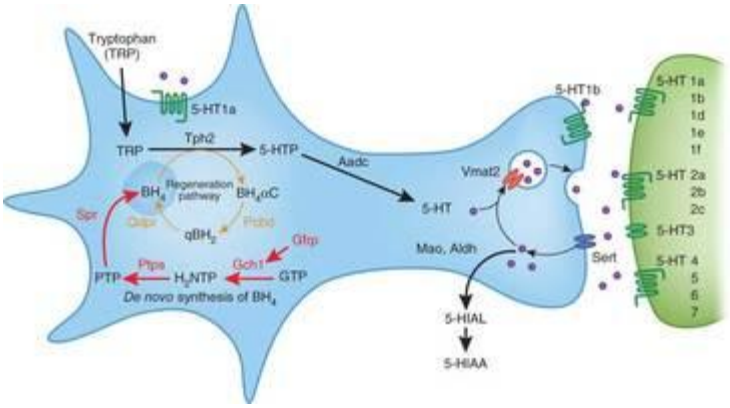

Figure 2. The pre- and post-synaptic serotonergic neuron and the distribution of 5-HT receptors. The 5-HT1a receptor is expressed both pre- and post-synaptically[44].

Increased 5-HT1a autoreceptor levels in mice results in decreased 5-HT neuron firing, 5-HT release and an increase in depressive-like behavior[45]. Consequently, clinically depressed patients show decreased post-mortem postsynaptic 5-HT1a receptors in PFC tissue[46]. Conversely, post-mortem studies show increased 5-HT1a autoreceptor levels in raphe tissue[47]. These findings are in line with the 5-HT deficiency hypotheses due to the autoinhibitory function of 5-HT1a autoreceptors.

5-HT1a receptors play a large role in antidepressive drug treatment function. SSRIs show a delayed onset of effectiveness, as shown in Figure 4. They increase serotonergic activity by blocking reuptake, thus inhibiting neuronal activity due to the activation of the 5-HT1a autoreceptor. SSRIs treatment, however, desensitizes the 5-HT1a autoreceptor whilst not desensitizing the post-synaptic receptors[48,49]. This indicates a large role for 5-HT1a activation in antidepressant drug function and a possible drug target for future drugs.

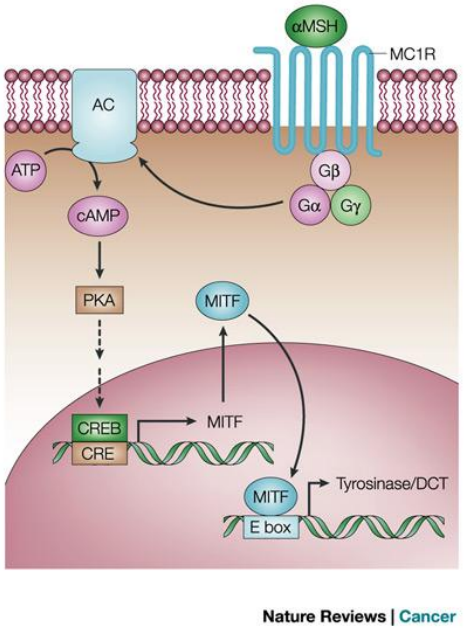

Figure 3. The cAMP-dependent pathway. Activation of PKA by cAMP induces CREB-regulated transcription factors increasing cellular activity[50].

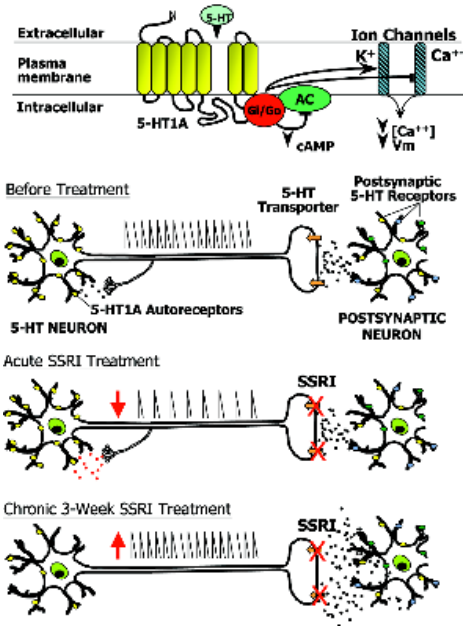

Figure 4. Effects of SSRI treatment on the firing rates of serotonergic neurons, showing the delayed onset of antidepressive treatments[51].

