Towards Omics-based, Systems Biomedicine and Path and Drug Discovery Methodologies for Depression-Inflammation Research.

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Abstract

Meta-analyses confirm that depression is accompanied by signs of inflammation including increased levels of acute phase proteins, e.g. C-reactive protein, and pro-inflammatory cytokines, e.g. interleukin-6. Supporting the translational significance of this, a meta-analysis showed that anti-inflammatory drugs may have antidepressant effects. Here we argue that inflammation and depression research needs to get onto a new track. Firstly, the choice of inflammatory biomarkers in depression research was often too selective and did not consider the broader pathways. Secondly, although mild inflammatory responses are present in depression, other immune-related pathways cannot be disregarded as new drug targets, e.g. activation of cell-mediated immunity, oxidative and nitrosative stress (O&NS) pathways, autoimmune responses, bacterial translocation, activation of the Toll-like Receptor and neuroprogressive pathways. Thirdly, anti-inflammatory treatments are sometimes used without full understanding of their effects on the broader pathways underpinning depression. Since many of the activated immune-inflammatory pathways in depression actually confer protection against an overzealous inflammatory response, targeting these pathways may result in unpredictable and unwanted results. Furthermore, this paper discusses the required improvements in research strategy, i.e. path and drug discovery processes, omics-based techniques and systems biomedicine methodologies. Firstly, novel methods should be employed to examine the intracellular networks that control and modulate the immune, O&NS and neuroprogressive pathways using omics-based assays, including genomics, transcriptomics, proteomics, metabolomics, epigenomics and microbiomics. Secondly, systems biomedicine analyses are essential to unravel the complex interactions between these cellular

networks, pathways and the multifactorial trigger factors and to delineate new drug targets in the cellular networks or pathways. Drug discovery processes should delineate new drugs targeting the intracellular networks and immune-related pathways.

Key words: depression, immune, inflammation, neuroprogression, oxidative and nitrosative stress, leaky gut, IDO, TRYCATs

Introduction

Depression, either unipolar or bipolar depression, is a life-long systemic and multifactorial disorder. Until recently, major elements of its pathophysiology were largely unknown and therefore targeted treatments (outside the monoamines) could not be developed. Classical antidepressants, which target monoamine systems, show a clinical efficacy that is only marginally better than placebo [1]. Recent meta-analyses summarize that depression is accompanied by mild systemic inflammation including increased levels of inflammatory cytokines, e.g. interleukin (IL)-6, IL-1 and tumor necrosis factor (TNF)α, and signs of an acute phase response [2,3]. Recently, a systematic review and a meta-analysis showed that anti-inflammatory drugs may have antidepressant effects [4,5]. The latter authors state that because there is an inflammatory state in depression, anti-inflammatory drugs were examined, and that the results of their meta-analysis are proof of concept that anti-inflammatory drugs are useful in depression. One of the major non-profit organizations supporting research on the causes of bipolar disorder, i.e. the Stanley Medical Research Institute, launched in 2014 a call to support research into the treatment of bipolar depression with anti-inflammatory drugs. Here it is contended that there are gaps in the way that inflammation in depression research is currently conceptualized and consequently we suggest a more powerful direction for future immune-related depression research.

Inflammation is not the only drug target in depression

As described by the Scientist (2003) “the first inkling that there is a connection between depression and inflammation came in 1990 when Michael Maes, a Belgian psychiatrist, reported

that depression is accompanied by an activation of the inflammatory response system". Nevertheless, mild chronic inflammation [1,6,7] is only one very general concept in depression and describes its pathophysiology only superficially [8]. Since 1990, other and sometimes more important immune-related pathways were discovered, e.g. activation of cell-mediated immunity (CMI), including T cell activation, e.g. a T helper (Th)1 shift [9]; activation of oxidative stress pathways [10-12]; decreased levels of key antioxidants, including coenzyme Q10 [12,13]; hypernitrosylation [12,14]; autoimmune responses directed against oxidative and nitroso-specific epitopes [12,14]; increased bacterial translocation followed by increased immune responses to lipopolysaccharides (LPS) of gram negative bacteria [15]; and activation of the Toll-like Receptor (TLR) 2/4 Radical Cycle [16-19].

