The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes

Wouter Beumer,†,1 Sinead M. Gibney,†,‡,1 Roosmarijn C. Drexhage,*, Lorena Pont-Lezica,§ Janine Doorduin,†, Hans C. Klein,† Johann Steiner,§ Thomas J. Connor,† Andrew Harkin,‡ Marjan A. Versnel,*, and Hemmo A. Drexhage*†

*Department of Immunology, Erasmus MC, Rotterdam, the Netherlands; †Institut de Biologie de l’Ecole Normale Supérieure, INSERM, Paris, France; ‡Department of Physiology and School of Medicine and §School of Pharmacy and Pharmaceutical Sciences, Trinity College Institute of Neuroscience, Trinity College, Dublin, Ireland; †Department of Psychiatry and Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; and ‡Department of Psychiatry, University of Magdeburg, Magdeburg, Germany

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ABSTRACT

This review describes a key role for mononuclear phagocytes in the pathogenesis of major psychiatric disorders. There is accumulating evidence for activation of microglia (histopathology and PET scans) and circulating monocytes (enhanced gene expression of immune genes, an overproduction of monocyte/macrophage-related cytokines) in patients with bipolar disorder, major depressive disorder, and schizophrenia. These data are strengthened by observations in animal models, such as the MIA models, the chronic stress models, and the NOD mouse model. In these animal models of depressive-, anxiety-, and schizophrenia-like behavior, similar activations of microglia and circulating monocytes can be found. These animal models also make in-depth pathogenic studies possible and show that microglia activation impacts neuronal development and function in brain areas congruent with the altered depressive and schizophrenia-like behaviors. J. Leukoc. Biol. 92: 959–975; 2012.

MACROPHAGE/T CELL THEORY OF DEPRESSION AND SCHIZOPHRENIA

Although reports of an involvement of the immune system in major mental illnesses exist since the beginning of the 20th century, it has taken nearly 100 years before detailed studies of this involvement became more numerous. These studies reported aberrant levels of proinflammatory cytokines in the serum, plasma, and cerebrospinal fluid of patients with schizophrenia and major mood disorders [1–4] and were reviewed recently by us [5]. On the basis of these reports, it was hypothesized that a proinflammatory state of the cytokine network induces psychopathologic symptoms and is involved in the pathogenesis and pathophysiology of these major mental illnesses.

Proinflammatory cytokines are produced primarily by activated cells of the immune system, such as activated endothelial cells, cells of the mononuclear phagocyte system, and T cells. The realization that such cells must be involved led to the macrophage/T cell theory of depression and schizophrenia, which was proposed in 1992 and adapted in 1995 [6, 7]. This theory states that chronically activated macrophages (and their counterparts in the brain, i.e., microglia) and T cells produce cytokines and inflammatory compounds impacting brain development and predisposing the brain in such way that genetic and environmental influences are able to precipitate the symptoms of schizophrenia and mania/depression.

In this review, we will focus on the evidence from current research on patients and animal models using state-of-the-art techniques for the activation of cells of the mononuclear phagocyte system, particularly microglia and circulating monocytes, in major psychiatric disorders.

NEUROENDOCRINE REGULATORY ROLE OF MICROGLIA

Microglial cells are the mononuclear phagocytes of the brain and are important for the cross-talk between the immune sys-
tem and, e.g., serotonergic and glutamatergic neurotransmission [8]. Animal studies have shown that microglia do not differentiate from circulating monocytes, as thought originally, but from primitive myeloid progenitors that emigrate from the yolk sac into the brain parenchyma [9–11]. Thus, microglia are present in the brain rudiment early during brain development (from E8 in the mouse) [10] and more importantly, participate in various aspects of brain development, including developmental cell death, axon remodeling, synaptogenesis, and synaptic pruning [12–15].

Microglia and programmed neuronal cell death

One of the best-known developmental functions of microglia is the phagocytosis of neurons undergoing programmed neuronal cell death (reviewed in refs. [13, 16, 17]. Microglia are found associated with neurons undergoing developmental cell death in various CNS regions, including the hippocampus [18, 19], the cerebellum [20], the retina [21–26], and the spinal cord [27–30]. In fact, in vivo imaging of the zebra fish embryo revealed that microglia engulf dying neurons with their processes [31]. In addition microglia direct cells to undergo programmed neuronal cell death via various pathways, including the production of NGF or the production of a respiratory burst in the retina [18, 20, 21], CD11b and DAP12 in the neonatal hippocampus [18], and TNF-α in the spinal cord [30].

Microglia and neuronal development/differentiation

The phagocytic capacity of microglia has also been observed in relation to axon remodeling and synaptic pruning. For example, in kittens and neonatal rats, microglia in the corpus callosum were observed engulfing nonmyelinated fibers during the known postnatal period of transitory axon elimination [32, 33]. Moreover, in the juvenile mouse, it was shown that microglia processes contact synaptic elements in the visual cortex and that this apposition is regulated by sensory experience [34]. Developing microglia express the complement receptor CR3, and it has been proposed that they eliminate unwanted synapses marked by complement protein C1q [35]. Evidence in support of this hypothesis comes from Paolicelli et al. [36], who found synaptic elements within the phagocytic compartments of microglia. In addition, a transient increase in synaptic spine density and immature synapses was observed in the hippocampus of mice with altered microglial function (CX3CR1-KO) [36]. It remains unclear whether these changes have functional consequences on hippocampal neurotransmission, as studies grouping these mice have obtained conflicting results [37, 38]. Microglia may also participate in synaptogenesis via the secretion of neurotrophic factors, such as thrombospondin [39], a family of ECM proteins [40].

Finally, a role for microglia in neuronal development is also suggested by in vitro work with microglia-conditioned medium and primary neuronal cultures. It was shown that microglia-conditioned medium enhances neuronal survival [41–44], increases neurite growth and complexity [42, 43], and in the case of catecholaminergic neurons, promotes neuronal maturation [41]. More generally, microglia secrete an array of chemokines, cytokines, giotransmitters, and neurotrophic factors that have been implicated in various aspects of neuronal function [45–48].

