Trained innate immunity: a salient factor in the pathogenesis of neuroimmune psychiatric disorders

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Abstract

Historically, only cells of the adaptive immune system have been considered capable of retaining memory for infectious challenges. Recently however, cells of the innate immune system have been shown to be capable of displaying long-term functional memory following a single immunostimulatory challenge, leading to enhanced production of proinflammatory molecules upon other subsequent, and temporally distant, immunostimulatory challenges. This effect has been termed “trained innate immunity”, and is underwritten by stable epigenetic changes in immune and metabolic pathways. Importantly, the long-term training of innate immune cells can ocurr as a result of infectious as well as and non-infectious challenges, including stress. Given the role that both stress and an activated immune system play in neuropathology, innate immune training has important implications for our understanding and treatment of neuropsychiatric disorders. This review fosuses on the evidence for trained innate immunity and highlights some insights into its relevance for psychiatric diseases.

Introduction

(Neuro)inflammation is increasingly recognised as a critical factor in the pathogenesis of numerous psychiatric illnesses. Indeed, research on immune dysregulation and psychiatric disorders has gained significant attention in recent years as data gathered from numerous experimental models have converged to provide support for the role of inflammatory mechanisms in the etiology of depression, schizophrenia and related psychotic disorders [1]. Over the last five years the number of publications on immunity and depression alone were almost doubled [2] and similarly, in the past seven years data supporting an immune-mediated cause in schizophrenia also escalated [3]. This advocates that research is now entering a new era of immunopsychiatric investigations that will profoundly change the understanding of brain disorders, in which immune impairments will be now acknowledged as potential underlying mechanisms of altered behavioural manifestations.

Historically, the brain has been thought of as an immunologically privileged site, shielded behind the blood–brain barrier (BBB)[4]. However, recent evidence proposes that immune components of the brain, such as proinflammatory molecules, are able to cross the BBB and directly exert negative effects on neuronal cells, ultimately contributing to the development and progression of major depressive disorder (MDD), schizophrenia and post-traumatic stress disorder, amongst others [5,6]. Cytokines such as IL-1β, IL-6, TNF-α, IFN-α and INF-y, as well as reactive oxygen species (ROS) and reactive nitrogen species (RNS), cause disruptions in neurogenesis, neuronal excitability, synaptic transmission, synaptic plasticity and neuronal survival [5-7], disturbances which have been implicated in the development and progression of neuropsychiatric disorders [8]. Microglia, the resident innate immune cells of the CNS, are the major mediators of neuroinflammation [9], although monocytes and macrophages are also important innate immune sources of sources of proinflammatory molecules that can act in the CNS [10,11]. These cells are strongly implicated in the pathogenesis of neuropsychiatric disorders therefore [12-14].

Over the past decades, the major difference between the adaptive and innate immune systems has been considered the ability of the adaptive immune system to retain memory for specific infectious agents, whilst the innate immune system has been considered neither capable of memory nor specificity [15]. This memory function of the adaptive immune system is critical to immunity in vertebrates, often offering lifelong protection against reinfection. Recently however, cells of the innate immune system, including monocytes and macrophages, have been discovered

capable of long-term functional memory following a single short lived immunostimulatory event, resulting in dramatically enhanced production of proinflammatory molecules in response to a subsequent, temporally distant, immunostimulatory event. This has been termed “trained innate immunity” or “innate immune memory” [16,17]. In animal models of dysfunctional or absent adaptive immunity, trained innate immune cells can protect against lethal infection with various pathogens [18,19]. Trained innate immunity represents a paradigm shift therefore in our understanding of innate immunity, with important implications for disease pathogenesis and immunotherapeutics [16,20].

Trained innate immunity is distinct from priming. Priming is a well described phenomenon during certain infections or stimulations [16,20,21]. Often, pre-exposure of an innate immune cell to a cytokine such as IFN-y is necessary for the cell to be able to fully activate and produce a robust immune response when exposed to a pathogen [22,23]. The cells are said to be pre-activated or “primed” in response to stimulation with the cytokine. Typically, primed cells produce little, if any, cytokines in the absence of a contemporaneous co-stimulation by a pathogen [24,25]. Priming effects also rapidly decline. In contrast, training is typically induced by pathogen associated molecular patterns (PAMPs), and involves long-term changes in innate immune cell function that persist well beyond the primary immunostimulatory event. Unlike primed cells, trained cells often have an elevated baseline proinflammatory cytokine production. The mechanisms underlying training are distinct from priming. Priming often involves the mobilization of pre-formed receptors in the cytoplasm to the plasma membrane as a result of a stimulus, leading to short-term term changes in receptor number or affinity [16,20]. In contrast, training is underwritten by stable epigenetic changes in immune and metabolic pathways [16,20,21]. This long term memory effect can persist for months, and possibly years, and is not exclusive or specific to the type of primary immune stimulating event [26]. Recent studies suggest that innate immune cells also retain long-term memory for non-infectious immunostimulatory challenges, such as stress [27].

Despite the widespread interest in immune function and psychiatric illness, there has been no exhaustive discussion relating to the implications of trained innate immunity on mental health pathology. Considering that trained innate immunity represents a paradigm shift in our understanding of immune function [8], the theoretical contributions of trained innate immunity to illnesses such as depression are potentially dramatic and worth of a comprehensive analysis. Here, we review recent evidence for innate immune training and examine why it is likely to be a major contributing factor to the pathogenesis of neuropsychiatric disorders. We concentrate primarily on

monocytes, macrophages and microglia, though there is also extensive evidence for trained immunity in NK cells [16], which have themselves been recently hypothesized to play a role in psychiatric illness [28].