Moreover, depression is characterized by immune-serotonin interactions, including activation of indoleamine 2,3-dioxygenase (IDO) with increased levels of tryptophan catabolites (TRYCATs) and lowered levels of tryptophan [20-22]; immune-endocrine interactions, including cytokine-associated glucocorticoid resistance [6,9]; and immune-metabolic (in particular lipids) interactions, including inverse associations between immune activation and lowered high density cholesterol, □3 polyunsaturated fatty acid (PUFAs) levels and the reverse cholesterol transport [11,23]. Interestingly, animal depression models based on psychosocial stress show that depression-like behaviors are associated with activated immune-inflammatory and oxidative and nitrosative stress (O&NS) pathways [24,25]. New animal models of depression have been developed based on chronic (repeated intermittent) administration of LPS and peripheral and central activation of the TLR complex and [26-28]. The latter studies suggest a bifunctional role of TLR-4 signaling pathway by triggering neuroinflammation at prefrontal cortex level and by

regulating gut barrier function and permeability [27,28]. Moreover they also show that bacterial translocation is responsible, at least in part, for the TLR-4 activation found in the brain after the exposure to the depression model [27,28].

Finally, all abovementioned immune- and O&NS-related pathways and interactions may explain the presence of neuroprogressive processes in depression, that is neuronal changes including reduced bioenergetic mitochondrial functions, neuroplasticity and neurogenesis, cell signaling dysfunctions, apoptosis, etc. [29-32]. Immune-inflammatory, O&NS, autoimmune and neuroprogressive pathways are involved in shaping depressive phenomenology, staging of depression (e.g. treatment resistance, recurrence, chronicity and sensitization) and the comorbidity with many (neuro)inflammatory or (auto)immune disorders [29-32]. Shared immune and O&NS pathways in part explain the robust comorbidity of depression with medical conditions, e.g. a) cardiovascular disorder; chronic obstructive pulmonary disease; systemic lupus erythematosus; HIV infection; inflammatory bowel disease; obesity; the metabolic syndrome; diabetes type 2; rheumatoid arthritis; chronic kidney disease; alcohol dependence; tobacco use disorder; and b) central nervous system (CNS) disorders characterized by neuroinflammation, e.g. Alzheimer's and Parkinson's disease; multiple sclerosis; and stroke [33,34]. Moreover, depression when suffered with other medical and CNS disorders may increase morbidity and mortality of these comorbid disorders [34]. The postnatal period, interferon-(IFN)α-based immunotherapy, nicotine dependence, hemodialysis and psychosocial stressors (life events, chronic stress) are other immune- and O&NS-related conditions that may trigger the onset of depression in some vulnerable subjects [33,34].

Gaps and required improvements: biomarkers

Measuring a few selected biomarkers, e.g. IL-6 without measurement of its soluble receptor (sIL-6R) or C-reactive protein (CRP), became the gold standard in inflammation and depression research [35,36]. However, appropriate acute phase protein biomarkers of clinical depression other than CRP were already established in the 1991-1992, e.g. haptoglobin, albumin and transferrin [2]. Nevertheless, most research focused on CRP despite the important role of for example haptoglobin in the immune response, bacterial translocation and O&NS pathways and a possible association between haptoglobin polymorphism (Hp1 and Hp2) and depression [2,37-39].

As an exemplar of the broader issue, IL-6 binds to the IL-6 receptor (IL-6R), and the combined complex of IL-6 and IL-6R binds to glycoprotein (gp) 130, which triggers intracellular signaling. The protein gp130 is expressed ubiquitously on cells, but IL-6R is expressed only on a few cells. A soluble form of IL-6R (sIL-6R) also binds IL-6, and this complex also binds to gp130 on cells that don't express the IL-6R; this mechanism is called IL-6 trans-signaling. IL-6 trans-signaling is pro-inflammatory, while classic IL-6 signaling has anti-inflammatory or regenerative effects [40]. Thus, results on plasma IL-6 levels in isolation of the wider pathway without sIL-6R measurements does not allow one to make any inferences on the inflammatory consequences of IL-6 findings. IL-6 trans-signaling is probably increased in unipolar and bipolar depression and may underpin many of the immune-related pathways in depression [41,42]. Surprisingly, most if not all papers reporting on plasma IL-6 did not measure sIL-6R levels. All in all, this exemplar suggests that future research should focus on the broader pathways including IL-6 trans-signaling (and other cytokine networks) and other acute phase protein biomarkers in depression (zinc, albumin, transferrin, etc). More importantly, future research should consider

the role of the intracellular networks that control immune-inflammatory, O&NS, autoimmune and neuroprogressive pathways [8].