2.5 Kynurenine Pathway

The availabity of tryptophan is one of the rate-limiting factors of serotonin turnover. Thus, the usage of tryptophan in different pathways lowers the amount of tryptophan available for serotonin production, thus lowering serotonergic activity. The kynurenine pathway, shown in figure 5, is such a pathway. Indoleamine 2,3-dioxygenase (IDO) metabolizes tryptophan into kynurenine[52]. Kynurenine itself is an inactive metabolite of tryptophan. Kynurenine is further metabolized by microglia or astrocytes, creating different end-products. Astrocytes create kynurenic acid via kynurenine aminotranferases[53]. Kynurenic acid is neuroprotective, possibly because of the antagonistic properties on the NMDA receptor hindering NMDA induced neurodegeneration.

Conversely, microglia produce 3-hydroxykynurenine,3-hydroxyanthranilic acid and ultimately quinolinic acid, all of which produce oxygen free radicals. Quinolinic acid is also an NMDA receptor agonist, which also induces neurodegenerative damage[54]. Indeed, quinolinic acid exerts neurodegenerative effects on brain tissue via calcium extrusion[55].

Thus, the kynurenine pathway does not only limit the amount of tryptophan for the production of serotonin, it can also produce neurotoxic substances.

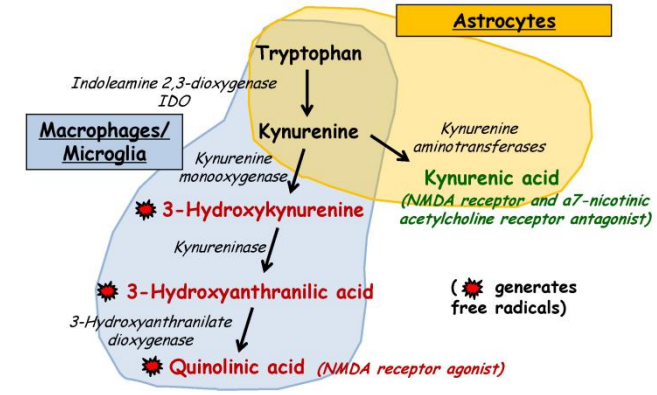

Figure 5. The differential pathways of the kynurenine metabolism, showing degredation by microglia and astrocytes[56].

3. Immunodysfunction

3.1 Cytokines

Cytokines play a large role in mediating regular immune function and are invaluable to our well-being. Yet, they can play a large role in the pathophysiology of neurodegenerative diseases as well. Cytokines serve as propagators and mediators and inhibitors of the immune response, indicating a diverse role in pathophysiology. There are both pro-inflammatory cytokines – such as IL-1β, IL-6, TNF-α, Interferons: IFN-α, IFN-γ – and anti-inflammatory cytokines – such as IL-4, IL-10 – either propagating or inhibiting immune response. These cytokines are produced and secreted by astocytes[57] and microglia[58] in the CNS. However, $CD4^+$ and $CD8^+$ Lymphocytes can also be recruited into the CNS possibly via active recruitment over the Blood-Brain Barrier (BBB)[59]. Seizure induction, a non-pharmacological treatment for depression, also results in recruitment of leukocytes[60] . The recruitment of lymphocytes into the CNS is also possible, via a CXCR3-dependent pathway[61]. CXCR3 is most pronounced in the recruitment of Th1-cells via IFN-γ inducible ligands[62]. This pathway can induce a feed-forward loop via the secretion of Th1-attracting ligands.

3.2 Interleukins

Interleukins are a family of both pro- and anti-inflammatory ligands. The most pronounced Interleukins in depression are the pro-inflammatory IL-1β and IL-6 and the anti-inflammatory IL-4 and IL-10.

IL-6 exerts its actions via binding of the IL-6 Receptor (IL-6R) and two gp130 signal-transducing receptor subunits. This creates the IL6-IL6R-gp130 complex[63]. The IL-6R is expressed on hepatocytes, neutrophils and macrophages[64]. However, gp130 is expressed is most of the human cells. However, cells that do not express the IL-6R can still be responsive to IL-6 production. In contrary to other cytokines, soluble IL-6R (sIL-6R) agonizes the IL-6R and binds to the gp130 complex inducing the same response as IL-6R activation[65]. Using sIL-6R from other cells, IL-6R deficient cells still bind too and activate the gp130 complex[66]. Activation of the IL-6R/IL-6-gp130 complex induces leukocyte infiltration[67], and prohibits T-cell apoptosis[68]. Strikingly, the suppression of apoptosis seems to be sIL6-R dependent. Blockade of the sIL-6R via sgp130Fc prevents IL-6 induced suppression of apoptosis[69].

