Pharmacogenomics of neuroimmune interactions in human psychiatric disorders

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There is bidirectional communication between the brain and the immune system. Overproduction of interleukin-1β (IL-1β) leads to systemic inflammatory response syndrome (SIRS). The crucial role of IL-1β in inflammation has been highlighted by studies performed in caspase-1 knockout mice (casp1$^{-/-}$), transgenic mice that lack mature IL-1β and survive lethal doses of lypopolysaccharide (LPS). We have previously shown that IL-1β, its receptor IL-1 receptor I (IL-1RI) and caspase-1 are expressed within the brain. Moreover, we documented that peripherally injected LPS triggers a specific spatiotemporal pattern of expression of IL-1β mRNA within the brain, suggesting that IL-1β could be a major regulator of the central inflammatory cascade. Therefore, we studied brain transcriptional patterns that occur during LPS-induced SIRS in wild-type and casp1$^{-/-}$ mice. We showed patterns of gene expression in wild-type and casp1$^{-/-}$ mice that included differential expression of several genes, such as those for cytokines, chemokines, nitric oxide synthase 2 and cyclo-oygenase 2. A key component of the neuroimmune-endocrine axis that is increased by IL-1β is corticotrophin releasing hormone (CRH). We found increased response to antidepressants in patients homozygous for the GAG haplotype of CRH receptor-1. Our results support the hypotheses that the CRH receptor-1 gene and possibly other genes in stress–inflammatory pathways are involved in the response to antidepressant treatment. Since dysregulation of the neuroimmune–endocrine axis appears to be one of the fundamental biological mechanisms that underlie psychiatric disorders, our findings might contribute to increase the understanding of the molecular pathways that are altered in these diseases.

The field of neuroimmunomodulation has evolved dramatically during the last 20 years. It is now very clear that peripheral cytokines modulate brain functions such as regulation of the hypothalamic–pituitary–adrenal (HPA) axis, fever, feeding, sickness behaviour and sleep (Vitkovic *et al.* 2000). Peripheral cytokines influence brain activity through at least four different routes: (1) peripheral organs synthesize and release cytokines that, by paracrine action, act on their receptors present in nerve fibres of the autonomic nervous system to modulate brain function; (2) circulating cytokines act within the brain vasculature to increase the expression of prostanoids and nitric oxide, which in turn diffuse through the brain parenchyma to trigger the inflammatory cascade; (3) cytokines might signal into the brain through specific areas that lack the blood–brain barrier (BBB), such as the circunventricular organs; or (4) alternatively, cytokines might enter the brain through a saturable transport mechanism. One of the most potent and well-described pro-inflammatory cytokines that modulates physiological and pathophysiological processes within the brain is interleukin-1β (IL-1β; Licinio & Wong, 1997*a*; Vitkovic *et al.* 2000; Allan & Rothwell, 2003; Elenkov *et al.* 2005).

Systemic administration of IL-1β produces several behavioural changes that include decreased locomotor activity, exploration and feeding (Swiergiel & Dunn, 2006). The crucial role exerted by IL-1β during lypopolysaccharide (LPS)-induced inflammation was previously demonstrated in knockout (KO) and transgenic mice with altered components of the IL-1β network (Li *et al.* 1995; Hirsch *et al.* 1996). Mice with a KO of the caspase-1 (casp1) gene, a cysteine-protease that cleaves

biologically inactive pro-IL-1β to render the biologically active 18 kDa IL-1β, are resistant to a lethal dose of LPS (Li *et al.* 1995). In contrast, mice with a KO of the endogenous IL-1 receptor antagonist (IL-1RA) were more sensitive to the lethal effects of LPS (Hirsch *et al.* 1996). Although in these two major reports the action of IL-1β within the brain was not differentiated from that shown in the periphery, the evidence indicating the major participation of IL-1β in neuroimmunomodulation is overwhelming (Licinio & Wong, 1997*b*; Wong *et al.* 1997; Licinio & Frost, 2000; Vitkovic *et al.* 2000; Elenkov *et al.* 2005; Swiergiel & Dunn, 2006). The action of IL-1β is regulated by a complex network of molecules that includes multiple ligands (IL-1α, IL-1β and IL-1RA), IL-1β accessory protein and casp1. We and others have previously shown that biological components of IL-1β are expressed within the brain (Wong & Licinio, 1994; Wong *et al.* 1995, 1996*a*, 1997; Licinio & Wong, 1997*b*) as well as in the pituitary (Licinio & Wong, 1997*b*).