Intracellular signaling pathways, such as glycogen synthase kinase-3 (GSK-3), nuclear factor-‿B (NF-‿B), Janus kinase / signal transducers and activators of transcription (JAK-STAT), nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) and the TLR2/4 complexes and other networks, modulate the immune-inflammatory, O&NS and neuroprogressive pathways [8]. Considering the broad-spectrum immune-inflammatory, O&NS and neuroprogressive state it may be hypothesized that these intracellular signaling networks are dysfunctional in depression [8]. There is, however, a lack of knowledge about how these networks critically interact with the immune-related pathways and/or undergo changes in activity or topology to lead to depression and to explain depression's multiple comorbidities. None of these pathway and network markers exists in isolation and there are several complex and reciprocal interactions between the pathways and network molecules. Thus, it is needed to screen the whole system, i.e. pathways and networks, not the isolated components. Phrased differently, what is needed is to pinpoint the defects in the dynamic crosstalk between those pathways and networks.

Contemporary nosologic classifications of disorders derive from associations between pathological analyses and clinical syndromes and sometimes simple biomarkers [43]. This diagnostic strategy, however, has many limitations including lower sensitivity and specificity in correctly diagnosing the disorder [43]. With the growing new possibilities of –omics-based data sets, allowing researchers to measure and analyze network and pathway alterations, these older diagnostic strategies, combining syndromal phenomenology with simple biomarkers, will soon become a historical footnote [43]. As a comparison, a home inspection should focus on

evaluating the whole building not the bricks. The inspection should pinpoint major condition problems and hazardous defects in the building structure including rotten or cracked foundations, serious corrosion or roof structure issues, etc. Defect recognition and the consequent structural diagnosis are consequently used to repair or prevent structural building failures.

Gaps and required improvements: new treatments

In psychiatry history the usual and logic path from "defect recognition" to "structural diagnosis" to "targeted repair" is actually reversed. First it is observed what clinically effective treatments do physiologically and then these medication effects are used as proxy pathophysiological markers for reverse engineering of other pharmaceuticals. One highly relevant example is the discovery and further development of the classical antidepressants drugs. Iproniazid was initially employed in the treatment of tuberculosis before it was discovered to be useful in the treatment of depression [44]. Also the discovery in the 1950s that imipramine, developed as an antihistamine, has antidepressant efficacy was by chance. The effects of these drugs on the monoaminergic systems were used to postulate the monoaminergic theories, including the serotonin hypothesis of depression [8]. This in turn fostered the (at first sight) pathophysiologically-guided development of selective serotonin reuptake inhibitors (SSRIs) [44]. Only later it was established that the efficacy of these drugs is much lower than first thought and probably only slightly better than placebo [1]. Thus, when all studies, either published or unpublished, submitted to the FDA were considered, the number of positive randomized controlled trials was only 51% [45,46]. This lack of effect of serotonergic drugs might be regarded as proof that antidepressants such as SSRIs cannot be regarded as "disease-centred" drugs, i.e. drugs that normalize defects in 5-HT, which were thought to underpin the

pathophysiology of depression [45]. The proposed "drug-centred model" similarly attempts to explain the working mechanism of antidepressants, i.e. SSRIs increase 5-HT and therefore may improve depression via effects on other systems. Recently, we argued that the effects of antidepressants, including SSRIs, do not neatly fit either the serotonin disease-centred or the drug-centred models [8]. Indeed, antidepressants exert at least part of their clinical efficacy by attenuating inflammatory, immune (Th1), O&NS and neuroprogressive pathways [8,12,24,25,41,47-52]. Moreover, the efficacy of antidepressants may be augmented by administration of compounds targeting immune-inflammatory, O&NS and neuroprogressive pathways [4,8]. Nevertheless, for at least 30 years the 5-HT hypothesis of depression has guided research into the pathophysiology of depression and fostered the development of new, usually similar antidepressant drugs. Given the new possibilities offered by -omics-based and systems biomedicine methodologies, this old reversed path of discovery in psychiatric research should be augmented to make room for a more logical and pathophysiologically valid systems biomedicine based "path and drug discovery process" as we will explain below.

The role of multiple immune-related pathways in the onset and course of depression equally shows that it is too simplistic to consider that the inflammatory component in depression may be the only drug target [8]. There is as yet no compelling evidence that anti-inflammatory agents improve the O&NS, autoimmune and neuroprogressive pathways in depression. More importantly, many of the activated immune-inflammatory components in depression actually confer protection against an overzealous inflammatory response. Previously, we have described that depression is not only accompanied by a pro-inflammatory response but also by counter-regulatory processes which tend to limit an overzealous immune-inflammatory response, i.e. the