Il-1β is synthesized from pro-IL-β under influence of caspase-1 activity of inflammasomes[70], protein complexes that play a role in the activation of inflammatory cytokines. However, this is not the only pathway via which IL-1β is produced, as caspase-1 deficient mice still produce IL-1β-dependent inflammatory responses[71]. IL-1β binds to the Interleukin-1 Receptor (IL-1R). Activation of the IL-1R induces nuclear factor-kappa B (nf-κB) via the pathway described in Figure 6. The most important step is the degradation of IκB via IKK-α and IKK-β[72]. This degradation of IκB leads to the release of nf-κB. IKK is regulated by nf-κB Inducing Kinase (NIK)[73], which in term is regulated by IRAK and TNF-receptor Associated Factor 6 (TRAF6)[74]. TRAF6 does not interact with TNF-α but is essential in the signaling transduction of the IL-1R induced nf-kβ response[75].The nf-κB –pathway induces the production of pro-inflammatory cytokines. Nf-κB regulates the pro-inflammatory cytokines IL-6, IL-2 and TNF-α[76,77].

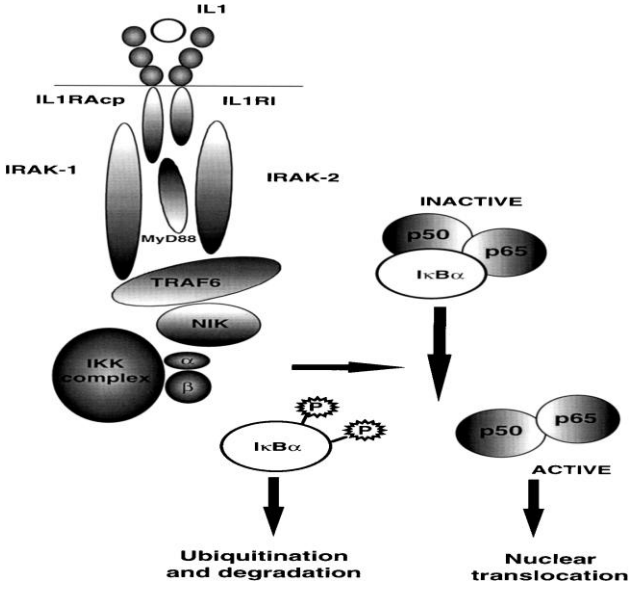

Figure 6. The IL-1R-pathway inducing activation of nf-κB via IRAK-TRAF6-NIK dependent activation of IKK which subsequently degradates IκB into nf-κB[78].

Interleukin-4 is produced by T-helper 2 cells and binds to the IL-4Rα[79]. IL-4 stimulates other cells into differentiating into T-helper 2 like cells, producing IL-4 and IL-10 as well as other cytokines[80]. IL-4 seems to play a role in the protection against autoimmunity, as demonstrated by clinical studies[81].

The IL-4 receptor is expressed in hematopoietic cells, endothelial, muscle, hepatocyte and brain tissue cells[82]. Binding of IL-4 to the IL-4Rα chain of the receptor leads to recognition by the γc-chain, resulting in heterodimerization and cell-signaling[83]. In γc-deprived cells, the IL-13R' chain can also serve as a heterodimer for IL-4Rα, thus replacing the γc-chain[84]. The IL-4Rα- γc heterodimer does not possess kinase activity. Thus, it recruits the Janus protein tyrosine kinase (JAK) family proteins, jak-1

jak-2 and jak-3[85]. Il-4 induces cell-proliferation via an Insulin Receptor Substrate (IRS) dependent pathway, shown in Figure 7. Using 32D cell lines, cell lines that do not possess detectable levels of IRS-1/IRS-2, the importance of IRS-1 and IRS-2 have been demonstrated. Cells lacking these proteins did not proliferate under the effects of IL-4[86,87]. Phosporylated IRS1/2 activates the phosphoinositide-3-kinase (PI-3-K) complex[88,89] and activates its catalytic subunit p110[89]. This p110 subunit produces phosphoinositides[90]: second messengers that activate Protein Kinase C (PKC) and Protein kinase B (Akt), proteins that play a large role in cell survival[91,92].

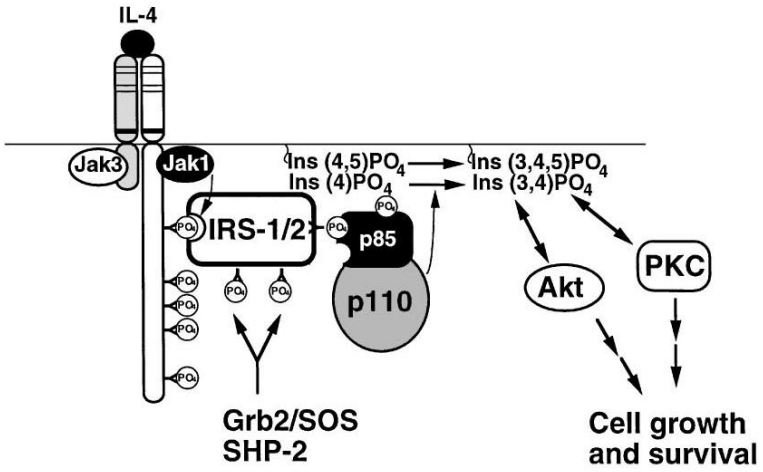

Figure 7.The IRS-1/2 dependent PI-3-K PKB/PKC pathway induced by IL-4R ligand binding[93].