Trained innate immunity

The innate immune system and training

The innate immune system recognizes invading pathogens through receptors called pathogen recognition receptors (PRR), which are present on the surface, in the endosome or in the cytoplasm of innate immune cells [29]. PRR recognize conserved microbial molecules including lipids, proteins, nucleic acids and carbohydrates. These microbial molecules are known as pathogen-associated molecular patterns (PAMP) [29]. A diverse range of PRR types exist, including toll-like receptors (TLR), C-type lectin receptors, nucleotide-binding oligomerization domain-like receptors (NOD), and retinoic acid-inducible gene I (RIG-I)-helicases [29]. A specific PRR may be capable of recognizing a single or multiple different PAMP. TLR-4, for example, recognizes lipopolysaccharide (LPS), an endotoxin present in the cell wall of multiple different gram negative bacteria [30]. Often, microorganisms will have multiple PAMP, which will together stimulate a suite of PRR on host cells. *Herpes* Simplex virus (HSV) PAMP activate TLR2 on the cell surface and TLR9 in the endosome [29] for example .

Typically, stimulation of a PRR by a PAMP leads to immune activation of the cell and the release of various proinflammatory cytokines, chemokines, ROS and RNS [29]. The exact response may vary dependent on the type of PAMP-PRR interaction. However, many different PAMP-PRR interactions activate the same or similar downstream signaling pathways. Activation of a PRR by a PAMP can sometimes lead to an altered cellular immune response upon subsequent reactivation of the same PRR or the activation of a different PRR that feeds into similar signaling pathways. This has been known about for some time in the context of innate immune tolerance [31,32]. Innate immune tolerance was first described in relation to the immunoparalysis that occurs after Gram-negative sepsis in critically ill patients [31]. Here, cells of the innate immune system fail to mount an appropriate proinflammatory response to re-stimulation by LPS or Gram-negative bacteria following Gram-negative sepsis. Thus, innate immune cells appear to retain an inhibitory memory for high dose LPS

stimulation under certain conditions. Innate immune cells have also recently been shown to be capable of retaining a long-term memory for certain PAMP or pathogens, resulting in an exaggerated immune response upon subsequent re-stimulation with similar or dissimilar PAMP or pathogens. This heterogeneous proinflammatory memory effect, "trained innate immunity", appears to be prolonged, lasting sometimes for months, and possibly even years [16]. Whether tolerance or training occurs is dependent on a number of factors, including the amount, nature, duration of exposure of the PAMP. For example, whilst high dose LPS results in decreased production of proinflammatory molecules upon re-exposure to LPS, low dose LPS exposure results in increase production of proinflammatory molecules upon re-exposure to LPS [33].

That this memory effect is heterogeneous and long lasting has important implications, as it may set the stage for a proinflammatory responsive state in response to a diverse array of temporally distant immunostimulatory events. Table 1 lists some of the main features of trained innate immunity, as well as potential consequences for neuropsychiatric disorders.

Training of monocytes and macrophages

Given that the adaptive immune system is highly specific, and the long held view that the innate immune system displays no memory for pathogens, we would not expect an enhanced immune response upon re-infection at a distant time with dissimilar organisms. Nor would we expect an enhanced immune response upon re-stimulation in the absence of a functional adaptive immune system. Several studies performed decades ago demonstrated just this though. Mice immunized with Bacillus Calmette–Guérin (BCG) displayed enhanced immunity and protection against lethal infection with non-tuberculous organisms such as *Candida* Albicans and *Schistosoma* Mansoni [18,34]. T-lymphocyte depletion failed to dampen this protective effect, which was subsequently found to be mediated by activated tissue macrophages [18]. Mice previously infected with the fungus *Candida* Albicans also showed enhanced immune responses and increased survival in response to infection with the Gram-positive bacterium *Staphylococcus* Aureus [35], an effect which was again found to be dependent on macrophage responses [19].

The importance of these findings and their implications were largely ignored however, until two recent studies replicated and extend these findings in human innate immune cells. Kleinnijenhuis et al (2012) isolated monocytes from adult humans before and two weeks after BCG

vaccination, and stimulated them ex-vivo with BCG, *Staphylococcus* Aureus, *Candida* Albicans, or LPS [36]. Post BCG vaccination, monocytes showed dramatically enhanced production (up to 7 fold) of various proinflammatory cytokines, including IFN-γ, TNF-α and IL-1β upon homogenous and heterogeneous stimulation, relative to monocytes pre BCG vaccination. This upregulation in proinflammatory function was accompanied by increased expression of various activation markers and PRRs, including CD11b, CD14, and TLR4, and was still present one year after BCG vaccination [26]. Primary stimulation of NOD2, a PRR for mycobacteria, with the NOD2-specific ligand MDP, mimicked the training effects of BCG, which was prevented when inhibiting NOD2 during primary stimulation, as well as its downstream signaling pathway, Rip2 kinase. Such training effects were also seen in human monocytes following neonatal BCG vaccination [37].