Interestingly, after peripheral injection of LPS, there is a specific spatiotemporal pattern of expression of IL-1β mRNA that is initially found within brain vasculature and brain areas that are predominantly outside the BBB and that later progresses towards regions within the brain parenchyma (Wong *et al.* 1997). A similar spatiotemporal pattern of expression was also described for the gene for the inducible isoform of nitric oxide synthase (iNOS; Wong *et al.* 1996*b*), a gene whose expression is increased by IL-1β. The biological relevance of IL-1β to control key elements of the CNS response to peripheral inflammation has been substantiated by the discovery that those responses could be attenuated or abolished by specific inhibition exerted by central injection of IL-1RA. In fact, centrally injected IL-1RA was able to block the peripherally injected LPS-induced increase expression of corticotrophin releasing hormone (CRH) mRNA within the paraventricular nucleus (PVN; Licinio & Wong, 1997*a*). Similarly, centrally or peripherally injected IL-1β produces a decrease in social behaviour, anhedonia and decreased food intake, symptoms that are also attenuated or abolished by centrally but not by peripherally injected IL-1RA (Licinio & Wong, 1997*a*). There is evidence that IL-1β expression within the brain is increased in response to acute and chronic insults (Loddick *et al.* 1997; Rothwell, 2003). In a model of experimentally induced stroke, our research team has shown that IL-1RA acts as an endogenous neuroprotective agent (Loddick *et al.* 1997). Indeed, it was shown in rats that were subjected to middle cerebral arterial occlusion (MCAo) that the stroke volume of their brains was increased to a greater extent in the animals that were pretreated with a centrally injected neutralizing antibody against IL-1RA (Loddick *et al.* 1997; Rothwell, 2003). However, since the expression of the genes that exert negative control of the expression of IL-1β, such as IL-10, IL-13 and IL1-RA, is much lower

within the brain than in the periphery, it suggests that the brain is more vulnerable to the deleterious effects exerted by IL-1β (Licinio & Wong, 1997*a*; Wong *et al.* 1997).

It has been hypothesized that dysregulation of the expression of pro-inflammatory cytokines, such as IL-1β, may be one of several factors underlying the pathophysiological conditions of neuropsychiatric and neurodegenerative diseases such as major depressive disorders (MDD) and Alzheimer's disease (Licinio & Wong, 1999; Elenkov *et al.* 2005; Mrak & Griffin, 2005). Interleukin-1β is capable of inducing several events that also occur in Alzheimer's disease, such as stimulation of the amyloidogenic pathway of amyloid precursor protein processing and impairment of cholinergic signal transduction (Combs *et al.* 2001). With regard to MDD, accumulating evidence suggests that biological components of the innate immune system play a major role in the pathophysiological conditions that underlie MDD (Maes *et al.* 1995; Elenkov *et al.* 2005). There is interesting evidence that different classes of antidepressants decrease the synthesis and release of IL-1β (Castanon *et al.* 2002) and the activity of cyclo-oxygenase 2 (COX-2; Muller *et al.* 2006), suggesting that normalization of components of the innate immune system might constitute a prerequisite to accomplish remission. The causative relationship between IL-1β and neuropsychiatric disorders is also supported by the recent findings that suggest that increased IL-1β might be responsible for the secondary effects shown during the therapeutical use of interferon-α (IFN-α) to treat viral hepatitis and several malignancies (Kaneko *et al.* 2006). Interferon-α possesses immune-activating, antiviral and antiproliferative properties but, in addition to its clinically beneficial effects, long-term and high-dose use of IFN-α has been noted to be frequently associated with various neuropsychiatric adverse effects, such as insomnia, agitation, cognitive dysfunction, depression and memory disturbance (Raison *et al.* 2005). In particular, MDD observed as a side-effect of IFN-α treatment has been reported in up to 30–45% of patients. Interruption of treatment is recommended in most of the cases (Kaneko *et al.* 2006). Recently, it was demonstrated in rats that administration of IFN-α decreased hyppocampal neurogenesis by a mechanism that required IL-1β (Kaneko *et al.* 2006), suggesting that IL-1β could be implicated in some of the adverse neuropsychiatric side-effects described above. The possibility exists that pronounced expression of IL-1β within the brain in the context of limited expression of the regulatory genes, such as IL-10, IL-1RA and IL-13 (Wong *et al.* 1997), could result in deleterious and irreparable effects within the CNS.

In order to better understand the molecular events that occur within the brain during inflammation and the specific roles of IL-1β in this inflammatory process, we have studied the molecular pathways triggered by LPS within the brain by performing microarray studies