Interleukin-10 is a suppressor of the inflammatory response via indirectly suppressing T-cell activity[94]. IL-10 works via repressing antigen-presenting abilities of macrophages[95] and dendritic cells and via inhibiting pro-inflammatory cytokine production[94]. IL-10 is produced by T-cells and macrophages and is essential for the regulation of chronic inflammation. Mice deficient of IL-10 lack this regulation and are susceptible to inflammatory illnesses[96]. In fact, IL-10 deficient mice succumb to an infection challenge. This is, however, not due to rapid growth of the pathogen but due to the immune response produced[97], notably the overproduction of TNF-α and IFN-γ. When not acutely challenged, IL-10 deficient mice develop chronic inflammatory syndromes such as chronic intestinal inflammation[98]. Binding of IL-10 to the IL-10R recruits the Jak-1 protein[99]. Jak-1 phosphorylates two tyrosine residues of the IL-10R which recruits STAT3[100] and induces the anti-inflammatory response. IL-10 inhibits the expression of IL-1β, IL-6, TNF-α and other cytokines/chemokines[101,102,103], thus strongly inhibiting the pro-inflammatory response.

3.3 TNF-α

TNF-α was first discovered as a protein that induces haemorrhagic necrosis in transplanted sarcomas in mice in 1975[104]. However, TNF-α also plays a pivotal role in the immune system. There are two receptors for TNF-α: TNF-R1[105] and TNF-R2[106]. Binding of TNF-α to the TNF-R1 recruits the TNFR-associated-death-domain protein (TRADD)[107]. TRADD can induce two different pathways: The Fas-

associated death domain (FADD) dependent, cell-death signaling pathway and the Receptor intering protein-1 (RIP-1) dependent cell-survival pathway.

FADD can bind to the death domain of TRADD creating the TRADD-FADD complex[108]. The TRADD-FADD complex recruits pro-caspase 8, which in term cleaves and activates pro-caspase 3, inducing apoptosis.

RIP-1 can also bind to the death-domain of TRADD[109]. This creates the TRADD-RIP-1 Complex. This complex binds TRAF-2[110]. The TRADD-RIP-1-TRAF-2 complex recruits MEKK-3[111] and TAK1[112], which leads to phosphorylation and ubiquination of IκB and formation of nf-κB. The TRADD-RIP-1-TRAF-2 complex also recruits inhibitor of cellular apoptosis proteins (cIAPs)[113] cIAP-1 and cIAP-2. cIAPs also have ubiquiting ligase protein activity and participate in the degradation of IκB[113]. nf-κB induced cFlip expression also inhibits the FADD-dependent apoptotic pathway[115].

The TNF-R1 response can also induce cell-death signaling pathways. Fas-associated death domain protein (FADD) can bind to the death domain of TRADD creating the TRADD-FADD complex[113]. The TRADD-FADD complex recruits pro-caspase 8, which in term cleaves and activates pro-caspase 3, inducing apoptosis. This pro-caspase dependent process can be inhibited by nf-κB induced cFlip expression[114].

In contrary to the widely expressed TNF-R1, the TNF-R2 expression is limited to a specific subset of cells[115]: $CD4^+$ and $CD8^+$ lymphocytes, endothelial cells, microglia, specific neurons – most notably in the hippocampus[116] – and oligodendrocytes. The TNF-R2 does not have a death domain[117]. The TNF-R2 recruits TRAF-1[118] and TRAF-2[119] which both lead to nf-κB activation via IKK-dependent pathways. As seen in Figure 8, there is a large amount of overlap in the functions of TNF-R1 and TNF-R2. Though generally, TNF-R1 activation leads to apoptosis and TNF-R2 activation leads to proliferation[115], both receptors can exert both proliferative and pro-apoptotic effects[120]. Both receptors can induce nf-κB-

