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An altered peripheral IL6 response in major depressive disorder

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Abstract

Major depressive disorder (MDD) is one of the most prevalent major psychiatric disorders with a lifetime prevalence of 17%. Recent evidence suggests MDD is not only a brain dysfunction, but a systemic disease affecting the whole body. Central and peripheral inflammatory changes seem to be a centerpiece of MDD pathology: a subset of patients show elevated blood cytokine and chemokine levels that partially normalize with symptom improvement over the course of antidepressant treatment. As this inflammatory process in MDD is poorly understood, we hypothesized that the peripheral tissues of MDD patients will respond differently to inflammatory stimuli, resulting in an aberrant transcriptional response to elevated proinflammatory cytokines. To test this, we used MDD patient- and control-derived dermal fibroblast cultures to investigate their response to an acute treatment with IL6, IL1 β , TNF α , or vehicle. Following RNA isolation and subsequent cDNA synthesis, quantitative PCR was used to determine the relative expression level of several families of inflammation-responsive genes. Our results showed comparable expression of the tested genes between MDD patients and controls at baseline. In contrast, MDD patient fibroblasts had a diminished transcriptional response to IL6 in all the gene sets tested (oxidative stress response, mitochondrial function, and lipid metabolism). We also found a

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significant increase in baseline and IL6 stimulated transcript levels of the IL6 receptor gene. This IL6 receptor transcript increase in MDD fibroblasts was accompanied by an IL6 stimulated increase in induction of *SOCS3*, which dampens IL6 receptor signaling. Altogether our results demonstrate that there is an altered transcriptional response to IL6 in MDD, which may represent one of the molecular mechanisms contributing to disease pathophysiology. Ultimately we hope that these studies will lead to validation of novel MDD drug targets focused on normalizing the altered IL6 response in patients.

Keywords

major depressive disorder; fibroblasts; inflammation; oxidative stress; mitochondria function; lipid metabolism; IL1b; TNF α ; IL6; cytokine

Introduction

Major depressive disorder (MDD) is a major public health concern with a lifetime prevalence of 17% in the United States and a leading cause of disability worldwide (Kessler et al., 2005; Moussavi et al., 2007). Up to 65% of those who suffer from a major depressive episode will have recurrent episodes (Eaton et al., 2008; Yiend et al., 2009), and only about a third of adults with MDD are successfully treated with initial therapies (Alexopoulos, 2005; Roose and Schatzberg, 2005). A potential reason for therapeutic failure is that dysfunction in MDD is not restricted to the brain. MDD is a systemic illness with central and peripheral inflammatory changes at the core of MDD pathology (Erdem et al., 2011; Lisi et al., 2013; Noto et al., 2014; Powell et al., 2014; Setiawan et al., 2015; Shimizu et al., 1996; Slavich and Irwin, 2014). Patients with inflammatory diseases have a higher incidence of MDD than the general population (Dickens et al., 2002; Graff et al., 2009; Krishnadas et al., 2011; Lo Fermo et al., 2010), and 20-82% of patients treated with pro-inflammatory cytokines like interferon develop MDD (Capuron et al., 2002; Krishnadas et al., 2011; Musselman et al., 2001; Reichenberg et al., 2005). Additionally, TNF α antagonists used to decrease systemic inflammation in psoriasis also attenuate depressive symptoms, suggesting a potential causative role for inflammation in the development of MDD (Krishnan et al., 2007; Tyring et al., 2006).

Altered levels of inflammation and immune response have been reported in numerous MDD studies. MDD patients have increased blood levels of acute phase proteins and pro-inflammatory cytokines/chemokines such as IL6, IL1 β , and TNF α (Dahl et al., 2014; Dowlati et al., 2010; Erdem et al., 2011; Maes et al., 1997; Maes et al., 1993b; Raison et al., 2006). These increases in pro-inflammatory cytokines positively correlate with MDD symptom severity (Howren et al., 2009; Yirmiya et al., 2000). Inflammation in MDD also occurs in the brain: a recent PET imaging study demonstrated increased numbers of activated microglia in the prefrontal cortex, anterior cingulate cortex, and insula of MDD patients (Setiawan et al., 2015). Most importantly, systemic inflammation markers decline with successful treatment and symptom improvement (Dahl et al., 2014; Himmerich et al., 2010; Lisi et al., 2013). These data indicate that inflammatory mediators at the tissue level may be involved in the pathophysiology of at least some forms of depression. Better

understanding these mechanisms at a cellular level might lead to improved treatment targets for depression accompanied by increased markers of inflammation.