compensatory anti-inflammatory reflex system (CIRS) [53]. For example, increases in sIL-1 receptor antagonist (sIL-1RA) and sIL-2R indicate immune-inflammatory responses in depression, but are part of the CIRS downregulating the primary immune response. Induction of the TRYCAT pathway through CMI, inflammatory and oxidative processes confers protection against the primary immune-inflammatory and oxidative responses through lowered levels of tryptophan and increased levels of TRYCATs, such as kynurenine, and kynurenic and xanthurenic acid [54,55]. Some acute phase proteins, including haptoglobin, have anti-inflammatory effects and act as antioxidants [37]. Elevated IL-6 levels may be anti-inflammatory and protective by increasing IL-1RA, IL-10 and glucocorticoid production [42,53]. Hypernitrosylation may downregulate intracellular signaling pathways including NF-κB and the TLR4 complex and thus may have restorative effects [56]. IgM-mediated autoimmune responses directed to oxidative specific epitopes are part of natural autoimmune responses that actually have restorative effects [57]. Peroxisome proliferator-activated receptor-gamma (PPARγ), a transcription factor up-regulated in brain following stress exposure [58], has been directly implicated in the regulation of the neuroinflammatory response because several of its ligands (such as 15d-PGJ_2) inhibit pro-inflammatory and O&NS mediators [59] as well as prevent excitotoxicity and energy compromise in the brain [60]. Thus, these CIRS responses have negative immunoregulatory, anti-inflammatory and/or antioxidant effects and may favor a Th2 or T regulatory responses thereby providing protection against immune-inflammatory and O&NS responses and neuroprogression [53]. Interfering with the equilibrium between the more detrimental and more protective forces may have unpredictable and unwanted effects. More

precise knowledge on the immune-inflammatory response and the CIRS in depression is needed before using drugs that interfere with this equilibrium.

Moreover, there are other caveats and lessons to learn. Anti-inflammatory drugs not only attenuate some specific inflammatory pathways but also unexpectedly drive other immune pathways to detrimental effects. A first example is COX-2 inhibition. Trials with selective COX-2 inhibitors in depression were initiated before it was known that COX-2 expression is increased in depression and thus that COX-2 is a new possible drug target in depression [61,62]. It was known that selective COX-2 inhibitors may cause neuro-inflammation, aggravate Th1 responses, decrease the levels of key antioxidants, increase lipid peroxidation, cause bacterial translocation, damage mitochondria, and aggravate neuroprogression and cardiovascular disorder [61]. Thus, while COX-2 inhibitors may downregulate an initial increase in COX-2 expression, these drugs could have unwanted effects on other pathways associated with depression [61]. A possible cause of these effects may be that the inhibition of COX-2 also produces a decrease in the levels of 15-PGJ_2, an endogenous ligand of transcription factor PPARγ. In this regard, a recent randomized double-blind placebo-controlled trial has shown that pioglitazone, a ligand of PPARγ, is a safe and effective adjunctive short-term treatment in patients with moderate-to-severe depression even in the absence of metabolic syndrome and diabetes [63].

Statins are another example. These drugs are thought to have a potential anti-depressive effect related to their anti-inflammatory properties [8,64]. A number of epidemiological studies show that statin use reduces depression risk [65,66], and the only randomized controlled trial to date suggests that lovastatin reduced symptoms of depression [67]. However, there are conflicting data suggesting that statins may actually worsen depression [68]. Tuccori et al. [69]

review that statins may cause many neuropsychiatric “side effects”, including depression, irritability, paranoia, alienation, sleep disorders, neurocognitive impairments and muscle weakness. These negative effects of statins could possibly be driven through effects on other pathways, including lipid and serotonin metabolism, the key antioxidant coenzyme Q10 and induction of autoimmune responses [68-70]. Infliximab, a monoclonal antibody directed against TNF□ with anti-inflammatory and anti-oxidative effects, was recently shown to have antidepressive effects in patients with initially increased CRP levels [71]. Nevertheless, Etanercept and Infliximab, two anti-TNF agents used in juvenile arthritis and psoriasis, arthritis and inflammatory bowel disease in adults, have a long list of potentially severe side effects including depression, suicide, induction of mania, nervousness, anxiety, panic, aggressiveness, fatigue, sleep disorders [72-75]. While anti-TNF□ agents have anti-inflammatory and antioxidative effects and reduce Th1 and Th17 responses these drugs may cause infections and autoimmune responses [76].