Microglia and neurogenesis

Microglia are present in the neurogenic niches of the embryonic and adult brains [49, 50], and their role has been found to be beneficial or detrimental depending on the paradigm (enriched environment, injury, inflammation) [51]. In support of a permissive role in neurogenesis, several in vitro studies carried out in noninflammatory conditions have shown that microglia, or microglia-conditioned medium, stimulate proliferation and differentiation of embryonic and adult neural progenitor/stem cells [50, 52, 53].

Adult mice with altered microglial function (CX3CR1 KO) were found to have impaired neurogenesis compared with WT [37, 54], also supporting the role for microglia in neurogenesis.

In the adult hippocampus, only a subset of the subgranular zone-generated neurons is integrated into the mature circuitry; the remaining apoptotic newborn neurons are eliminated by microglia [49]. These findings show the importance of the microglial phagocytic function within the neurogenic niche. Despite the in vitro and in vivo evidence for a functional role of microglia in neurogenesis, the signals governing these mechanisms remain to be uncovered.

INFLAMMATORY-ACTIVATED MICROGLIA IN PATIENTS WITH MAJOR PSYCHIATRIC DISORDERS

An abnormal inflammatory activation of microglia can be detrimental for neurogenesis and synaptogenesis by lack of provision of neuronal growth factors or by producing neurotoxic factors and cytokines [51]. In mutant mice in which microglia are in an activated state during prenatal development (CD200KO and DAP12KO mice), there are altered levels of glutamate receptors, resulting in impaired, long-term potentiation and hippocampal transmission [55–57]. In favor of a direct detrimental action of inflammatory cytokines on neuronal development, in vitro work has shown that cytokines, such as IL-6, TNF-α, and IL-18, can affect neuronal proliferation, survival, and aspects of differentiation-like neurite outgrowth and gene expression patterns [58, 59].

Histomorphological studies showing activated microglia in patients with psychiatric disorders

Although there are histomorphological signs of an abnormal inflammatory activation of microglia in postmortem studies on patients with major psychiatric disorders (Table 1), studies are limited and controversial.

A postmortem study on brains from schizophrenia patients, who had committed suicide during acute psychosis, revealed increased density of microglia [64]. Three other studies reported increased microglial activation in schizophrenia patients [61, 63, 68], whereas another three did not find an activation state of microglia [60, 62, 67]. A drawback of some of these postmortem studies is that they were performed on old
Reduced primarily by microglial cells, and is an endogenous product of the tryptophan pathway (see pathway, Fig. 1B), preserved in bipolar disorder. QUIN is a terminal breakdown product of the tryptophan pathway (see pathway, Fig. 1B), produced primarily by microglial cells, and is an endogenous modulator of the NMDA glutamate receptor. A key enzyme for the production of QUIN in the microglia is IDO, which is activated by proinflammatory cytokines, including IFN-α, IL-1, IL-6, and TNF-α.

The authors chose the subregions of the anterior cingulated cortex in the brains of suicide victims as regions of interest to study the QUIN+ microglia [66], as recent MRI studies observed a relationship between disturbances in glutamatergic neurotransmission in these brain areas with the severity of depression, including anhedonic features [70, 71]. The observed relative increase in QUIN in the sACC and aMCC of depressed patients compared with controls (particularly in MD but not in BD) Quinolinic acid immunoreactivity increased in microglial cells of sACC and aMCC of depressed patients compared with controls (particularly in MD but not in BD)

### TABLE 1. Summary of Histological Postmortem Studies on Microglia

<table>
<thead>
<tr>
<th>Study</th>
<th>Origin of brain samples</th>
<th>Analyzed brain regions</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnold et al. [60]</td>
<td>Laboratory for Cellular and Molecular Neuropathology, Department of Psychiatry, University of Pennsylvania (Philadelphia, PA, USA)</td>
<td>Hippocampus, prefrontal, orbitofrontal, and calcarine cortex</td>
<td>CD68 expression unchanged in SZ compared with controls</td>
</tr>
<tr>
<td>Bayer et al. [61]</td>
<td>Institute of Neuropathology, University of Bonn (Germany), and Neuropathological Laboratory, Institute for Nervous and Mental Diseases (Butlapest, Hungary)</td>
<td>Hippocampus and prefrontal cortex</td>
<td>“Stronger” HLA-DR expression (qualitative evaluation) in SZ and MD compared with controls</td>
</tr>
<tr>
<td>Falke et al. [62]</td>
<td>Laboratory for Cellular and Molecular Neuropathology, Department of Psychiatry, University of Pennsylvania</td>
<td>Mediodorsal thalamus and caudate nucleus</td>
<td>CD68 unchanged in SZ compared with controls</td>
</tr>
<tr>
<td>Radewicz et al. [63]</td>
<td>Department of Neurodegenerative Disorders and Division of Neuroscience, Charing Cross Hospital (London, UK), and State Psychiatric Hospital (Wiesloch, Germany)</td>
<td>Prefrontal, anterior cingulate, and temporal cortex</td>
<td>Increased HLA-DR expression in elderly patients with SZ compared with controls</td>
</tr>
<tr>
<td>Steiner et al. [64, 65]</td>
<td>Laboratory for Experimental Psychiatry, Department of Psychiatry, University of Magdeburg (Germany)</td>
<td>Hippocampus, mediodorsal thalamus, prefrontal, anterior cingulate cortex</td>
<td>HLA-DR unchanged in the whole group of SZ and depression cases compared with controls but increased in acutely ill suicidal patients</td>
</tr>
<tr>
<td>Steiner et al. [66]</td>
<td>Laboratory for Experimental Psychiatry, Department of Psychiatry, University of Magdeburg</td>
<td>Subregions of the anterior cingulate cortex (sACC, aMCC, pACC)</td>
<td>Quinolinic acid immunoreactivity increased in microglial cells of sACC and aMCC of depressed patients compared with controls (particularly in MD but not in BD)</td>
</tr>
<tr>
<td>Togo et al. [67]</td>
<td>Institute of Psychiatry (Tokyo, Japan), and Department of Psychiatry, University of Yokohama (Japan)</td>
<td>Hippocampus and temporal cortex</td>
<td>CD40 and HLA-DP/DQ/DR unchanged in SZ compared with controls</td>
</tr>
<tr>
<td>Wierzba-Bobrowicz et al. [68, 69]</td>
<td>Department of Neuropathology, Institute of Psychiatry and Neurology, University of Warsaw (Poland)</td>
<td>Anterior cingulate and temporal cortex</td>
<td>Stronger HLA-DP/DQ/DR expression (qualitative evaluation) in SZ compared with controls</td>
</tr>
</tbody>
</table>

SZ, Schizophrenia; MD, major depression; BD, bipolar disorder.

to very old individuals (after the process of dying) and might not reflect the pathophysiology of acute exacerbations, as they might when the patients were dying during a stable disease phase.