The systemic nature of MDD provides the opportunity for use of peripheral human cell line models, such as human dermal fibroblasts (HDFs), to study altered cellular functions associated with inflammation. HDFs have the same genetic variance as other tissues and express many of the same receptors and signal transduction proteins as the brain (Manier et al. 2000; Shelton et al. 1996). In addition, HDFs produce reliable results with no differences attributable in HDF experiments based on the number of passages (Akin et al. 2005). Recent studies from our lab and others using cultured HDFs from MDD patients and healthy controls have shown altered cell signaling, cell cycle regulation, apoptosis, energy production pathways, and oxidative stress both at baseline and in conditions of metabolic stress (Akin et al., 2005; Garbett et al., 2015a; Garbett et al., 2015b; Gibson et al., 2012; Manier et al., 2000). Importantly, numerous post mortem studies of gene expression, lipid content, and protein level alterations associated with MDD have found differences in these pathways, making HDFs an easily maintained, accessible, and relevant model for studying cellular changes relevant to the CNS (Karry et al., 2004; Lalovic et al., 2010; Manji et al., 2012; Müller et al., 2015; Shelton et al., 2011; Tobe, 2013). Our current study used this system to investigate differential gene expression in response to acute cytokine stimulation. We hypothesized that MDD patients respond differently to inflammation than healthy controls.

In this manuscript, we present evidence to support this hypothesis and discuss the implications of an altered inflammation-response.

Materials and Methods

Patient population

This study was approved by the Vanderbilt University Institutional Review Board. The diagnosis of each patient was set as current major depressive episode according to the Structured Clinical Interview for DSM-IV-TR (First et al., 2002). Exclusion criteria included other primary DSM-IV-TR (Association., 2000) diagnoses, a history of bipolar disorder or non-mood psychotic disorder, known systemic inflammatory disorder or unstable medical condition, any condition that would preclude the biopsy (e.g., hemophilia) (Garbett et al., 2015b; Manier et al., 2000). Written informed consent was obtained from all the participants. HDFs were generated from samples taken from seven female MDD patients and seven age-matched female controls (CTR). Demographic data are presented in Supplementary Table 1.

Harvesting and culturing HDFs

Harvesting procedures and culturing methods have been described previously (Garbett et al., 2015a). The HDF cultures underwent 5-10 passages in full DMEM (Dulbecco's Modified Eagle's medium). The medium contained 250 mM glucose, 1mM sodium pyruvate, and 2mM L-glutamine, antibiotic/antimycotic solution and 10% fetal bovine serum. Cultures were kept on 37°C at 5% CO₂ in 100 mm, non-coated plastic flasks with 0.75-1×10⁶ starting

density. Culture maintenance was performed as previously described (Garbett et al., 2015b; Manier et al., 2000).

Cytokine exposure and RNA collection/isolation

Briefly, when HDF cultures reached 12-16 million cells/sample, they were subcultured and plated onto 60 mm non-coated plastic plates with a starting cell density of $0.7-1.2 \times 10^6$. Treatment was always performed a day after the subculturing, after an overnight adherence. To eliminate the effects of cytokines that might be present in the serum-containing media, all cytokine stimulations were performed in serum-free media. All the cytokine and vehicle treatments lasted for 8 hours in 6 mL DMEM containing 250 mM glucose and 1mM sodium pyruvate, which was supplemented with 2mM L-glutamine and antibiotic/antimycotic solution. Treatment conditions were either 20 ng/mL IL6, 30 ng/mL IL1 β , 20 ng/mL TNF α , or media with no cytokine added. Dose was based on concentrations used in previously published cell culture studies (Guo et al., 2006; Liu et al., 2011; Niemand et al., 2003; van Zuiden et al., 2012).