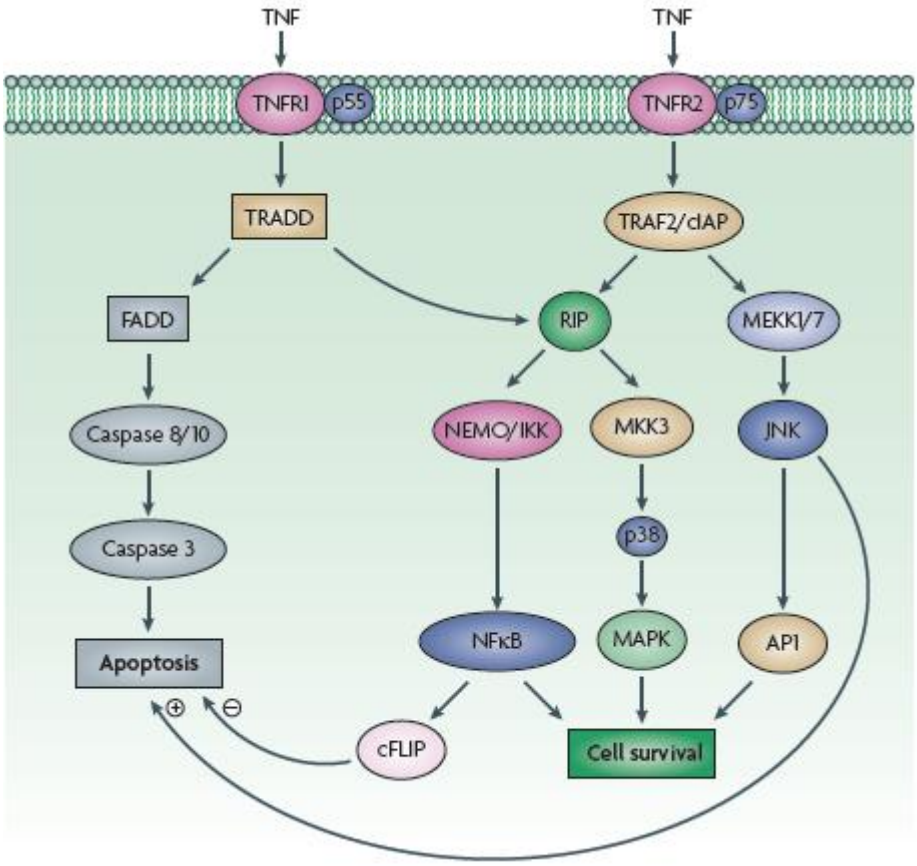

Figure 8. The TNF-R1 and TNF-R2 pathways showing cross-talk between the two different receptors types[115].

dependent cell-proliferation. This, however, seems to be largely dependent on the type of cell and situation. Therefor more research is required.

3.4 Interferons

Interferons are pleiotropic cytokines that activate different cells of the immunesystem. There two types of Interferons: Type-1 interferons (IFNα, β and IFNω) and Type-2 interferons (IFNγ). Both types of interferons share common effects: Inhibition of growth and immunomodulation[121].

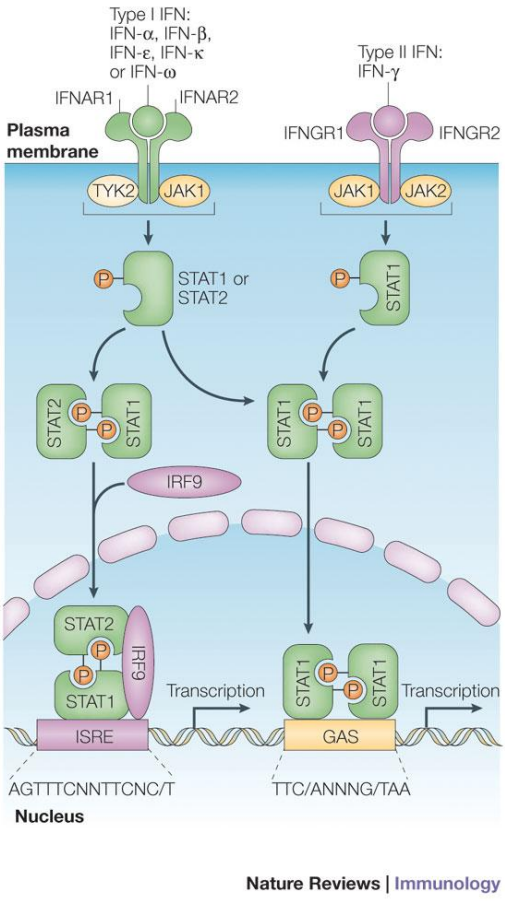

Figure 9. The Jak-Stat dependent pathways of both the IFNAR and the IFNGR[123].

Type-1 interferons exert their effects on the shared Interferon alpha receptor (IFNAR), whilst IFNγ exerts its effect via the interferon gamma receptor (IFNGR). The IFNAR is composed out of two subunits: IFNAR1 and IFNAR2[122]. Binding of the IFNAR-ligand creates a heterodimer of the two receptors which subsequently recruit the Jak1 and Tyk2 proteins[123]. The Jak1 and Tyk2 proteins phosphorylate the IFNAR1 and IFNAR2 receptor subunits which recruit the STAT-enzyme[124] creating STAT1-STAT2 heterodimers. This heterodimer induces a signaling cascade, regulating the gene expression of IFN-regulated genes. This process is shown in Figure 9. Type-1 interferons also regulate the IRS1/2-PI3K[125] dependent nf-κB pathway[126], via the activation of Akt and PKC, in primary astrocytes[127], neutrophils[128], B-lymphocytes[129], reducing apoptosis and promoting survival in these cells.