Thus far, only two histological studies have analyzed patients with affective disorders. A qualitative study of HLA-DR expression showed increased expression of this surface marker on microglia of the hippocampus and prefrontal cortex of depressed patients [61].

More recently, increases in the expression of QUIN have been identified in ramified microglia in subregions of the anterior cingulate cortex of severely depressed patients [66]. The production of microglial QUIN from tryptophan was increased in the sACC and aMCC but not in the pACC of patients with major depression (also, see Fig. 1A). A similar trend was observed in bipolar disorder. QUIN is a terminal breakdown product of the tryptophan pathway (see pathway, Fig. 1B), produced primarily by microglial cells, and is an endogenous modulator of the NMDA glutamate receptor. A key enzyme for the production of QUIN in the microglia is IDO, which is activated by proinflammatory cytokines, including IFN-α, IL-1, IL-6, and TNF-α.
depletion may secondarily lead to deficiency of its metabolite serotonin, which has antidepressant properties (Fig. 1B).

Future studies on the expression of QUIN in other depression-relevant brain areas, such as the amygdala, basal ganglia, hippocampus, or certain brainstem areas, are under way.

**Brain scans showing activated microglia in patients with psychiatric disorders**

Currently, one does not need to rely solely on postmortem studies to study the activation of microglia. Developments in the field of PET allow researchers to study microglia activation in patients in real time. A PET-tracer ([11C]-PK11195) binds to the mitochondrial TSPO, whose expression is increased in activated microglia and interestingly, also in proinflammatory-activated cells of other lineages [74]. This technique has already been applied successfully in several patient and animal studies of neuropsychiatric disorders [75]. These studies show that immune activation (“inflammatory”) lesions occur in brain regions that are related to the specific disease process. For example, in schizophrenia, microglia activation is found in the hippocampal area, where functions (immediate memory, sensory/emotional integration) are impaired. Interestingly these focal changes are found only in acute psychotic patients, in which cognitive impairment is most prominent [76], and not in patients that recovered from psychosis [77], who showed a global brain inflammatory effect.

The [11C]-PK11195 tracer has the disadvantage of a poor signal-to-noise ratio as a consequence of high, nonspecific binding to brain tissue. For that reason, new and more-sensitive PET tracers for the TSPO have been developed or are under development [75]. At this moment [11C]-DPA713 and [11C]-PBR28 seem to be the most promising candidates, although further evaluation and a direct comparison with [11C]-PK11195 need to be performed. Furthermore, it was found recently that the new PET tracers have different binding affini-
tities for the polymorphisms of the TSPO in humans, resulting in low-, mixed-, and high-affinity binding [78, 79].

In addition to the novel PET tracers for the TSPO, PET tracers are under development that allow imaging of other components of the immune system, such as other markers of neuroinflammation (β-glucuronidase by 18F-FEAnGA) [80], and activated T and B cells. For example, it was shown recently that activated microglia and macrophages can also be imaged with the PET tracer [11C]-ketoprofen methyl ester, which binds to COX-1 (and not to COX-2), an enzyme that plays an important role in the regulation of neuroinflammation [81].

Using PET, microglia activation has also been found in brain disorders such as Alzheimer and HIV dementia, Parkinson’s disease, multisystem atrophy, multiple sclerosis, herpes encephalitis, traumatic brain injury, and stroke [82, 83]. In addition, microglia activation is found in peripheral disorders, such as hepatitis, thiamine deficiency, and hepatic encephalopathy [84–86], and even after sleep loss [87].

The mechanisms responsible for the activation of microglia in these various diverse disorders remain elusive, but apart from indirect activation of microglia, a direct microbe-driven activation of microglia is possible. Many routes of direct infection of the microglial cells are present in the human. Pathogens have various modes of entry into the CNS, including through the olfactory bulb (respiratory virus and HSV), by monocytes that enter the brain from the bone marrow (polyoma virus, causing progressive multifocal leukoencephalopathy), or macrophages (HIV) via the vagus route (via intestinal organs), as well as through nervous transmission (as, e.g., in herpes encephalitis). An excellent review on these means of direct transmission was published recently [88].

ABNORMALLY ACTIVATED CIRCULATING MONOCYTES IN PATIENTS WITH PSYCHIATRIC DISORDERS

Raised numbers of circulating monocytes
The activation of the microglia may be part of a systemic activation of the mononuclear phagocyte system in general, and there are presently strong indications for an activation of circulating monocytes in patients with psychiatric disease. There are early reports showing that the number of circulating monocytes is aberrant in patients with schizophrenia. Rothermundt et al. [89], reported a slight increase in the mean absolute and relative monocyte counts, whereas others [90–92] supported these observations, showing a monocytosis and a higher number of CD14+ cells in nonmedicated schizophrenia patients and children with psychosis.

In the cerebrospinal fluid of patients with schizophrenia, there is an accumulation of monocytes and macrophages during acute psychotic episodes [93]. Theodoropoulou et al. [94], showed an increased percentage of circulating PBMCs expressing ICAM-1 in patients with schizophrenia, but the authors did not make a distinction between circulating lymphocytes and monocytes. Nevertheless, their observation supports an activated state of immune cells in patients with schizophrenia, facilitating endothelial transmigration of these cells.