Similar training effects were observed following stimulation of adult human monocytes in-vitro with either *Candida* Albicans or β-glucan (a component of fungal cell walls) for 24 hours [38]. Upon a second stimulation with *Candida* Albicans, bacteria, or various PAMP/PRR ligands up to two weeks later, pre-stimulated monocytes showed significant increases (of up to 10-fold) in TNF-α and IL-6 production relative to naive monocytes. The magnitude of the training effects was dependent on the doses of both the primary and secondary stimulation. Again, specifically inhibiting the PRR for β-glucan and its downstream signaling pathways during primary stimulation, in this case Dectin-1 and Raf-1, prevented training. These training effects were accompanied by dramatic and long lasting changes in gene expression. Genes for multiple PRR unrelated to Candida/β-glucan were upregulated, including several TLRs, which might partly explain the heterogeneous effect of training. Other upregulated genes included chemokines and nitric oxide synthases, as well as several histone methyltransferases, suggesting a potential role for epigenetic remodeling in training.

For some PAMP, the dose can determine whether training or tolerance is induced, whereas for others, different doses appear to consistently induce training. For example, moderate to high doses of a variety of PAMP and PRR ligands, such as LPS (100 μg/ml), Pam3CSK4 (100 μg/ml), flagellin (10 μg/ml) and Poly(I:C) (100 μg/ml) (which all engage TLRs) induced heterogeneous tolerance in human monocytes [*33*]. Lower doses (0.1 pg/ml to 1 μg/ml) however, induced heterogeneous training. In contrast, low to moderate doses of β-glucan (1 μg/ml), Tri-DAP (10 μg/ml) and MDP (10 μg/ml) (which engage NODs), all induced training (up to 5 fold increases in TNF-α). The reason why some PAMP/PRR induce only training or tolerance across a range of doses, whilst for other PAMP/PRR the effect is dose-dependent, is currently unclear.

Training of microglia

Few studies have investigated the long-term upregulation of microglial function as a result of a single immunostimulatory challenge, and none in the context of the concept of training. Nevertheless, there are suggestions that microglia are capable of being trained. Hippocampal microglia isolated from adult mice that had been injected once intra-peritonealy with *Salmonella* typhimurium and then given an intra-hippocampal injection of LPS four weeks later showed an increase in the activation markers CD11c and MHCII relative to microglia from mice only exposed to *Salmonella* typhimurium [39]. *Salmonella* typhimurium injection alone did not result in an increase in CD11c and MHCII immunoreactivity, suggesting that the increase seen following LPS injection was not simply due to an increase in the number of microglia. Neonatal rats injected with *Escherichia* Coli subcutaneously and then given a peripheral injection of LPS in adulthood had increased microglial CD11b gene expression, as well as faster and more prolonged increases in microglial production of IL-1β protein, without there being any differences in microglial numbers [40,41]. These changes were accompanied by decreased neurogenesis in the hippocampus and memory impairments, which were prevented when caspase-1 (a protease that cleaves the precursors pro-IL-1β and pro-IL-18 into active IL-1β and IL-18) was inhibited prior to LPS challenge in adulthood. Although suggestive of training, it is possible, however, that interactions between the adaptive and the innate immune system were responsible for the observed effects in these studies. To specifically rule out interactions with other cell types, live microglia need to be isolated and tested in-vitro. Although animals with severe combined immunodeficiency could be used to rule out interactions with the adaptive immune system, this would still leave the possibility that other cell types such as astrocytes and neurons, which are known to have immunomodulatory properties [42,43], are responsible for any observed effects. A very limited number of studies have performed experiments on isolated microglia. Microglia isolated from adult rats that had been infected neonatally with *Escherichia* Coli showed increased production of IL-1β mRNA (on a per cell basis) when exposed to low dose LPS in-vitro [44]. Again, neonatally infected rats had memory impairments when administered LPS in adulthood, which were prevented by administering minocycline prior to adult LPS challenge (which also prevented increases in IL-1β mRNA). Microglia isolated from the fetuses of maternal sheep injected with LPS and then maintained in-vitro for 3 weeks, had significantly higher production of IL-1β (> 4 fold) in response to LPS in-vitro relative to controls [45]. The expression

of heme oxygenase (decycling) 1 (HMOX1), which is thought to have an anti-inflammatory role [46], was strongly downregulated in LPS/LPS microglia.

Training of innate immune cells by non-microbial stimuli

Upon cellular damage or stress, host cells release a variety of molecules. Some of these released molecules can activate PRR and initiate an inflammatory/immune response, much like PAMP. These endogenous PRR-stimulating non-microbial molecules are known as danger associated molecular patterns (DAMP) [47]. Examples of DAMP include high mobility group box-1 (HMGB1), S-100 proteins, heat-shock proteins (HSP), hyaluronan, surfactant protein, IFN-α, uric acid, fibronectin, beta defensin, and cardiolipin, amongst others [47]. Many of these DAMP are oxidized versions of proteins and other molecules present on apoptotic host cells or in cellular debris [48]. These molecules are oxidized as a result of cellular damage/death induced ROS generation, resulting in hydrophobic regions being exposed and available to be recognized by PRR as DAMP [47]. Many of these endogenous oxidized molecules and hydrophobic regions share molecular identity with microbial PAMP [47,48]. Thus, we might expect DAMP to be also capable of inducing training. Exposure of human monocytes to oxidized low density lipoprotein (LDL) (1-10ug/ml) for 24 hours, but not LDL, for example results in increased protein production (up to 5 fold) of TNF-α, IL-6, IL-8, upon re-stimulation 7 days later with various TLR-4 (e.g. LPS) and TLR-2 agonists (e.g. Pam3Cys) [49]. Blocking these TLR receptors or their downstream pathways inhibited the training effects.