Interestingly, type-1 IFNs can also induce apoptosis via the phosphorylation of p38 via activation of the MAPK kinase cascade[130]. This cascade activates the effector proteins: MSK1/2[131], which are important for the suppression of hematopoietic cells[132].

The IFNGR is also a heterodimeric receptor, consisting out of the IFNGR1 and IFNGR2 subunits. The IFNGR also activates the JAK family proteins[85] Jak1 and Jak2[133] which activate STAT1[134], creating a heterodimer via which IFN-γ induces effects on gene expression. Similar to type-1 interferons is the activation of the PI3K pathway by IFNγ[135]. IFNγ, however, does not induce IRS1/2 phosphorylation[136] indicating a possible role for other, until now, unknown phosphoproteins. This pathway regulates PKC and Akt activity indicating a role in the regulation of cell proliferation[137].

3.5 Activation of Microglia & Lymphocytes

Microglia and T&B Lymphocytes are among the effector cells of the immune system that are active in the CNS and they play a major role in the pathophysiology of neurodegenerative and autoimmune diseases.

Microglia are involved in the removal of degenerating neurons. Microglia are activated by various pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β[138]. When activated microglia change morphologically[139] and their expression pattern of membrane receptors alters[140]. Microglia are capable of producing an array of pro-inflammatory cytokines: IL-1β, IL-6, TNF-α and many others[141,142,143], which aid in creating an inflammatory loop. Activated microglia can be neurotoxic. The mechanisms of microglia neurotoxicity are shown in Figure 10. Activated microglia produce intracellular reactive oxygen species (ROS) [144] which regulate the expression of the pro-inflammatory factors excreted by microglia[145]. The activation of NADPH oxidase, via the production of the superoxide anion (O_2^{-*}) further enhance the expression of cytokines[146].

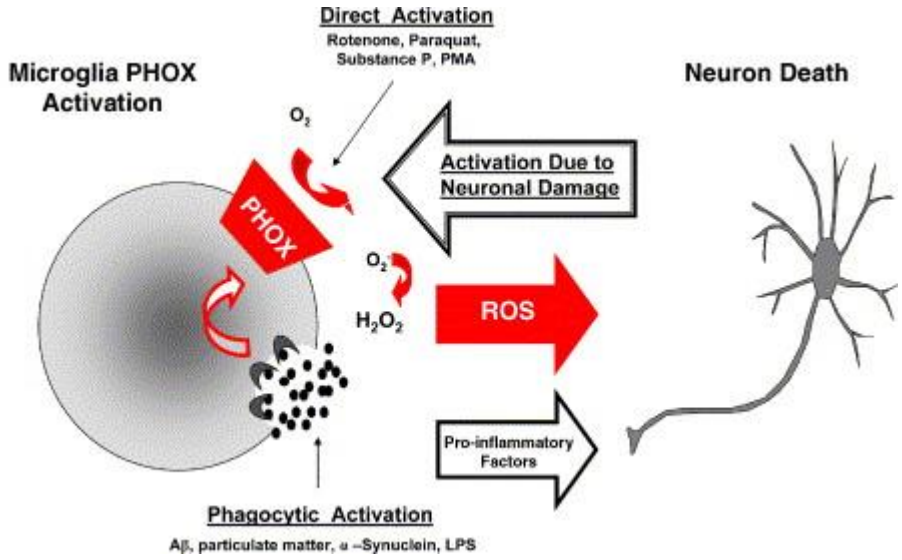

Figure 10. Activation of microglia induces neuronal death via ROS and pro-inflammatory cytokine production[138].

Lymphocytes play a great part in the regulation of the immune response in the central nervous system. Especially T-lymphocytes play an important, direct, role in the regulation of the immune response. Different types of T-cells secrete different kinds of cytokines. In normal homeostasis there is a balance within different types of T-cells, such as Th1/Th2 cells. However, a disbalance of these T-helper cell subtypes is implicated in the pathophysiology of depression[16,17]. Th1 cells primarily produce cytokines that promote a cellular immune response such as TNF-α and IFN-γ whilst Th2 cells produce cytokines that inhibit the cellular immune response and promote the humoral immune response, such as IL-4 and IL-10[147]. Thus, Th1 cells secrete cytokines that are neurodegenerative whilst Th2 cells secrete cytokines that are neuroprotective. Due to the observed disbalance in not only MDD, but also other neurodegenerative diseases, this implicates that T-helper cell disbalance is a key player in the pathophysiology and possible pathogenesis of depression.