After the treatment, samples were washed twice with 5 mL ice-cold PBS then collected in 1.5 ml Trizol and stored -20°C for 24 hours until RNA was isolated, following the protocol given by the manufacturer. For each sample and treatment, RNA was isolated from ~1,000,000 cells.

cDNA synthesis and quantitative Real Time-PCR

The concentration of isolated RNA was measured by Nanodrop. Agilent 2100 Bioanalyzer was used to determine the quality of the samples. Total RNA integrity for RNA samples was between 9.5 and 10. From 1000 ng of the previously isolated RNA, cDNA was generated with random primers using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

The primers used for quantitative Real Time PCR (qPCR) are shown in Supplementary Table 2. Primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) was used to design primers. Multiple primers were designed for each target gene, and primers were tested using multiple dilutions of cDNA before use. The primer pair with most optimal (>90%) efficiency and lowest cycle number to reach the detection threshold was used. Each primer pair was run in at least triplicate for each sample, and SYBR Green was used for product detection. No reverse transcriptase cDNA reactions were used to verify the lack of genomic DNA contamination in each RNA sample. The cycle at which the PCR amplification product reached the detection threshold (Ct) was obtained for each gene. Scaled Ct (normalized to *PGK1*) and Ct (normalized to *PGK1* expression and vehicle condition) were calculated for each sample as previously published (Arion et al., 2007; Garbett et al., 2012; Kalman et al., 2014).

Statistical approaches

Average expression values for each group (MDD and CTR) were calculated for each gene tested. Gene expression difference between groups was considered significant at $p < 0.05$ in a two-tailed, unpaired student t test. Difference in gene expression between treatment

conditions within a group was considered significant at $p < 0.05$ in a two-tailed, paired student t test.

Results

Baseline expression of target genes in MDD and CTR HDFs was comparable

MDD ($n = 7$) and healthy CTR ($n = 7$) HDFs treated with vehicle were not visibly different in cultures (data not shown). After 8 hours of vehicle treatment, there was also no difference in baseline gene expression between MDD and CTR in any of the 46 target genes tested (Figure 1, Supplementary Table 3). The target gene set focused on aberrant cellular functions associated with inflammation in the context of MDD and included genes that play a role in tryptophan metabolism, lipid metabolism, mitochondrial function, and oxidative stress response. The target genes were chosen based on pathways found to be disrupted in multiple blood and brain MDD studies (Quak et al. 2014; Lalovic et al. 2010; Czarny et al., 2015; Spinazzola and Zeviani, 2009; Shelton et al., 2011), and gene sets were grouped together based on the functional roles of the target gene products. Importantly, all these genes were expressed in MDD and CTR HDFs at baseline, providing additional evidence that HDFs express genes relevant to cellular dysfunction observed in neuropsychiatric disease as seen in previous studies (Garbett et al., 2015b; Manier et al., 2000; Shelton et al., 2004).

Transcriptome response to cytokine stimulation in HDFs

In a within subject comparison, both MDD and CTR HDFs displayed altered expression of the genes tested in response to cytokine stimulation with a unique set of changes observed for IL1 β , TNF α , and IL6 (Table 1). To IL1 β and TNF α stimulation, both MDD and CTR HDFs responded with considerable induction of immune and oxidative stress response genes such as *NFKB1*, *SOD2*, and *IRF1*. IL1 β stimulation produced increased repression of the gene encoding early growth response 1 in both MDD and CTR HDFs compared to other cytokine stimulation conditions (Figure 2, Supplementary Table 4) whereas TNF α caused a greater induction of *PIMI* in both MDD and CTR HDFs (Figure 3, Supplementary Table 4). TNF α also produced a subtle repression of *SKA2* in CTR HDFs that was not altered in MDD HDFs.

Differential responses to IL6 stimulation in MDD HDFs

Treatment with IL1 β or TNF α did not produce any discernable differences in the relative expression level of the tested genes between CTR and MDD HDFs. On the contrary, IL6 stimulation resulted in a differential MDD response compared to CTR (Table 2). In general, expression of the tested genes was reduced in CTR HDFs treated with IL6 compared to vehicle. In sharp contrast to CTR, the expression of the tested genes remained mainly unchanged in MDD HDFs with the exception of *NFKB1* and *SOD2*, which were changed in the opposite direction compared to CTR. (Figure 4, Supplementary Table 4). In other words, on a transcriptional level, MDD HDFs did respond differently to IL6 stimulation compared to CTR HDFs, and we interpret these data as a diminished IL6 response in MDD.