The study above is the only study that we are aware that has directly investigated whether DAMP can induce training. There are, however, a few studies that provide indirect evidence for DAMP induced training. Sterile traumatic brain injury (TBI) releases a suite of DAMP [50], and might therefore be a systemic non-infectious immunostimulatory event capable of inducing microglial training. In humans, TBI is associated with microglial activation on PET decades later [51], and is a risk factor for depression [52]. Microglia isolated from mice that had been injected peripherally with LPS one month after TBI showed increased IL-1β and TNF-α mRNA expression relative to microglia from LPS exposed only mice [53]. This heightened microglial activity was accompanied by depressive-like behaviors in the mice. Although suggestive, this is not direct evidence for DAMP induced microglial training however, as TBI is a complex multifaceted phenomenon and the microglia were not stimulated ex-vivo, thus raising the possibility that interactions with other cell types may have been responsible for the upregulation of microglial proinflammatory function. Microglia isolated from

mice 24 days after repeated social defeat stress (RSD) also had higher IL-1β, IL-6, and TNF-α mRNA expression and IL1-β protein production in response to ex-vivo LPS stimulation relative to non stressed controls [27]. This upregulation in microglial proinflammatory function was accompanied by an increase in social avoidance behavior in RSD mice. Whether, specifically, stress induced released of DAMPs resulted in microglial training was not investigated however. Psychological and physical stress results in the release of a variety of DAMP both peripherally and centrally, including heat shock protein 72, uric acid and high mobility group box-1 [54-56]. Stressor exposure also releases microbial associated molecular patterns (MAMP) from the gut microbiota into the blood and/or extracellular environment [57]. It seems likely that DAMP and MAMP, like PAMP, are capable of training innate immune cells. Given the strong association between stress, inflammation and neuropsychiatric illnesses, investigating whether stress induced release of DAMP or MAMP train microglia in a homogeneous and heterogeneous manner is an intriguing avenue of future study.

Mechanisms underlying training

Several studies have specifically investigated whether training is underwritten by epigenetic remodeling. Human monocyte training as a result of BCG or Candida/β-glucan exposure results in an increase in H3K4me3, a histone modification associated with the regulation of immune-related genes, at the promoters of target genes including TNF-α, IL-6, IL-18 [36,38]. Consequently, blocking histone methylation by inhibition of histone methyltransferases prevented training. Training of monocytes by non-microbial stimuli, such as oxidized LDL, is also dependent on the enrichment of H3K4me3 at the promoters of various immune related genes [49]. β-glucan training in human monocytes has also been associated with epigenetic remodeling of various metabolic pathways [58,59]. β-glucan trained monocytes display reduced oxygen consumption and increased glucose consumption, consistent with a switch from oxidative metabolism to glycolysis. This metabolic shift is characteristic of activated monocytes and macrophages, and, as result, disrupting these changes in cellular metabolism inhibits training. Further, in mice, LPS induced macrophage training results in phosphorylation of the stress-response transcription factor ATF7 [60]. ATF7 normally suppresses a group of genes encoding factors involved in innate immunity in macrophages by recruiting the histone H3K9 dimethyltransferase G9a. Training leads to the release of ATF7 from chromatin and a decrease in repressive histone H3K9me2 marks, and thus increased expression of immune related genes. Interestingly, ATF7 is also phosphorylated as a result of social isolation stress and various other stressors [61].

Whilst to our knowledge there have been no studies investigating whether epigenetic remodeling is responsible for training effects in microglia, there is evidence that epigenetic remodeling does underlie tolerance in microglia. In response to a high dose in-vivo LPS exposure, isolated mouse microglia displayed reduced proinflammatory cytokine production upon a secondary LPS stimulation in-vitro [62]. These effects were mediated by a reduction in the levels of H3K4me3 at the promoters of various immune related genes. Whilst proinflammatory cytokine production was diminished, phagocytic activity and nitric oxide production were enhanced however. This study is interesting, not only because it demonstrates that microglia are capable of functional epigenetic remodeling, but also because it shows that the dichotomy between innate immune tolerance versus training is potentially simplistic. Whilst some proinflammatory and neurotoxic innate immune cell functions may be downregulated, others may be simultaneously upregulated. Since proinflammatory cytokines, ROS and RNS all have the potential to be neurotoxic, it will be important to delineate the extent to which production of these different molecules are affected as a result of training.

Implications for neuropsychiatric diseases

There remain a lot of unanswered questions regarding the details of trained innate immunity. We have listed some of these in Table 2. Most important, given the association between neuroinflammation and psychiatric disease, is whether trained innate immunity contributes to the onset and progression of neuropsychiatric disorders. A key unknown is also the duration for which training can last. That training in human monocytes has been detected up to a year post primary exposure suggests that training effects can be long-term. This is an important observation. Early-life immunostimulatory events such as stress and infection are risk factors for the development of neuropsychiatric disorders, and possibly neurodegenerative disorders, in adulthood [63]. Indeed, children prenatally exposed to elevated levels of the stress hormone cortisol reported persistent changes in the innate immunity with higher level of proinflammatory cytokines and subsequent development of emotional, behavioral and cognitive psychopathologies later in life [9-12]. Similarly, mice prenatally exposed to a bacterial infection with LPS showed depression- and anxiety-related behaviors during adulthood. In addition, LPS-treated mice had reduced serotonin and noradrenaline levels in the hippocampus, a brain region very well-known for its neurogenenic properties