In contrast to schizophrenia, higher numbers of CD14+ monocytes could not be found in patients with bipolar disorder [2, 95]. Neither were there differences between the number of mature (CD14+CD16−) and immature (CD14+CD16+++) circulating monocytes in these bipolar disorder patients.

Enhanced expression of immune genes in circulating monocytes
Recently, two gene-expression profiling studies [2, 96] were carried out on purified monocytes of psychiatric patients (56 bipolar and 27 schizophrenia patients) and matched healthy controls using Affymetrix analyses, followed by confirmatory quantitative real-time PCR. In sum, an aberrant expression of 34 genes was detected, mutually correlating and forming a monocyte gene expression signature (Fig. 2). Further, careful analysis of the gene expression detected within this signature two main subsets of strongly correlating genes—Clusters 1 and 2.

• Cluster 1 was composed primarily of proinflammatory cytokines and compounds, including IL-1β, IL-6, TNF, PTGS2, PTX3, various proinflammatory chemokines, and the inflammatory regulators PDE4B and DUSP2. This subcluster is likely driven by the transcription factors/regulators ATF3, EGR3, MXD1, and MAFF [2, 97].

• Cluster 2 was mainly made up of adhesion/motility factors and chemokines, such as CDC42, CCL2, CCL7, EMP1, and STX1A. PTPN7 and NAB2 are likely important transcription regulators for this subcluster.

The majority of patients with bipolar disorder showed an activated monocyte gene expression set-point involving Clusters 1 and 2 genes, whereas the majority of the schizophrenia patients showed an activated monocyte set-point of Cluster 1 genes only [96]. Also, the overexpression of monocyte activation genes was particularly evident in active cases, i.e., in patients with mania, an active depression, or an active psychosis [2].

The origin of the activated monocytes in psychiatric disease: genes or environment?
Genome-wide association studies have been performed over the last decade in large cohorts of patients with bipolar disorder and schizophrenia with overall disappointing results. It turned out to be virtually impossible to consistently find specific genes linked to these disorders. Large meta-analyses were needed to complete the genome-wide association studies, and presently, a few genetic markers with a limited risk have been identified. Among these markers is the MHC complex in schizophrenia [98] and the TNF gene in major depression [99]. A new approach using the genome-wide association study data to identify the molecular pathways (and not the separate genes) that are involved in psychiatric disease has been (somewhat) more successful and found, for instance, an involvement of the glutamate metabolism pathway and the TNFR1 pathway in schizophrenia [100]. These genome-wide association study data thus strengthen the concept that there is a role for an activated mononuclear phagocyte system but also demonstrate that the contribution of genetic polymorphisms to the activation of this system is limited.
Padmos et al. [101] carried out a case-control, gene-expression study using the monocytes of bipolar twins to determine the relative contribution of genetic and environmental factors to the aforementioned monocyte gene-expression signature. They also found that the association of bipolar disorder with all Subcluster 1 genes was primarily the result of a common, shared environmental factor. Some of the Cluster 2 genes (e.g., CCL2, CCL7, EMP1, and CDC42), on the other hand, were determined genetically.

There are various environmental factors that could play a role in the overexpression of Cluster 1 genes, and many articles highlight the role of the microbiome, acute and chronic stress, and dietary factors in a putative activation of proinflammatory mechanisms in circulating monocytes [102].

Abnormally raised levels of proinflammatory cytokines, chemokines, and adipokines in patients with psychiatric disorders

Most of the available literature about psychiatric patients has not focused on the cells of the mononuclear phagocyte system, but on levels of proinflammatory cytokines, chemokines, and other compounds produced by these cells. The resulting work/literature indicates, albeit inconsistently, that the IL-1β, IL-6, and TNF-α cytokine networks are activated in schizophrenia, bipolar disorder, and severe depression [1, 2, 94, 105–127]. In a previous review, we reported that generally, these cytokines were often found to be increased (in 28 out of 52 studies when taking schizophrenia and bipolar disorder studies together). This notion points to a variable, proinflammatory activity of the mononuclear phagocyte system in these psychiatric disorders. In addition, many antidepressant and antipsychotic medications are shown to have anti-inflammatory properties and reduce the concentration of proinflammatory cytokines in serum [128]. Several meta-studies, however, have shown that there is not a complete normalization of all proinflammatory cytokines during and after treatment, although the treatment was beneficial [129, 130].

What is the origin of these cytokines? It is possible that these cytokines are produced in the brain, leaking to the periphery, or are produced by peripheral immune cells and have their effect in the brain. One must take into account that increased levels of proinflammatory cytokines are not only found in psychiatric disease but also in cardiovascular disease, autoimmune disease, and cancer. In addition, it is important to note that even in healthy individuals, cytokine levels in serum or plasma are strongly confounded by a number of conditions, such as age, gender, socioeconomic status [131], metabolic syndrome and visceral obesity [132, 133], smoking [134] after exercise [135], poor-rated self-health [136, 137], and medication [138].

These confounders are illustrated nicely in a recent study of ours. In this study the levels of proinflammatory cytokines...
To take leptin as an example, the adipokine acts directly on microglia by activating the microglia and inducing the production of the proinflammatory cytokines IL-1β and TNF-α [156]. Pretreatment of microglia with leptin before LPS exposure results in even greater production of IL-1β and TNF-α. In contrast, addition of leptin after LPS exposure reduced IL-1β and TNF-α mRNA expression and IL-1β production. In addition, leptin treatment resulted in a unique morphological phenotype of the microglia, different from that after treatment with LPS or untreated cells [156]. These findings regarding the role of leptin on microglia should be taken into account when studying microglia, especially in schizophrenic patients suffering from the metabolic syndrome.

Another mechanism by which cytokines can act directly on the brain cells after entering the brain is that they can induce the activation of IDO, resulting in depletion of tryptophan for serotonin synthesis (also see Histomorphological studies showing activated microglia in patients with psychiatric disorders), facilitating the development of mood disorders [157–160].

WHAT CAN WE LEARN FROM MONONUCLEAR PHAGOCYTE SYSTEM ACTIVATION IN ANIMAL MODELS OF PSYCHIATRIC BEHAVIOR?