In MDD HDFs, all of the tested gene sets demonstrated differential relative expression in response to IL6. The differentially changed oxidative stress related mRNA transcripts included *NFKB1*, *IKBKB*, *IKBKG*, *NFE2L2*, *KEAP1*, *SOD2*, *STAT1*, and *P38*. *NFKB1*, *IKBKG*, and *IKBKB* gene products all promote signaling of the transcription factor NFκB (Liu et al., 2012; Tak and Firestein, 2001). *NFE2L2* and *KEAP1* encode the transcription factor Nrf2 and its cytoplasmic repressor, respectively; and *STAT1* and *P38* produce stress related signaling proteins whereas Mn-dependent superoxide dismutase (*SOD2*) neutralizes oxidants (Bresciani et al., 2015; Levy and Darnell, 2002; Zarubin and Han, 2005). From the mitochondrial gene set, several genes were strongly repressed in CTR, but not in MDD HDFs. These were genes crucial for ATP synthesis (*MTATP6*), mitochondrial fission and transport (*MFN2*, *MIRO1*), and mtDNA synthesis (*POLG*) (Al-Damluji, 2004; Degos et al., 2014; Hayashi et al., 2009; Las and Shirihi, 2014). Similar differences were observed in the lipid metabolism-related genes *DHCR7* and *HMGCR*, both crucial in cholesterol metabolism (Sharpe and Brown, 2013). The IL6 induced expression level of target genes important for tryptophan breakdown was not different in MDD compared to CTR HDFs. Spindle and kinetochore association complex subunit 2 (*SKA2*) was found to be differentially regulated in response to IL6 in MDD compared to CTR HDFs.

Due to the substantial alteration in relative gene expression changes observed with IL6 stimulation, next we measured the levels of the IL6 receptor (*IL6R*), its signal transducer gp130 (*IL6ST*), and suppressor of cytokine signaling 3 (*SOCS3*) (Figure 5) (Babon et al., 2014; Fischer et al., 2004). Interestingly, we found that the relative gene expression of *IL6R* was higher not only at baseline, but also after IL6 stimulation in MDD HDFs compared to CTR. Additionally, *SOCS3* was considerably induced in response to IL6 stimulation in MDD HDFs whereas no change from baseline expression was observed in CTR HDFs. This pattern of expression suggests an increase capacity of MDD HDFs to respond to IL6 stimulation as well as an active dampening of IL6 signaling by induction of *SOCS3*.

Discussion

Our results demonstrate that 1) MDD and CTR HDFs expressed cytokine-responsive genes, 2) MDD and CTR HDFs responded to IL1β, IL6, and TNFα stimulation, and this response was unique for the various cytokines tested, 3) MDD HDFs had an altered response specifically to IL6, but not to IL1β and TNFα, and 4) the differential IL6 response between the MDD and CTR HDFs might be due to active dampening of IL6 signaling by induction of *SOCS3*. Additionally, this is the first demonstration of the utility of HDFs to understand the effects of IL6 on a molecular level in MDD patients.

HDFs are easy to maintain, carry the same genetic variance as other tissues, including brain, and provide an easily maintainable, relevant system to interrogate the effects of different conditions and/or treatments on cellular function in systemic diseases (Bahn and Chan, 2015; Manier et al., 2000). Unlike peripheral blood mononuclear cell cultures, the use of HDFs after several passages negates the majority of effects that arise from medications and/or humoral factors, as only a small portion of the cells in culture are actually from the original biopsy sample when the experiment is performed. HDFs express many of the same receptors and signal transduction proteins as neural tissue, and the signal transduction

changes observed in HDFs might facilitate our understanding of signaling changes in the brain (Shelton et al., 1996). This has provided a much needed model for our laboratory and others to study various brain disorders, including Fragile $\times$ disorder (Kumari et al., 2014), Huntington's disease (Fernandez-Estevez et al., 2014; Jia et al., 2015), bipolar disorder (Gaspar et al., 2014; Johansson et al., 2013; McCarthy et al., 2013), and MDD (Akin et al., 2005; Garbett et al., 2015a; Shelton et al., 1996).