Finally, it was reviewed that multi-targeting the abovementioned pathways, i.e. inflammation and Th1 activation and O&NS and neuroprogressive pathways may have therapeutic promise by exploiting the synergy between these pathways [8;77]. This is corroborated by reports that nutraceuticals with anti-inflammatory and anti-O&NS effects, which multi-target these pathways [e.g. ω3 PUFAs, zinc, N-acetyl cysteine and curcumin] may have a clinical efficacy in depression and reduce depressive-like behaviors in animal models [4,78-81]. Fond et al. [4], in their systematic review, conclude that of all possible anti-inflammatory treatments examined in depression ω3 PUFAs have the best benefit/risk ratio profile. Many other nutraceuticals with anti-inflammatory and anti-O&NS effects or their combinations (e.g.

coenzyme Q10, lipoic acid, green tea extract) are good drug candidates to be tested in randomized controlled trials because of their well known safety record and their properties multi-targeting the pathways involved in depression [8]. Different drugs that multi-target the different abovementioned networks and pathways are already developed, e.g. kinase inhibitors and Nrf-2 activators, but their efficacy and safety profile should first be screened in diverse (depression) models [8].

The message is that without exact knowledge of the detailed pathways and networks involved in depression and the exact effects of classical anti-inflammatory drugs on these pathways/networks, the outcome is unpredictable [8,82]. Thus, new trials with anti-inflammatory drugs should be preceded and guided by detailed understanding of these agents on the networks and pathways [8,82]. Using "path and drug discovery processes" in combination with systems biomedicine methodologies allows one to better delineate these pathways and networks and the effects of different drugs on these pathways [82].

The way forward: path and drug discovery, omics-based assays and systems biomedicine methodologies

State-of-the-art, omics-based assays, either high throughput or targeted, using standardized operational procedures, including network-focused Multi-Elisarrays and PCR Arrays measuring the NF-κB, JAK-STAT, GSK-3, Nfr2 and TLR networks in relation to immune-inflammatory, O&NS and neuroprogressive pathways, serotonin turnover, metabolic and endocrine functions and the microbiome should be carried out. This research should be translational including depressed patients with and without comorbid disorders and controls

(healthy volunteers and subjects with the comorbid disorders) and ideally include examination of plasma, cerebrospinal fluid (CSF) and stool microbiome samples and should examine –omics-based biomarkers that assess these networks and pathways, including genomics, transcriptomics, proteomics and immunopreoteomics, metabolomics, epigenomics, etc. . Proof of –omics-based research in patients should be checked in post-mortem brain in both patients and control groups.

Consequently, the complex systems medicine mechanisms underpinning depression and comorbid disorders and the sex-and gender-related differences, according to the gendered innovations approach [83] should be delineated using system medicine based methodologies [42,84-90]. Systems biomedicine approaches performed on omics-based high throughput and targeted measurements will likely be essential to a) unravel the non-linear and complex interactions between the networks, pathways, multifactorial trigger factors and comorbid disorders; b) pinpoint the molecular defects in these networks and pathways that cause depression and maintain the comorbid disorders; and c) develop a mathematical model that delineates the molecular signature and new biomarkers of depression. The identification of the peripheral and central cell signaling networks and immune-inflammatory, O&NS and neuroprogressive pathways that cause depression and maintain the comorbid disorders by means of systems biomedicine methods is likely to be a much needed advance compared with the present state of the art, which only considers the role played by a few inflammatory biomarkers. Visualization, machine learning and data mining tools should be used to explore and interpret the networks and pathways and a causal reasoning approach [86,87] should be applied to identify and rank trigger factors for depression and its phenotypes, including melancholic, physio-somatic, anxiety and atypical symptoms, suicidal ideation and behavior, neurocognitive disorders and staging characteristics. To prioritize trigger factors and delineate new intervention strategies,

existing knowledge databases, consisting of pre-compiled information on molecular and pathway analysis (e.g. the ResNet databases), could be used. Causal reasoning analysis and dedicated visualization tools can be used to delineate trigger and pathophysiological factors as well as new drug targets for therapeutic intervention [88,89]. State-of-the-art bioinformatic analyses could be employed to model the complex dynamic processes and predict the phenotypic effects of perturbations in the networks/pathways using their static structure [90]. Drug discovery processes could consequently identify the effects of new putative antidepressants. Towards this end, different families of new and selective kinase inhibitors, e.g. p38 MAPK, JAK 1,-2,-3 inhibitors and GSK-3 inhibitors, Nrf-2 activators and TLR2/4 antagonists should be tested in immune-inflammatory depression models.

All in all, the combination of path and drug discovery processes, omics-based assays and systems biomedicine methodologies will enable the generation of new systems biomedicine models in depression, to visualize the complex architecture of these networks and pathways whose alterations cause depression and maintain or worsen the comorbid disorders. Based on these assays, new omics-based biomarker tools may be developed that will be useful to diagnose depression. The systems biomedicine approach will hopefully delineate new drug targets and new combinatorial treatments that will aid in the prevention and the treatment of depression also when comorbid with (auto)immune or neuro-inflammatory disorders.

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