In addition to patient studies, there is evidence from animal models that an activation of the mononuclear phagocyte system influences the brain, causing an altered behavior. These models include simple peripheral immune challenge, the MIA models, psychological stress models, and the NOD mouse model. These animal models allow us to monitor activation of the mononuclear phagocyte system in the periphery as well as in the brain and study the effect on behavior.

Peripheral immune challenge and relevance to behavioral changes associated with psychiatric disorders

Several animal models exist that characterize the depressive-like effects of peripheral immune stimulation in adult animals, including exposure to the bacterial endotoxin LPS [161–163], exposure to the viral mimetic poly I:C [164, 165], and inoculation with BCG [166, 167]. Each of these induces an acute episode of sickness behavior, which is followed by behavioral deficits that can be interpreted as a depressive-like phenotype.

In response to LPS, a TLR4 agonist, depressive-like symptoms have been observed 24 h postadministration, a time by which sickness behavior has dissipated. This depressive-like behavior was defined as increased immobility, frequently interpreted as despair or helplessness in the tail suspension test and the forced swim test and also a decrease in sucrose preference, indicative of anhedonic behavior that was sustained up to 48 h postadministration [162, 163, 168]. Moreover, antidepressant treatment has been shown to alleviate these LPS-induced, depressive-like symptoms [161, 169, 170]. Increased microglial activity, as indicated by morphological change and increased OX-42 immune reactivity, is found in the brain in response to LPS, and whereas this activation peaks at 8–24 h,
it is still apparent several days postadministration [171]. In this animal model, the microglial inhibitor minocycline has been shown to have antidepressant properties in this animal model [168], suggesting a role for microglia and the corresponding induction of cytokines in the development of the depressive phenotype.

Poly I:C is a TLR3 agonist that has been shown to initiate inflammatory responses comparable with that of a systemic viral infection. A single systemic injection of poly I:C has been reported to induce depressive-like behaviors, which are accompanied by increased expression of IFN-α in a wide variety of brain regions for up to 8 days following administration [172]. Moreover, the behavioral deficits observed following poly I:C administration are accompanied by increased expression of the serotonin transporter and reduced synaptic availability of serotonin, as assessed using in vivo microdialysis [172]. Based on these findings, we suggest that poly I:C administration may represent a model of postviral depression. It is particularly noteworthy that expression of IFN-α, a cytokine that is a known inducer of depression in humans [173], remains elevated in the brain for at least 8 days following a single administration of poly I:C.

As with LPS and poly I:C, peripheral immune stimulation with BCG also induces depressive-like behaviors, including immobility in the tail suspension test and forced swim test, as well as a deficit in sucrose preference [166, 167]. In this animal model, however, the behavioral abnormalities are apparent for up to 3 weeks postinfection, allowing for a more appropriate discrimination between sickness behavior and depression. The depressive-like behaviors observed after infection are associated with increases in plasma IFN-γ and TNF-α levels. The role of these cytokines in BCG-induced depression was confirmed further by the absence of a behavioral deficit in BCG-inoculated IFNγR−/− mice and those that had been pretreated with the TNF-α antagonist etanercept [167]. These findings suggest that targeting these cytokine pathways may have therapeutic effects in the treatment of psychiatric illnesses associated with immune activation.

The MIA model

Epidemiological studies in humans have revealed a strong, positive correlation between prenatal infection and an increased risk of developing psychiatric disorder. For example, the children of mothers who suffered from influenza in the first trimester of pregnancy have a seven times higher chance of developing schizophrenia; this was three times higher for an infection in the second trimester. Another study showed that mothers who are seropositive for HSV-2 during pregnancy have a two times higher chance that their offspring develops schizophrenia. Moreover, in a cohort study, IgG antibodies to Toxoplasma were twice as high in mothers who gave birth to a child with schizophrenia [174]. Generally, it has been proven that Toxoplasma, HSV, rubella, and CMV cross the placenta and cause congenital brain anomalies directly. However, a role for inflammation and immune activation in general should not be neglected. Indeed, it has also been shown that levels of proinflammatory cytokines were higher in the serum of mothers during the pregnancy of a child who later developed a psychiatric disorder [174].

Studies using the MIA model vary widely in what species and immunogens are used (LPS for TLR4 activation or poly I:C for TLR3 activation), how the immunogen is administered, at what dose, as well as the timing and length of exposure. Similarly, there are numerous behavioral, anatomical, and molecular readouts that have been evaluated and this at various postnatal ages. Several detailed and comprehensive reviews of the results obtained from rodent MIA models have been published recently [175–177]. In addition, studies where the immunogen was administered directly into the uterus or the fetus have also been reviewed [58, 178].

Studies using these various versions of the MIA model resulted in offspring that display behavioral deficits reminiscent of symptoms associated with psychiatric disorders, such as impaired prepulse inhibition, impaired latent inhibition, increased anxiety, impaired locomotor activity, altered social behavior, and deficits in learning and memory [175–177, 179].

The particular power of the MIA models is that they can be used to investigate the mechanisms that lead to the reported changes in behavior and brain function and in particular, how the maternal and fetal immune systems might influence fetal brain development. Initially, studies focused on identifying the characteristics of the activated immune response in the mother and fetus. Thus, it was observed that MIA results in increased levels of various proinflammatory molecules, not only in the pregnant dam but also in the placenta, amniotic fluid, and the fetus itself [58, 176–179]. Moreover, there is evidence that this fetal immune response has long-lasting effects on the immune system of the offspring as they grow [176, 180]. More specifically, LPS- and poly I:C-induced MIA in the rat and mouse have been linked to elevated circulating levels of TNF-α, IL-1β, IL-6, iNOS, IL-10, MCP1, VEGF protein, and/or mRNA after stimulation [176, 177, 181–185]. In addition, there are reports that MIA can change the levels of neurotrophic and other neuronal development factors, such as NGF, BDNF [181, 186], semaphorin 5B, and groucho [183] in the neonatal brain.