We used MDD and CTR HDFs to test gene expression changes in 46 genes with a variety of cellular functions in response to several cytokines. With IL6 stimulation, we observed a unique gene expression response in MDD, including genes encoding proteins related to crucial stress response transcription factors (*STAT1*, *P38*, NFKB subunit- *NFKB1*, *IKK* subunits- *IKBKB* & *IKBKG*, *Nrf2*- *NFE2L2*, and *KEAP1*) (Bryan et al., 2013; Levy and Darnell, 2002; Tak and Firestein, 2001; Zarubin and Han, 2005). The JAK/STAT pathway is the main signaling cascade activated by IL6 with *STAT1/3* producing the first wave of gene expression changes (Heinrich et al., 2003). NFKB interacts both synergistically and competitively with *STAT* transcriptional regulation to promote inflammation response gene expression (Grivennikov and Karin, 2010; Heinrich et al., 2003). Moreover, *P38* MAP kinase produces NFKB activation and induction of transcripts crucial for regulating numerous inflammatory diseases (Badger et al., 1998; Guan et al., 1998; Hollenbach et al., 2004; Perregaux et al., 1995; Zarubin and Han, 2005). The transcription factor *Nrf2* can also be activated by *P38* MAP kinase through the release its cytoplasmic repressor *Keap1*. *Nrf2* regulates genes with antioxidant response elements and is strictly part of the defensive response to oxidative stress (Bakunina et al., 2015). In addition, *SOD2* (Mn-dependent superoxide dismutase), a target gene of NFKB and *Nrf2*, was induced with IL6. Our results suggest that MDD fibroblasts have a diminished IL6 response with the exception of *NFKB1* and *SOD2* induction. Our findings, in concordance with previous studies in MDD patients, support a role for NFKB expression in MDD pathogenesis (Lukic et al., 2014; Pace et al., 2006).

The genes demonstrating an altered relative expression in response to IL6 stimulation in MDD HDFs also included transcripts crucial for cellular metabolism. The altered genes involved in mitochondrial function (*POLG*, *MIRO1*, *MFN2*, and *MTATP6*) and lipid metabolism (*HMGCR* and *DHCR7*) suggest IL6 normally represses genes necessary for metabolic processes (Al-Damluji, 2004; Cyster et al., 2014; Graham and Allen, 2015; Ness, 2015). Interestingly, altered mitochondrial function has been associated with disturbed cholesterol metabolism (Allen and Graham, 2012), and both of these processes appear to play a role in MDD pathophysiology (Zorumski et al., 2013). Altered cholesterol biosynthesis may even exacerbate inflammation in MDD by causing increased release of pro-inflammatory cytokines and altering NFKB signaling (Andres et al., 2012; Ghisletti et al., 2007; Lawlor and Vince, 2014; Shibata and Glass, 2010; Traversari and Russo, 2012). In addition, we found *SKA2* to be subtly less repressed in response to IL6 stimulation in MDD, which may reflect inefficiency in the anti-proliferative response to IL6 (Hanisch et al., 2006). Altogether our results suggest a lack of repression of growth and energy production processes in the MDD HDF IL6 response.

It was perhaps not surprising that the gene encoding the IL6 receptor was expressed at a higher level both at baseline and after IL6 stimulation in MDD. IL6 signals through an IL6 receptor/gp130 complex that phosphorylates JAK and causes subsequent phosphorylation/activation of STAT transcription factors as well as MAPK and ERK signaling pathways (Babon et al., 2014). Suppressor of cytokine signaling 3 (*SOCS3*) transcription is induced by STAT3 and binds both the IL6 receptor/gp130 complex and JAK to inhibit further signaling and to mark both JAK and the receptor for degradation. *SOCS3* inhibition of IL6 signaling often returns signaling induced by IL6 to baseline (Babon et al., 2012; Kershaw et al., 2013). *SOCS3* was found to be significantly induced in MDD but not in CTR in response to IL6.

This finding in combination with the increased baseline expression of the IL6 receptor transcript suggests an increased capacity to respond to IL6 with a concomitant increase in feedback inhibition by *SOCS3*. Importantly, IL6-mediated *SOCS3* dampening was not observed in CTR HDFs.