associated with cognitive and memory functioning [13]. Therefore, evidence seems to suggest that specific long-term changes in the inflammatory environment is alone sufficient for the occurrence of several psychopathologies later in life. One way in which these events are thought to contribute to the development of neuropsychiatric illnesses is through the induction or a proinflammatory predisposed phenotype which can lead to neuroinflammation, in particular following a subsequent, temporally distant, immunostimulatory event. This ultimately results in the disruption of key processes involved in CNS development and regulation throughout adolescence and adulthood [63]. These secondary immunostimulatory events can be completely unrelated in nature to the primary immunostimulatory event. In animal models for example, the combination of prenatal polyI:C (which activates TLR-3 [64]) followed by peripubertal stress [65], or TBI followed by LPS [53], induce depressive-like behaviours. These findings cannot be easily explained by adaptive immunity or classical understandings of innate immunity. That trained innate immunity is heterogeneous in nature, likely because different PAMP and DAMP can activate the same PRR or downstream signaling pathways, resulting in the reprogramming of similar epigenetic pathways, offers a potential mechanistic explanation. Adult microglia arise almost exclusively from a founding population of bone marrow-derived primitive macrophages during development and are maintained throughout life [66]. Events early in life may lead to microglia later in life having a lower threshold for activation and/or the enhanced production of proinflammatory molecules that can cause impairments in neuroplasticity and neurogenesis.

Furthermore, after accounting for genetic factors, it is not clear why some individuals develop inflammation and psychiatric illness upon exposure to the same environmental risk factors whilst others do not [1]. Trained innate immunity, specifically individual variation in the epigenetic landscape for immune-related genes and pathways as a result of previous inflammatory events, might account for some of the variation in how individuals respond to risk factors. It is possible that the presence of one inflammatory risk factor may train the innate immune system to react more vigorously in response to a subsequent, temporally distant, or even contemporaneous, inflammatory risk factor.

Once cells of the innate immune system such as microglia are trained, this could lead to a cycle in which (neuro)inflammation begets (neuro)inflammation (Figure 1). (Neuro)inflammation can result in cell damage and death, and thus the release of numerous DAMP. As suggested, DAMP may themselves be capable of inducing training. Thus, as a result of infection or stress induced

(neuro)inflammation and cell injury/death for example, the release of DAMP may train the innate immune system towards a proinflammatory phenotype, even if the initial (neuro)inflammatory insult did not. Upon secondary challenge at a distant time, the enhanced release of proinflammatory and neurotoxic molecules can then result in greater (neuro)inflammation, cell death, and DAMP release, and so on (Figure.1). This may explain, for example, how prior episodes of depression for example appear to sensitize immune responses to subsequent depressive episodes, with levels of proinflammatory cytokines and chemokines increasing with sequential depressive episodes [67,68]. Indeed, several studies have found that baseline depression and/or anxiety, even subclinical, predict a later development of depression in hepatitis C Virus patients receiving the standard treatment with the proinflammatory cytokine IFN-α [14-17]. One plausible hypothesis for such phenomenon is that upon primary challenge with the virus, DAMPs recognition by PRRs elicit antimicrobial responses that regulate facets of the innate immune system [8], which potentially predispose to the occurrence of a first episode of depression. In addition, such DAMPs-mediated training immunity implicates regulation of monocites to macrophages differentiation [8], which upon secondary challenge with IFN-α treatment may ultimately predispose the individual to a second episode of depression. Although several studies reported that a history of past psychiatric disturbance significantly increased the risk of depression during IFN-α therapy [18], on the other hand other studies did not find that a past history of depression significantly increases the risk of neuropsychiatric disturbance [14, 19-21]. This suggests that having a blunted response is also plausible and that the release of DAMP in presence of (neuro)inflammation could theoretically result in tolerance. However, whether tolerance or training occurs may well be dependent on the nature, doses, duration and frequency of immunostimulatory events.

Conclusion

Many questions relating to the contribution of trained innate immunity to disease onset and progression in neuropsychiatric disorders need to be addressed. The discovery of trained innate immunity has opened up a new dimension in the field of immunotherapeutics, such as vaccine development. It is likely that trained innate immunity will also have a significant impact on our understanding of disorders in which the innate immune system is implicated, including neuropsychiatric illnesses, as well as the development of novel treatments for such disorders.

Conflicts

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References

1. Berk M, Williams LJ, Jacka FN, Neil AO, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013 Sep 12;11(1):1–1.

2. Perry VH, Cunningham C, Holmes C. Systemic infections and inflammation affect chronic neurodegeneration. Nat Rev Immunol. 2007 Feb;7(2):161–7.

3. De Chiara G, Marcocci ME, Sgarbanti R, Civitelli L, Ripoli C, Piacentini R, et al. Infectious agents and neurodegeneration. Mol Neurobiol. 2012 Dec;46(3):614–38.

4. Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am J Psychiatry. 2010 Mar;167(3):261–80.

5. Jones KA, Thomsen C. The role of the innate immune system in psychiatric disorders. Mol Cell Neurosci. 2013 Mar;53:52–62.

6. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. J Neuroinflammation. 2013;10:43.

7. Lull ME, Block ML. Microglial activation and chronic neurodegeneration. Neurotherapeutics. 2010 Oct;7(4):354–65.

8. Bakunina N, Pariante CM, Zunszain PA. Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology. 2015 Mar;144(3):365–73.

9. Streit WJ, Mrak RE, Griffin WST. Microglia and neuroinflammation: a pathological perspective. J Neuroinflammation. 2004 Jul 30;1(1):14.