A direct role for many of these cytokines has been established using techniques, such as the administration of blocking antibodies or injection of the pregnant dam with the purified cytokine itself. Much attention has focused on IL-6, as exposure of pregnant dams to IL-6 late during pregnancy results in deficits similar to those observed in LPS and poly I:C-induced MIA models. These deficits include impaired spatial learning and other hippocampal abnormalities, such as neuronal loss, astrogliosis, and changes in neurotransmitter receptor expression [187]. In support for a central role of IL-6, treatment of inflamed dams with anti-IL6 antibodies protects against the development of these abnormal behaviors [188], and IL-6KO animals that were exposed to prenatal immune activation have normal behavior [175–177]. Another important cytokine is TNF-α, a gene implicated in the risk of schizophrenia [179]. Elevated concentrations of TNF-α have been linked to fetal loss and growth restriction by showing that these effects could be limited by the administration of anti-TNF-α antibodies and reproduced by direct treatment of pregnant dams [177, 189].
190]. Other cytokines might also mediate the effects of prena-
tal inflammation; for example, prenatal exposure to EGF, IL-
1β, or leukemia inhibitory factor leads to reduced prepulse
inhibition and impaired social interaction in rats [179]. Fi-
ally, some cytokines have been found to be neuroprotective:
treatment with IL-10 can protect against the white matter dam-
age observed in MIA models [191].

Apart from the changes in cytokine levels found, MIA mod-
els have often been associated with increased neuronal cell
death (regional or whole brain) and/or decreased neurogen-
esis, resulting in region-specific or whole brain-size reduction
[176–178]. Another common feature of MIA is white matter
lesions with reduced numbers of oligodendrocytes and hypo-
myelination [176–178]. Finally, a characteristic found in these
animal models and that is common to psychiatric disorders is
increased astrogliosis and microglial activation [176–178]. In-
terestingly, some of the most affected areas of the brain are
those innervated by the dopaminergic system and the pyrami-
dal cells of the cortex and the hippocampus.

Regarding the dopaminergic system, the majority of studies
shows that prenatal inflammation leads to dopaminergic hy-
perfunction in animal models, similar to what is observed in
schizophrenia [175, 177]. The majority of work has been done
using the viral mimic model (poly IC), where immune stain-
ing showed increased numbers of tyrosine hydroxylase and
dopamine-transporter+ cells in mesencephalon, as well as in-
creased tyrosine hydroxylase immune reactivity in dopamine-
innervated regions. Interestingly, although investigators found
raised dopamine levels in the prefrontal cortex, this area had
decreased dopamine 1 and 2 receptor immune reactivity [175–
177]. In the case of LPS-mediated MIA, several studies in the
rat have shown that chronic LPS-induced MIA results in in-
creased levels of dopamine and tyrosine hydroxylase reactivity
in the nucleus accumbens of the offspring [180, 192, 193],
whereas a more acute treatment has the opposite effect, de-
creased levels of dopamine and tyrosine hydroxylase reactivity,
but also increased the expression of inflammatory cytokines, in-
cluding IL-1β, TNF-α, and IL-6. Moreover, social defeat stress
increased trafficking of macrophages to the CNS and in-
creased the inflammatory profile of these macrophages. These
findings support the hypothesis that stress can increase the
proinflammatory state of the CNS. At least in this paradigm,
the stress-induced behavioral and microglial changes seem to
be mediated by activation of β-adrenergic receptors and de-
pendent on IL-1, as microglial activation and behavioral
changes were ameliorated following pretreatment with the
β-adrenergic receptor antagonist propranolol or in IL-
1R−/− mice. Similarly, a role for IL-1 has been demonstrated in
mediating the depressive-like behavioral changes and reduc-
tion in hippocampal neurogenesis observed in the chronic
mild stress model of depression [204, 205]. Whereas many ani-
mal models support a role for inflammatory mediators in the
induction of depression-like behaviors in animals, these find-
ings emphasize the importance of stress-induced microglial
activation and most particularly, the inflammatory cytokine
IL-1β in the development of depression that may occur in the
absence of a direct immune stimulus.

On the other hand, axes other than the β-adrenergic-IL-1
axis might be present in the chronic stress models. Acute
stress increases the cortisol levels, activating TDO activity in
the liver, resulting in increased tryptophan breakdown prod-
ucts associated with neuronal cell death and depression (see
also Fig. 1B).

When administered on the backdrop of a psychosocial stres-
sor, the behavioral and cytokine responses to poly I:C are ex-
acerbated [165]. Similar results were found when LPS was ad-
ministered on the backdrop of social defeat stress [206]. In
this study, it was demonstrated that stress not only exaggerated
the LPS-induced behavioral and microglial alterations but also
increased the trafficking of inflammatory macrophages into
the CNS. One possible mechanism by which this may occur is
via stress-induced activation of TLRs and their endogenous
ligands. Recent evidence suggests that TLR4, which is activated
by LPS, can also be up-regulated in the brain in response to
chronic mild stress [207]. This stress-induced activation of
TLR4 could therefore prime the brain to any subsequent ef-
fects of LPS. The TLR4 endogenous ligand heat shock pro-
tein-72 has also been shown to be up-regulated in response to
stress [208–210], and it has been suggested that this may en-
hance LPS binding to TLR4 and thus, the cytokine response
induced [211]. These findings are consistent with the view that
the activation of the immune system and psychological stres-

Animal models of stress-induced microglial activation
It is widely accepted that exposure to psychological stress plays
a causal role in the etiology of depression; however, the pre-
cise mechanisms underlying the relationship between them
remain to be elucidated. Stress has also been shown to be asso-
ciated with altered immune function in the periphery as well
as in the brain, and it is has been suggested that immune acti-
vation may provide a link between stress exposure and the de-
velopment of depressive symptoms [196]. The use of animal
stress models has been central to the study of this comorbidity.
Several paradigms of stress-induced depression have been
characterized for their immune-modulating effects, including
acute and chronic restraint stress, chronic mild stress, foot
shock, maternal separation, and social disruption [197–203].