Strong evidence exists for a primary role for IL6 in the aberrant inflammatory profile of MDD. Chronic stress-induced depressive symptoms in animal models have frequently been associated with increases in serum, CSF, and brain concentrations of IL6 (LeMay et al., 1990; Monje et al., 2011; Sukoff Rizzo et al., 2012; Voorhees et al., 2013). Successful amelioration of these depressive symptoms with antidepressants or IL6 receptor antibodies are accompanied by normalization of IL6 levels, reminiscent of that observed in human studies (Hodes et al., 2014; Reus et al., 2015). Concordantly, IL6 knockout mice are resistant to develop depressive symptoms in models of chronic stress (Chourbaji et al., 2006), and blockade of IL6 receptor trans-signaling decreases the induction of depressive symptoms by IL6 (Sukoff Rizzo et al., 2012). In line with animal studies, MDD patient studies also find consistent increased concentrations of IL6 (Dowlati et al., 2010; Maes et al., 1993a; Moieni et al., 2015; Quak et al., 2014). These IL6 elevations in MDD correlate with factors associated with depression, such as acute stress and childhood trauma (Carpenter et al., 2010). Furthermore, IL6 has the capacity to alter processes associated with depression such as increasing tryptophan catabolism and stimulating the HPA axis (Besedovsky et al., 1991; Fujigaki et al., 2006; Li et al., 2003; Raison et al., 2009). Interestingly, the MDD HDF IL6 response in our experiments are unlikely to be influenced by most humoral (i.e. elevated serum IL6), medication, and epigenetic effects due to the minimum 5-10 culture passages after the initial biopsy culture before use in our experiments. We believe our data primarily represents a genetically encoded disruption of IL6 signaling as opposed to a transient adaptation, which is likely due to chronic elevation of IL6 levels in MDD. When taken together with the above discussed studies, it appears that MDD is characterized by both a chronic elevation of IL6 and a genetically encoded, summative altered IL6 responsiveness. Therefore, we propose that the association of increased IL6 and an aberrant IL6 response in MDD might represent a molecular end-point of genetic predisposition to MDD and is in agreement with previously reported data (Su et al., 2009).

In summary, we observed MDD and control HDFs to have a divergent response to IL6 stimulation. Here we were able to identify oxidative stress and cellular metabolism related genes as differentially expressed in MDD patient HDFs compared to CTR only with IL6

stimulation and not with IL1 β or TNF α . The mostly unaltered relative gene expression with IL6 stimulation in MDD as well as the increased relative baseline expression of *IL6R* and IL6 stimulated induction of *SOCS3* provides strong evidence that there is an aberrant inflammatory response in MDD. It is notable that we observe a blunted response in MDD fibroblasts to IL6 exposure. Yet, previous findings suggests that IL6 signaling produces depressive symptoms (Sukoff Rizzo et al., 2012). While this might appear contradictory at first glance, these data are not mutually exclusive: our findings might be related to an adaptive suppression feedback mechanism which prevents excessive stimulation in response to elevated IL6 levels. Additionally, we must emphasize that the molecular phenotype is only revealed after a challenge with cytokines. Simply measuring basal levels of mRNA transcripts cannot reveal the phenotype we describe here. Yet our study is not without limitations. We selected a limited gene set, used 3 cytokines for stimulation, and tested a single response time, leaving considerable room for follow-up studies.

Conclusions

The idea that not only is inflammation increased in MDD but the response to that inflammation is different in MDD opens a new avenue to explore in MDD pathogenesis. An altered response to inflammation may serve as a vulnerability factor for an individual to progress towards MDD when internal and/or external stressors arise, and perhaps inflammatory biomarkers and response to inflammation could assist in identifying effective treatments for patients. This strengthens the argument that inflammation needs to be addressed with MDD treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- HDFs express genes altered in blood cells and neural tissue of patients with MDD.
- MDD patients have a blunted transcriptional response to IL6 but not IL1 β or TNF α .
- The IL6 signaling suppressor SOCS3 is induced by IL6 in MDD but not controls.
- • Altered *SOCS3* levels might be critical for the blunted IL6 response in MDD.

Cytokine-responsive genes are expressed in fibroblasts

Figure 1. Both MDD and CTR HDFs expressed inflammation-responsive genes at baseline

Gene expression was assessed by qPCR. MDD and CTR HDFs treated with vehicle express cytokine-responsive genes. At baseline, MDD and CTR HDFs had comparable expression. X-axis shows the tested gene transcripts whereas Y-axis shows data as a scaled qPCR threshold cycle (Ct). Smaller Ct values indicate higher abundance of transcripts. Ct values >30 (dashed line) indicate unexpressed transcripts. Bars denote standard error of means (SEM).