10. Beumer W, Gibney SM, Drexhage RC, Pont-Lezica L, Doorduin J, Klein HC, et al. The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. J Leukoc Biol. 2012 Oct 31;92(5):959–75.

11. Perry VH, Nicoll JAR, Holmes C. Microglia in neurodegenerative disease. Nat Rev Neurol. 2010 Apr;6(4):193–201.

12. Yirmiya R, Rimmerman N, Reshef R. Depression as a Microglial Disease. Trends Neurosci. 2015 Oct 1;38(10):637–58.

13. Soczynska JK, Mansur RB, Brietzke E, Swardfager W, Kennedy SH, Woldeyohannes HO, et al. Novel therapeutic targets in depression: Minocycline as a candidate treatment. Behav Brain Res. 2012 Dec 1;235(2):302–17.

14. Zheng X, Zhang X, Wang G, Hao H. Treat the brain and treat the periphery: toward a holistic approach to major depressive disorder. Drug Discov Today. 2015 May;20(5):562–8.

15. Delves PJ, Martin SJ, Burton DR, Roitt IM. Roitt's essential immunology. John Wiley & Sons; 2011 Oct 7.

16. Netea MG, Latz E, Mills KHG, O'Neill LAJ. Innate immune memory: a paradigm shift in understanding host defense. Nat Immunol. 2015 Jul 1;16(7):675–9.

17. Netea MG, Quintin J, van der Meer JWM. Trained Immunity: A memory for innate host defense. Cell Host & Microbe. 2011 May 19;9(5):355–61.

18. van 't Wout JW, Poell R, van Furth R. The role of BCG/PPD-activated macrophages in resistance against systemic candidiasis in mice. Scand J Immunol. 1992 Nov;36(5):713–9.

19. Bistoni F, Verducci G, Perito S, Vecchiarelli A, Puccetti P, Marconi P, et al. Immunomodulation by a low-virulence, agerminative variant of Candida albicans. Further evidence for macrophage activation as one of the effector mechanisms of nonspecific anti-infectious protection. J Med Vet Mycol. 1988;26(5):285–99.

20. Condliffe AM, Kitchen E, Chilvers ER. Neutrophil priming: pathophysiological consequences and underlying mechanisms. Clin Sci. 1998 May;94(5):461–71.

21. Netea MG. Training innate immunity: the changing concept of immunological memory in innate host defence. Eur J Clin Invest. 2013 Jul 20;43(8):881–4.

22. Schroder K, Sweet MJ, Hume DA. Signal integration between IFNγ and TLR signalling pathways in macrophages. Immunobiology. 2006 Sep;211(6-8):511–24.

23. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. Nat Rev Neurol. 2014 Mar 18;10(4):217–24.

24. Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol. 2009 Apr;27(1):119–45.

25. Wynne AM, Henry CJ, Godbout JP. Immune and behavioral consequences of microglial reactivity in the aged brain. Integr Comp Biol. 2009 Aug 22;49(3):254–66.

26. Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LAB, Jacobs C, et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. J Innate Immun. 2014;6(2):152–8.

27. Ramirez K, Shea DT, McKim DB, F RB, Sheridan JF. Imipramine attenuates neuroinflammatory signaling and reverses stress-induced social avoidance. Brain Behav Immun. 2015 May 1;46(C):212–20.

28. Poli A, Kmiecik J, Domingues O, Hentges F, Blery M, Chekenya M, et al. NK cells in central nervous system disorders. J Immunol. 2013 May 17;190(11):5355–62.

29. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006 Feb;124(4):783–801.

30. Lien E, Means TK, Heine H, Yoshimura A, Kusumoto S, Fukase K, et al. Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. J Clin Invest. 2000 Feb;105(4):497–504.

31. Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol. 2009 Oct;30(10):475–87.

32. Martin SF. Adaptation in the innate immune system and heterologous innate immunity. Cell Mol Life Sci. 2014 Jul 6;71(21):4115–30.

33. Ifrim DC, Quintin J, Joosten LAB, Jacobs C, Jansen T, Jacobs L, et al. Trained immunity or tolerance: opposing functional programs induced in human monocytes after engagement of various pattern recognition receptors. Clin Vaccine Immunol. 2014 Mar 26;21(4):534–45.

34. Tribouley J, Tribouley-Duret J, Appriou M. Effect of Bacillus Callmette Guerin (BCG) on the receptivity of nude mice to Schistosoma mansoni. C R Seances Soc Biol Fil. 1978;172(5):902–4.

35. Bistoni F, Vecchiarelli A, Cenci E, Puccetti P, Marconi P, Cassone A. Evidence for macrophage-mediated protection against lethal Candida albicans infection. Infect Immun. 1986 Feb;51(2):668–74.

36. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LAB, Ifrim DC, Saeed S, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A. 2012 Oct 23;109(43):17537–42.

37. Jensen KJ, Larsen N, Biering-Sørensen S, Andersen A, Eriksen HB, Monteiro I, et al. Heterologous immunological effects of early BCG vaccination in low-birth-weight infants in Guinea-Bissau: a randomized-controlled trial. J Infect Dis. 2015 Mar 15;211(6):956–67.

38. Quintin J, Saeed S, Martens JHA, Giamarellos-Bourboulis EJ, Ifrim DC, Logie C, et al. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. Cell Host & Microbe. 2012 Aug;12(2):223–32.

39. Püntener U, Booth SG, Perry VH, Teeling JL. Long-term impact of systemic bacterial infection

on the cerebral vasculature and microglia. J Neuroinflammation. 2012;9:146.