Another major T-lymphocyte important for autoimmunity and disease is the Th17 cell, IL-17 secreting T cells. IL-6 induces Th17 cell differentiation and inhibits differentiation of Treg cells, inhibitors of the immune response[148]. Th17 cells propagate the pro-inflammatory immune response via production of IL-6 and TNF-α. Thus this is another part of the immune response that is self-propelling when disregulated.

Concluding, the activation of the cellular immune response is upregulated in depression, producing neurodegenerative pro-inflammatory cytokines that also influence the cellular immune response themselves. This leads to the overactivation of the immune system in a self-propelling manner,

leading to all sorts of tissue damage and direct neuronal death. However, it's possible that the production of cytokines and activation of immune cells also have different indirect effects on the different neurotransmitter systems implicated in depression, like serotonin, as the pathophysiology includes serotonergic dysfunction, hippocampal dysfunction and immunodysfunction. The question is: How much are these three processes related.

4. The influence of the immune system on serotonergic dysfunction

Due to the neurodegenerative nature of the over stimulated immune system in depression, its plausible that the immune cells attack serotonergic and hippocampal cells, thus inducing apoptosis. However cytokines produced by these cells can also have more direct effects on the serotonergic neurotransmission, possibly even causing the lowered serotonin transmission as seen in MDD. Figure 11 summarizes the effects of cytokines on the serotonergic neurotransmitter system.

Evidence for the proposed link is based on the therapies given to treat depression. Mostly these are SSRIs, however these SSRIs also have anti-inflammatory properties in addition to their serotonin raising properties[6,7]. Another piece of evidence is the induction of 'sickness-behaviour', depressive like behaviour in animal models by pro-inflammatory cytokines IFN-γ, TNF-α, IL-6, IL-1β[149,150]. Sickness behaviour is defined as animals showing less activity, in for example a forced-swim test, less exploration behaviour and less interest in pleasurable stimuli, such as food. One of the ways this sickness behaviour may be induced is by lowering serotonin levels in the brain. IFN-γ, TNF-α[149],IL-1β[151] and IL-6[150], have all been shown to affect the IDO-kynurenine pathway. These pro-inflammatory cytokine upregulate the expression of IDO, the propagator of the kynurenine pathway. IDO produces kynurenine from tryptophan, the essential amino acid crucial in the production of serotonin. Lowered amounts of tryptophan available for serotonin production result in lowered serotonin available for neurotransmission.

This is, however, not the only reason why the upregulation of IDO is detrimental and capable of inducing depressive-like symptoms. Microglia, also activated by pro-inflammatory enzymes[138], further metabolize kynurenine into 3-hydroxykynurenine and 3-hydroxyanthranilic acid, substances that produce ROS and thus further activating pro-inflammatory cytokine production from microglia, and ultimately quinolinic acid is produced. Quinolinic acid is a neurodegenerative substance, that damage cells via NMDA stimulation and calcium extrusion[54,55]. The production of kynurenine and Quinolinic acid represses hippocampal neurogenesis: IL-1β decreases the number of DCX-positive cells in the dentate gyrus by 28% and also decreases neuronal maturation, as measured by MAP-2 positive cells, of human progenitor cells by 36%[151]. Zunszain et al also measured the levels of IDO and kynurenine, which were increased by IL-1β. An kynurenine monooxygenase inhibitor,Ro 61-8048, abolished the effect of IL-1β on neurogenesis, indicating that the detrimental effects of IL-1β on neurogenesis are kynurenine-mediated. Since TNF-α, IFN-γ and IL-6 all activate the kynurenine pathway, they all are capable of decreasing neuronal maturation in the hippocampus and limit the tryptophan supply for serotonin production.

However, this pathway does not only affect neuronal progenitor cells, it also effects already mature cells in the dorsal raphe nucleus[152]. Indeed, activation of the kynurenine pathway, induced by IFN-γ or TNF-α in this experiment, induced neurodegeneration of the serotonergic neurons in the dorsal raphe nucleus, indicating widespread effects of the upregulation of IDO via pro-inflammatory cytokines.

Not only do pro-inflammatory cytokines decrease the maturation of hippocampal neuronal progenitor cells and survival of the serotonergic neurons in the dorsal raphe nucleus, they also directly affect the firing rate of serotonergic neurons in the dorsal raphe nucleus, however in different manners. IL-1β decreased serotonergic firing rates in the dorsal rat nucleus[153], as measured directly, whilst IL-6 increases serotonergic activity, as measured by extracellular 5-HIAA levels[154]. This may indicate that serotonin plays a role in the biological effects of IL-6, however this is not certain. And though IL-6 stimulates serotonergic neurotransmission in a direct manner, this may not be sufficient to protect neurons from other, negative, effects from IL-6 such as the propagation of the immune response and the induction of other, also neurotoxic, pro-inflammatory cytokines.