Evidence suggests that the depressive-like behaviors observed in
these animal models may be, in part, a result of the activation of
microglia. Restraint stress provokes changes in microglial activity
in stress-responsive brain regions, including the prefrontal cortex,
amygdala, hypothalamus, and hippocampus, as indicated by in-
creased Iba-1 immune reactivity [199, 201]. Furthermore, this
stress-induced microglial activation was associated with behavioral
deficits, including anhedonia and cognitive impairment, which
were reversed by the concurrent administration of minocycline,
an inhibitor of microglial activation [201].

A role for microglia has also been implicated in the depres-
sive phenotype elicited after social defeat stress [203]. Expos-
uire to this stressor not only increased the number of acti-
vated microglia in the brain, as indicated by increased number of
CD11b+ cells and increased Iba-1 immune reactivity, but
also increased the expression of inflammatory cytokines, in-
cluding IL-1β, TNF-α, and IL-6. Moreover, social defeat stress
increased trafficking of macrophages to the CNS and in-
creased the inflammatory profile of these macrophages. These
findings support the hypothesis that stress can increase the
proinflammatory state of the CNS. At least in this paradigm,
the stress-induced behavioral and microglial changes seem to
be mediated by activation of β-adrenergic receptors and de-
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changes were ameliorated following pretreatment with the
β-adrenergic receptor antagonist propranolol or in IL-
1R−/− mice. Similarly, a role for IL-1 has been demonstrated in
mediating the depressive-like behavioral changes and reduc-
tion in hippocampal neurogenesis observed in the chronic
mild stress model of depression [204, 205]. Whereas many ani-
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hance LPS binding to TLR4 and thus, the cytokine response
induced [211]. These findings are consistent with the view that
the activation of the immune system and psychological stres-
sors may act synergistically, where exposure to psychological stress may enhance the cytokine response elicted by an immune challenge, which in turn, may exacerbate the central response, leading to psychological deficits and depression [212].

The NOD mouse model

Patients with psychiatric disease and their first-degree relatives (parents, children) are more prone to develop autoimmune diseases, such as autoimmune thyroiditis and T1DM. Many of these first-degree relatives with an autoimmune disease do not have a psychiatric disorder [213–216]. These observations refute the concept that psychiatric disorders and autoimmunity are cause or consequence of each other. It rather suggests that there exists a shared common immune pathogenesis for psychiatric disease and these autoimmune diseases.

The NOD mouse is a widely used model for the spontaneous development of autoimmune diabetes, similar to T1DM found in humans. This includes the presence of pancreas-specific autoantibodies, autoreactive T cells and genetic aberrancies in MHC and various non-MHC alleles. In addition to T1DM, the NOD mouse spontaneously develops autoimmune thyroiditis, when on a high iodine diet. Interestingly, there are also signs of an abnormal behavior and neurodevelopment of the NOD mice. Several reports indicate an abnormal behavior in NOD mice as compared with C57BL6 mice. An early study by Amrani et al. [217] found increased activity of NOD mice in the open field. In addition, NOD mice showed a decreased response to a psychological stressor but not to a metabolic stressor as compared with C57BL6/6 mice. The finding of increased activity in the open field was supported by a study of Bette et al. [218], where they compared 14 inbred mouse strains in the open field, and the NOD was among the most active strains. Limitation of these studies is that they do not take into account the different genetic background of the strains as a possible cause of this abnormal behavior. Nevertheless, the NOD mouse model opens avenues to study the association of autoimmunity and psychiatric disease and gene-environment interactions in this association.

Are there shared common abnormalities in NOD macrophages in the pancreas/thyroid, in circulating monocytes, and the microglia in the brain?

Although fate-mapping studies have shown that microglia and macrophages arise from a common primitive macrophage precursor and belong to different myeloid lineages [9], many similarities are present between the cell types. Both express the surface markers CD11b, CD14, and CFS1R and require the transcription factor PU.1 function [219]. In addition, gene-expression profiling showed a close relationship between bone marrow-derived macrophages and microglia in C57BL/6 mice [220].

The earliest local histological sign of the insulitis/thyroiditis process of the NOD mouse is an accumulation of macrophages and DCs in the glandular tissues, later followed by a lymphocyte accumulation and a destruction of endocrine cells [221–224]. There are signs that these early accumulating macrophages and DCs tend to have an immune-activated set-point, they lack surface CCR5 expression (CCR5 ligation is essential to dampen IL-12 production) [225], and the composition of the accumulating population of macrophages and DCs lacks a prototypic, tolerogenic DC population, the CD8α+CD103+Langerin+ DGS (unpublished results).

With regard to circulating monocytes in the NOD mouse, there is abnormal maturation and differentiation of monocytes from precursors, resulting in an abnormally high number of mature Ly6Clow monocytes (the murine counterpart of CD14+CD16+ monocytes) [226]. This imbalance is also found in the nondiabetic strains NOR and NODH2b, suggesting that this feature is intrinsic of its genetic background and not a consequence of disease. Secondly, these mature monocytes display a high adhesion to fibronectin and ICAM-1. Third, results from in vitro cultures showed that NOD monocytes preferentially differentiate into inflammatory macrophage-like cells instead of tolerogenic DCs. This might be related to the dysregulation of STAT5 in the monocytes/macrophages of the NOD mouse, resulting in aberrantly high PGE2, COX-2, and GM-CSF production [227]. These findings were supported partially by a more recent study, where they found after in vitro LPS stimulation, an increased expression of COX-2 mRNA and PGE2 secretion by the monocytes obtained from NOD mice compared with C57BL/6 mice [228].