Figure 2. MDD and CTR HDFs responded to IL1 β stimulation, but with no differences across diagnosis observed in magnitude of response

IL1 β stimulation changed the relative expression of the cytokine-responsive genes tested in MDD and CTR HDFs, demonstrating the ability of HDFs to be stimulated by cytokines. This response was not significantly different for the oxidative stress (A), mitochondrial metabolism (B), tryptophan & lipid metabolism (C), or proliferation, apoptosis, & immune response (D) related gene sets. X-axis shows the tested gene transcripts whereas Y-axis denotes data as the normalized expression difference between cytokine and vehicle-treated conditions (ΔC_t). A ΔC_t value of 0 indicates that the gene was expressed at the same relative level with vehicle or cytokine treatment, while values < 0 indicates repression and > 0 indicates transcript induction by treatment. An absolute ΔC_t value on 1 corresponds to a 2-fold change. Error bars denote

Figure 3. MDD and CTR HDFs had a comparable response across diagnosis to TNF α stimulation

Figure layout is identical to that of Figure 2. TNF α stimulation changed the relative expression of the cytokine-responsive genes tested in MDD and CTR HDFs. The response was not significantly different by unpaired student t-test between MDD and CTR fibroblasts in the oxidative stress (A), mitochondrial metabolism (B), or tryptophan & lipid metabolism (C) related gene sets. (D) A significant difference ($p < 0.05$, *) was observed with SKA2 expression, being repressed in CTR HDFs but unchanged in MDD HDFs with TNF α .

Figure 4. MDD and CTR HDFs showed a different gene expression response to IL6 stimulation
 Figure layout is identical to that of Figures 2-3. The majority of gene transcripts showed a differential response to IL6 stimulation between MDD and CTR HDFs (A-D). Error bars denote SEM; * denotes statistical significance ($p < 0.05$) by unpaired student t test.

Figure 5. MDD HDFs had an increased *IL6R* and *SOCS3* expression

(A) MDD HDFs had a significantly higher relative gene expression of *IL6R* (IL6 receptor) than CTR HDFs with both vehicle and IL6 exposure. (B) IL6 stimulation resulted in a strong, MDD-specific induction in *SOCS3* levels, while the change in expression of *IL6R* and *IL6ST* were not significantly different between the MDD and CTR HDFs. Error bars denote SEM; * denotes significant response by unpaired student t test between the CTR and MDD HDF responses. Values with SEM and associated p-values are listed in C.

Table 1
MDD and CTR HDF responses to cytokine stimulation in comparison to vehicle treatment

	WITHIN SUBJECT COMPARISON OF CYTOKINE RESPONSE BY GENE SET					
	MDD (p-value)			CTR (p-value)		
	IL6	IL1 β	TNF α	IL6	IL1 β	TNF α
All gene sets	0.143	0.174	0.013	0.000	0.430	0.265
Oxidative stress	0.011	0.015	0.008	0.000	0.079	0.078
Mitochondrial function	0.522	0.052	0.329	0.000	0.094	0.031
Tryptophan/lipid metabolism	0.037	0.072	0.988	0.004	0.040	0.074
Proliferation/apoptosis/immune response	0.272	0.604	0.362	0.003	0.555	0.394

A paired student t test was used to compare the expression of the indicated gene sets between vehicle and each cytokine treatment condition for MDD and CTR HDFs. At least one gene set was altered by each cytokine stimulation compared to baseline expression for both MDD and CTR (p < 0.05 shaded).

Table 2
Comparison of response to cytokine stimulation between MDD and CTR

ACROSS DIAGNOSIS COMPARISON OF CYTOKINE RESPONSE BY GENE SET			
	MDD vs. CTR (p-value)		
	IL6	IL1 β	TNF α
All gene sets	0.000	0.729	0.357
Oxidative stress	0.000	0.742	0.539
Mitochondrial function	0.002	0.835	0.267
Tryptophan/lipid metabolism	0.000	0.330	0.090
Proliferation/apoptosis/immune response	0.032	0.967	0.997

An unpaired student t test was used to compare the relative expression difference from baseline of the indicated gene set between MDD and CTR with $p < 0.05$ considered significance (shaded). When comparing all tested genes or each gene set, only the relative gene expression response to IL6 was significantly different between MDD and CTR.