40. Bilbo SD. Neonatal infection-induced memory impairment after lipopolysaccharide in adulthood is prevented via caspase-1 inhibition. J Neuroscience. 2005 Aug 31;25(35):8000–9.

41. Bland ST, Beckley JT, Watkins LR, Maier SF, Bilbo SD. Neonatal Escherichia coli infection alters glial, cytokine, and neuronal gene expression in response to acute amphetamine in adolescent rats. Neurosci Lett. 2010 Apr 19;474(1):52–7.

42. Ramos HJ, Lanteri MC, Blahnik G, Negash A, Suthar MS, Brassil MM, et al. IL-1β Signaling Promotes CNS-Intrinsic Immune Control of West Nile Virus Infection. PLoS Pathog. 2012 Nov 29;8(11):e1003039–16.

43. Chauhan VS, Sterka DG Jr., Furr SR, Young AB, Marriott I. NOD2 plays an important role in the inflammatory responses of microglia and astrocytes to bacterial CNS pathogens. Glia. 2009 Mar;57(4):414–23.

44. Williamson LL, Sholar PW, Mistry RS, Smith SH, Bilbo SD. Microglia and Memory: Modulation by Early-Life Infection. Journal of Neuroscience. 2011 Oct 26;31(43):15511–21.

45. Cao M, Cortes M, Moore CS, Leong SY, Durosier LD, Burns P, et al. Fetal microglial phenotype in vitro carries memory of prior in vivo exposure to inflammation. Front Cell Neurosci. 2015 Aug 4;9(150):12.

46. Ye M, Wang Q, Zhang W, Li Z, Wang Y, Hu R. Oroxylin A exerts anti-inflammatory activity on lipopolysaccharide-induced mouse macrophage via Nrf2/ARE activation. Biochem Cell Biol. 2014 Oct;92(5):337–48.

47. Seong SY, Matzinger P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. Nat Rev Immunol. 2004 Jun;4(6):469-78.

48. Matt U, Sharif O, Martins R, Knapp S. Accumulating evidence for a role of oxidized phospholipids in infectious diseases. Cell Mol Life Sci. 2014 Nov 20;72(6):1059–71.

49. Bekkering S, Quintin J, Joosten LAB, van der Meer JWM, Netea MG, Riksen NP. Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. Arterioscler Thromb Vasc Biol. 2014 Aug;34(8):1731–8.

50. Manson J, Thiemermann C, Brohi K. Trauma alarmins as activators of damage-induced inflammation. Br J Surg. 2012 Jan;99 Suppl 1:12–20.

51. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, et al. Inflammation after trauma: Microglial activation and traumatic brain injury. Ann Neurol. 2011 Jun 27;70(3):374–83.

52. Jorge RE, Robinson RG, Moser D, Tateno A, Crespo-Facorro B, Arndt S. Major depression following traumatic brain injury. Arch Gen Psychiatry. 2004 Jan;61(1):42–50.

53. Fenn AM, Gensel JC, Huang Y, Popovich PG, Lifshitz J, Godbout JP. Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. Biolo

Psychiatry. 2014 Oct 1;76(7):575-84.

54. Fleshner M, Campisi J, Amiri L, Diamond DM. Cat exposure induces both intra- and extracellular Hsp72: the role of adrenal hormones. Psychoneuroendocrinology. 2004 Oct;29(9):1142–52.

55. Weber MD, Frank MG, Tracey KJ, Watkins LR, Maier SF. Stress induces the danger-associated molecular pattern hmgb-1 in the hippocampus of male sprague dawley rats: a priming stimulus of microglia and the nlrp3 inflammasome. J Neuroscience. 2015 Jan 7;35(1):316–24.

56. Maslanik T, Mahaffey L, Tannura K, Beninson L, Greenwood BN, Fleshner M. The inflammasome and danger associated molecular patterns (DAMPs) are implicated in cytokine and chemokine responses following stressor exposure. Brain Behav Immun. 2013 Feb 1;28(C):54–62.

57. Fleshner M. Stress-evoked sterile inflammation, danger associated molecular patterns (DAMPs), microbial associated molecular patterns (MAMPs) and the inflammasome. Brain Behav Immun. 2013 Jan 1;27(C):1–7.

58. Saeed S, Quintin J, Kerstens HHD, Rao NA, Aghajanirefah A, Matarese F, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science. 2014 Sep 25;345(6204):1251086–6.

59. Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, et al. mTOR- and HIF-1 -mediated aerobic glycolysis as metabolic basis for trained immunity. Science. 2014 Sep 25;345(6204):1250684–4.

60. Yoshida K, Maekawa T, Zhu Y, Renard-Guillet C, Chatton B, Inoue K, et al. The transcription factor ATF7 mediates lipopolysaccharide-induced epigenetic changes in macrophages involved in innate immunological memory. Nat Immunol. 2015 Aug 31;16(10):1034–43.

61. Maekawa T, Kim S, Nakai D, Makino C, Takagi T, Ogura H, et al. Social isolation stress induces ATF-7 phosphorylation and impairs silencing of the 5-HT 5B receptor gene. The EMBO Journal. 2009 Nov 5;29(1):196–208.

62. Schaafsma W, Zhang X, van Zomeren KC, Jacobs S, Georgieva PB, Wolf SA, et al. Long-lasting pro-inflammatory suppression of microglia by LPS-preconditioning is mediated by RelB-dependent epigenetic silencing. Brain Behav Immun. 2015 Aug 1;48(C):205–21.

63. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. Maternal immune activation and abnormal brain development across CNS disorders. Nat Rev Neurol. 2014 Oct 14;10(11):643–60.

64. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature. 2001 Oct 18;413(6857):732-8.

65. Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, et al. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science. 2013 Feb 28;339(6123):1095–9.

66. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis

reveals that adult microglia derive from primitive macrophages. Science. 2010 Nov 4;330(6005):841–5.

67. Maes M, Mihaylova I, Kubera M, Ringel K. Activation of cell-mediated immunity in depression: Association with inflammation, melancholia, clinical staging and the fatigue and somatic symptom cluster of depression. Prog Neuropsychopharmacol Biol Psychiatry. 2012 Jan 10;36(1):169–75.

68. Celik C, Erdem M, Caycı T, Ozdemir B, Akgul EO, Kurt YG, et al. The association between serum levels of neopterin and number of depressive episodes of major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2010 Mar 17;34(2):372–5.

1. Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *Lancet Psychiatry* 2015; **2**(3)**:** 258-270.

2. Jeon SW, Kim YK. Neuroinflammation and cytokine abnormality in major depression: Cause or consequence in that illness? *World J Psychiatry* 2016; **6**(3)**:** 283-293.

3. Aricioglu F, Ozkartal CS, Unal G, Dursun S, Cetin M, Muller N. Neuroinflammation in Schizophrenia: A Critical Review and The Future. *Klin Psikofarmakol B* 2016; **26**(4)**:** 429-437.

4. Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC. CNS immune privilege: hiding in plain sight. *Immunol Rev* 2006; **213:** 48-65.

5. Borsini A, Zunszain PA, Thuret S, Pariante CM. The role of inflammatory cytokines as key modulators of neurogenesis. *Trends in neurosciences* 2015; **38**(3)**:** 145-157.

6. Borsini A, Alboni S, Horowitz MA, Tojo LM, Cannazza G, Su KP *et al.* Rescue of IL-1beta-induced reduction of human neurogenesis by omega-3 fatty acids and antidepressants. *Brain, behavior, and immunity* 2017.

7. Lull ME, Block ML. Microglial activation and chronic neurodegeneration. *Neurotherapeutics* 2010; **7**(4)**:** 354-365.

8. Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, Matarese F *et al.* Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 2014; **345**(6204)**:** 1251086.

9. Talge NM, Neal C, Glover V, Early Stress TR, Prevention Science Network F, Neonatal Experience on C *et al.* Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry* 2007; **48**(3-4)**:** 245-261.

10. Glover V. Annual Research Review: Prenatal stress and the origins of psychopathology: an evolutionary perspective. *J Child Psychol Psychiatry* 2011; **52**(4)**:** 356-367.

11. Van den Bergh BR, Van Calster B, Smits T, Van Huffel S, Lagae L. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2008; **33**(3)**:** 536-545.

12. Sandman CA, Buss C, Head K, Davis EP. Fetal exposure to maternal depressive symptoms is associated with cortical thickness in late childhood. *Biological psychiatry* 2015; **77**(4)**:** 324-334.

13. Depino AM. Early prenatal exposure to LPS results in anxiety- and depression-related behaviors in adulthood. *Neuroscience* 2015; **299:** 56-65.

14. Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J *et al.* A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. *Molecular psychiatry* 2002; **7**(9)**:** 942-947.

15. Dieperink E, Ho SB, Thuras P, Willenbring ML. A prospective study of neuropsychiatric symptoms associated with interferon-alpha-2b and ribavirin therapy for patients with chronic hepatitis C. *Psychosomatics* 2003; **44**(2)**:** 104-112.

16. Capuron L, Ravaud A. Prediction of the depressive effects of interferon alfa therapy by the patient's initial affective state. *The New England journal of medicine* 1999; **340**(17)**:** 1370.

17. Van Thiel DH, Friedlander L, De Maria N, Molloy PJ, Kania RJ, Colantoni A. Treatment of chronic hepatitis C in individuals with pre-existing or confounding neuropsychiatric disease. *Hepato-gastroenterology* 1998; **45**(20)**:** 328-330.

18. Gohier B, Goeb JL, Rannou-Dubas K, Fouchard I, Cales P, Garre JB. Hepatitis C, alpha interferon, anxiety and depression disorders: a prospective study of 71 patients. *World J Biol Psychiatry* 2003; **4**(3)**:** 115-118.

19. Renault PF, Hoofnagle JH, Park Y, Mullen KD, Peters M, Jones DB *et al.* Psychiatric complications of long-term interferon alfa therapy. *Archives of internal medicine* 1987; **147**(9)**:** 1577-1580.

20. Schaefer M, Schmidt F, Folwaczny C, Lorenz R, Martin G, Schindlbeck N *et al.* Adherence and mental side effects during hepatitis C treatment with interferon alfa and ribavirin in psychiatric risk groups. *Hepatology* 2003; **37**(2)**:** 443-451.

21. Pariante CM, Orru MG, Baita A, Farci MG, Carpiniello B. Treatment with interferon-alpha in patients with chronic hepatitis and mood or anxiety disorders. *Lancet* 1999; **354**(9173)**:** 131-132.

Figure legend

Figure 1. Trained innate immunity and a resulting neuroinflammatory cycle. Red represents an immune stimulation, blue represents a consequence. The release of DAMP is a consequence of neuronal injury and death, but can serve as a potential secondary immune stimulation, as well as a reinforcing stimulation for pre-existing training.