Although there are many similarities between peripheral macrophages/DCs, monocytes, and microglia (see above), they nevertheless belong to different lineages. It remains therefore to be established whether the abnormalities found in the peripheral cells of the mononuclear phagocytes system in the NOD mouse are also present in the microglia. Current, preliminary data of ours obtained by Affymetrix whole genome gene expression profiling on cortical microglia showed an overexpression of gene networks involved in cell proliferation and growth and cell-cycle control in the NOD microglia. Genes involved in phagocytosis were clearly down-regulated. Evidence for immune activation, such as the overexpression of IFN-1β, was not found in the microglia of NOD mouse (unpublished results). With regard to environmental influences, it is of interest that a strong and prolonged reaction of NOD microglia to LPS stimulation was found. Twenty-four hours after LPS injection, the microglia of the NOD mouse was still activated by overexpression of IFN type I-inducible genes and inflammatory gene networks, whereas the CD-1 control strain did show reduced microglial activation, 24 h after stimulation (unpublished results). This is in accord with the observation that the NOD is, in particular, sensitive to react with a depressive-like behavior to LPS stimulation. A study of Bluhle et al. [229], where they injected NOD and CD-1 mice with IL-1β and LPS to induce sickness behavior, indicated that NOD mice are particularly sensitive to the behavioral effects of IL-1β compared with CD-1 mice, although the distribution of IL-1Rs in the dentate gyrus, choroid plexus, meninges, and anterior pituitary of NOD and CD-1 mice was found the same. Recent unpublished work of ours confirms these previous data. In addition, these data show an increased, depressive-like behavior in the forced swim test and a prolonged sickness behavior, 24 h after LPS injection (unpublished results).
In sum, the NOD mouse is probably a good model to study gene-environment relationships between peripheral and brain mononuclear phagocyte system activation and abnormal behavior. The present data suggest a rather “normal” behavioral phenotype and normal microglia in the NOD mouse under steady-state conditions. In addition, microglia did not show clear signs of activation. However, when the immune system of the NOD mouse is activated by LPS or IL-1, an unknown cascade of biological events induces an aggravated form of depressive-like behavior and prolonged microglia activation.

Interestingly, NOD macrophages and DCs have been described as defective for IDO activity after IFN-γ stimulation [230]. Excessive IDO activity has been linked to depressive-like behavior and is considered to be associated with chronic low-grade inflammation of the brain. Apparently, the IDO pathway does not play a role in the NOD mouse model under steady-state conditions but might be operative under LPS or IL-1 stress.

**Imaging of inflammation in animal models of depression and schizophrenia**

As mentioned above, animal models can be of great value in studying the interaction between an activated immune system and changes in behavior, i.e., appearance of depressive- or schizophrenic-like behavior. Moreover, in animal models, such as the MIA and the NOD model, it is of interest to study the immune system and behavior over time and in living animals. Indeed, activation of the immune system may occur long before any behavioral changes can be found and may progress over time. PET imaging nowadays also allows for noninvasive imaging of microglia and other components of the (activated) immune system in animals, making use of dedicated, high-resolution PET scanners.

Recently, a study was published by Dobos et al. [231], in which [11C]-PK11195 PET was used to study microglial activation during 4 consecutive days after intracerebroventricular injection of LPS in mice. It was found that microglial activation was increased at 2 days, peaking at 5 days after LPS injection. These mice showed depressive-like behavior on Day 3 after LPS injection, suggesting that there might be a relationship between microglial activation and depressive-like behavior.

In addition to LPS-induced, depressive-like behavior in mice, microglial activation was found in the MIA model. With the use of immunohistochemistry, an increase in the number of activated microglia in the offspring at 4 weeks of age was observed [232]. Although PET was not used in this study, a related study in rabbits showed that [11C]-PK11195 PET is a useful tool to study activated microglia in the MIA model. In the offspring of pregnant rabbits that were injected with LPS, [11C]-PK11195 PET revealed the presence of activated microglia on Postnatal Day 1 [233].

Schizophrenia-like behavior has also been observed in rats that were injected with HSV-1, providing evidence for the hypothesis that HSVs play a role in schizophrenia (unpublished results). Two days after infection, the rats displayed increased exploration, rearing behavior, and anxiety (i.e., schizophrenic-like behavior), which were increased at Day 4 after infection. [11C]-PK11195 PET imaging in these rats revealed that activated microglia were present at Day 4 after infection (unpublished results). Another study showed an increased number of microglia after 7 and 14 days [234] (also shown in Fig. 3).

**CONCLUSION**

In conclusion, studies in patient cohorts and in various animal models on microglia, monocytes, and their products strongly suggest a key role for these cells of the mononuclear phagocyte system in the pathogenesis of major psychiatric disorders, such as bipolar disorder, major depressive disorder, and schizophrenia. There is accumulating evidence for activation of microglia and circulating monocytes in psychiatric patients and animal models of depressive-, anxiety-, and schizophrenia-like behavior. Figure 4 gives a hypothetical scheme about how we view that such activations arise and how they have their impact on the growth, development, and function of the neuronal circuitry in the brain. Strikingly, activation of the mononuclear phagocytes in the periphery and microglia in the brain...
are also found in several nonpsychiatric disorders, including obesity. It remains puzzling how such a general activation of the mononuclear phagocyte system at the level of the brain (microglia), the circulation (monocytes), and the tissues (macrophages, DCs) as a key element in the pathogenesis of major psychiatric disorders. The abnormally immune activation set-points of these cells reached after development from (local) precursors are induced by gene-environment interactions, such as inflammatory influences during fetal life (e.g., MIA model), alone or in combination with a genetic background, predisposing to an aberrant proinflammatory differentiation tendency of myeloid precursors to monocytes/macrophages/DCs and microglia (e.g., in the NOD mouse model). Whether there exists a direct migration of activated monocytes to the brain in psychiatric disease needs further exploration. Also, the role of inflammatory cytokine/chemokine/adipokine exchange among the various compartments (circulation, brain, and peripheral tissues, such as adipose tissue and lymphoid tissue) needs clarification. A crucial concept of the model is that the normal physiological support given by normal microglia to normal growth, differentiation, and function of brain neuronal circuitry is perturbed by the abnormal activation set-point of the microglia. These abnormal interactions between microglia and neurons might have different consequences (leading to a variety of psychiatric symptoms) as a result of gene-environment interactions, in part, other than those playing a role in the actual immune activation set-point change of the microglia. These gene-environment interactions are thought to include gene variants playing a role in neuron development (e.g., BDNF polymorphisms), the timing (early or late in life), character and profile of the immune activation signals (infectious, systemic as a result of high-fat diet, and others), acute stressors letting the imbalanced system fail, and others.

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The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes

Wouter Beumer, Sinead M. Gibney, Roosmarijn C. Drexhage, et al.

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