in wild-type and casp1 KO mice during LPS-induced systemic inflammatory response syndrome (SIRS), a pathophysiological condition that causes the loss of more than 200 000 lives per year in the USA (Mastronardi *et al.* 2007). Our results suggested that LPS-induced IL-1β plays an important stimulatory role in the regulation of gene expression within the brain. We found that there were eight genes that had a lower level of LPS-induced expression in the casp1 KO mice, suggesting that IL-1β is a major factor to control their expression during SIRS. These genes were two GTPases (TGTP and GBP-2), two chemokines (CXCL-1 and CXCL-10), the metalloprotease ADAMTs1, IL-1RA, iNOS and COX-2 (Mastronardi *et al.* 2007). Thus, our results revealed the relevance of biologically active IL-1β to control the expression of all of the mentioned genes. Since it is well known that NO increases the synthesis of prostaglandins by stimulating the expression of the inducible COX-2, it appears that the lower stimulation of the DNA-directed synthesis of NOS2 in casp1$^{-/-}$ mice led to decreased expression of COX-2. Decreased level of expression of CXCL-1 and CXCL-10 in the casp1$^{-/-}$ mice during the LPS-induced inflammatory stress might account for decreased recruitment of monocytes and neutrophils within the brain. With regard to ADAMTS1, the diminished expression of this metalloprotease could also be neuroprotective, since metalloproteases were described to increase BBB permeability during inflammation. Collectively, these results suggest that the brain of the casp1$^{-/-}$ mice might be more refractory to the deleterious effects produced by LPS-induced SIRS and could thus explain, at least in part, the survival of casp1$^{-/-}$ and mutant mice that overexpressed IL-1RA following lethal doses of LPS (Li *et al.* 1995; Hirsch *et al.* 1996).

The fact that sickness-like behaviour can be elicited by both pro-inflammatory cytokines and MDD supports the concept that common molecular pathways are shared by inflammation and neuropsychiatric disorders. Interestingly, it was recently shown that the COX-2 inhibitor celecoxib has therapeutic effects in both MDD (Muller *et al.* 2006) and schizophrenia (Riedel *et al.* 2005). Thus, reduction of casp1 bioactivity (Dinarello, 2004; Loher *et al.* 2004) might be a therapeutic strategy to treat both SIRS and neuropsychiatric disorders with prominent inflammatory components. It is noteworthy that casp1 inhibitors are the first orally active agents that target cytokines. The casp1 inhibitor pranalcasan is in clinical trials. Such drugs reduce not only IL-1β but also the pro-inflammatory actions of IL-18 and IL-33 (Carriere *et al.* 2007).

One of the intriguing aspects of the mechanism of action of pro-inflammatory cytokines is how an acute exposure may develop into a long-term behavioural effect. For instance, early life exposure to an immune challenge may trigger long-term effects that modulate the HPA axis response to novel stress during adulthood function (Shanks *et al.* 2000). Since we have seen that IL-1β is clearly responsible for the regulation of the expression of several genes within the brain, it would be possible that some of these genes would also be epigenetically modulated to sustain long-term changes in their expression. Interestingly, it was recently demonstrated that in *in vitro* studies that inhibition of the DNA methylase DNMT3b in a messangial cell culture accentuated iNOS expression and consequently NO production, suggesting that IL-1β-induced increase of iNOS expression can be epigenetically downregulated (Yu & Kone, 2004). Another major component related to the inflammatory cascade, COX-2, was shown also to be subject to control by hypermethylation (Murata *et al.* 2004). These findings imply that gene expression of biological components of the innate immune system, besides being regulated acutely by an inflammatory stressor, could also undergo long-term alterations that would modulate their subsequent response to subsequent stress situations. Alternatively, it is also possible that certain types of acute stressors set in motion a number of spatiotemporal biological changes that sustain the sensitization of certain physiological systems, such as the HPA axis.

A key component of the neuroimmune–endocrine axis, namely CRH, has been implicated in depression and antidepressant treatment response by a series of independent lines of evidence, the latest of which are in the domain of pharmacogenomics. In a recent study, we examined the association of CRH receptor 1 (CRHR1) genotypes with the phenotype of antidepressant treatment response in 80 depressed Mexican-Americans in Los Angeles who completed a prospective randomized, placebo lead-in, double-blind treatment of fluoxetine or desipramine, with active treatment for 8 weeks. Using the haplotype-tag single-nucleotide polymorphisms rs1876828, rs242939 and rs242941, we tested for haplotypic association between CRHR1 and 8 week response to daily antidepressant treatment. We found increased response to antidepressants in patients homozygous for the GAG haplotype of CRHR1. Such work supports the hypotheses that response to antidepressant treatment is heterogeneous and that the CRHR1 gene and possibly other genes in stress–inflammatory pathways are involved in the response to antidepressant treatment (Licinio *et al.* 2004). Recently, these findings were independently replicated in a Chinese sample (Liu *et al.* 2007).

In conclusion, gene–environment interaction could dictate the organism susceptibility to respond to different types of stressors. High-throughput technologies, such as genome wide scan, genotyping, microarray expression and micorarray-based DNA methylation profiling, proteomics and metabolomics will certainly facilitate understanding

of the complexity of individual differences. Moreover, integration of all of the information obtained by these techniques would lead to the development of tailored drugs that eventually would increase efficacy in the response of the current treatments.

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