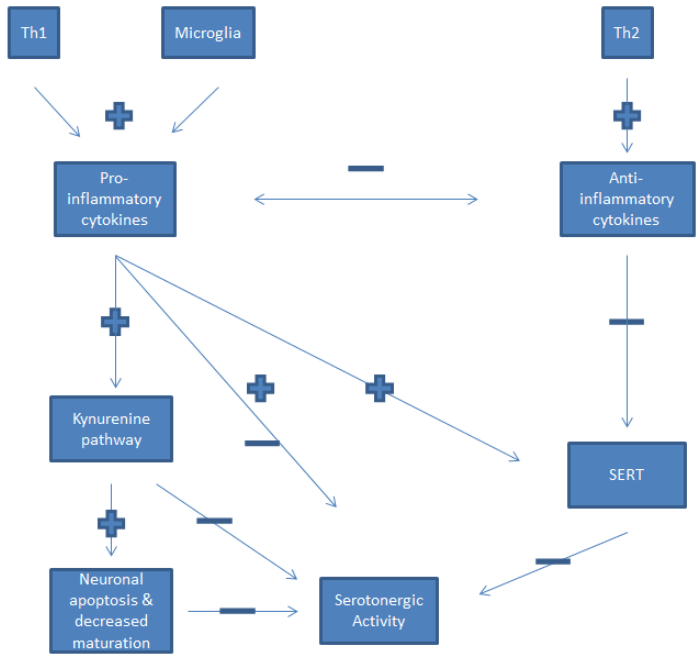

Figure 11. The effects of pro- and anti-inflammatory cytokine production on serotonergic neurotransmission, via direct, kynurenine and SERT pathways.

In addition to the effects of cytokines on neuronal survival, neuronal differentiation and maturation and direct effects on activity cytokines also further decrease serotonergic activity via the activation of the serotonin transporter, the protein that reuptakes serotonin after release. Indeed, pro-inflammatory cytokines such as IL-1β, TNF-α[155] and IFNα/γ[156] but again not IL-6[157] enhance serotonergic reuptake via SERT in a raphe cell line, RN45A[155], or a carcinoma cell line, JAR[157], decreasing the amount of time serotonin activates receptors in the synaptic cleft. Conversely, IL-4 decreases serotonin reuptake[158], increasing serotonergic neurotransmission. Since pro-inflammatory cytokines are upregulated in depression and anti-inflammatory cytokines such as IL-4 are downregulated in major depressive disorder, resulting in higher serotonin reuptake activity, is one of the processes involved in lowered serotonergic neurotransmission in MDD.

5. Discussion

As discussed above, the immune system plays a large role in serotonergic dysfunction in MDD. Pro-inflammatory cytokines lower serotonergic activity, as seen in MDD, via multiple processes: Lowering the availability of tryptophan for the production of serotonin via activating the kynurenine pathway whilst creating neurotoxic substances such as quinolinic acid via this same pathway; Via increasing serotonin reuptake, via inhibiting the firing rate of serotonergic neurons and via neurodegeneration of serotonergic neurons in the raphe nucleus. Thus pro-inflammatory cytokines have a whole array of effects on the serotonergic neurotransmitter system.

Anti-inflammatory cytokine IL-4 directly protects serotonergic function via decreasing serotonin reuptake, and inhibiting SERT. However, indirectly anti-inflammatory cytokines such as IL-4 and IL-10

protect the neurotransmitter systems and its neurons via inhibiting the pro-inflammatory cellular responses, decreasing the neurotoxicity of the immune response.

Thus, the upregulation of pro-inflammatory cytokines seen in MDD and the downregulation of anti-inflammatory cytokines have profound effects on the functioning of the serotonergic neurotransmitter system, either causing or mediating the symptoms of MDD.

The disbalance between pro- and anti-inflammatory cytokines is a feed-forward process: Pro-inflammatory cytokines stimulate the differentiation of T-lymphocytes into pro-inflammatory producing T-cells, such as Th1-cells, activate pro-inflammatory producing monocytes, microglia and other cells and inhibit the production of anti-inflammatory cytokines.

Treatments of depression are based on inactivating SERT and not on inhibiting the immune response. These treatments are also rather old and were discovered before the idea of inflammatory neurodegeneration became prominent in modern-day medicine.

However, these treatments have a 3 week onset time, possibly due to the receptor expression of 5-HT1a. This receptor, when stimulated, inhibits serotonergic neurotransmission. As it is upregulated in MDD, increasing serotonin levels without decreasing the 5-HT1a autoreceptor would only result in inhibition of neurotransmission. Thus, research should focus on the effectiveness of anti-inflammatory treatments or co-treating patients with SSRis and anti-inflammatory therapies. Due to the effects of the immune system on several parts of the serotonergic neurotransmitter system, this may prove to be a far quicker and more effective treatment of depression.

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