Published in final edited form as:
Eur Neuropsychopharmacol. 2008 March ; 18(3): 230–233.

A Detailed Examination of Cytokine Abnormalities in Major Depressive Disorder

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Abstract

Recent technological advances offer an opportunity to further elucidate the complex cytokine network in Major Depressive Disorder (MDD). Twenty cytokines were simultaneously assessed in 49 individuals with MDD and 49 age and gender matched controls. Multiple proinflammatory and two antinflammatory cytokines were significantly elevated in the MDD sample, including an antidepressant naïve subset. These data support a generalized chronic inflammatory state in MDD, and implicate additional cytokines and chemokines previously linked to cardiovascular disease.

Keywords

Cytokines; Inflammation; Depression; Major Depressive Disorder; Stress; Cardiovascular

Introduction

While well-established research has implicated inflammatory pathways in Major Depressive Disorder (MDD), including the longstanding cytokine hypothesis of depression (e.g. Maes et al., 1990, Maes et al., 1995, Smith, 1991, Dantzer and Kelley, 2007, Irwin and Miller, 2007), the hypothesis that chronic inflammation may represent a stress response secondary to or inherent in MDD has also been proposed (For review see Hayley et al., 2005, Raison et al., 2006). Regardless of their etiology, chronic inflammatory responses in individuals with MDD may increase "allostatic load," which may result in downstream biological damage and offer one potential contribution to the elevated cardiac and other excess medical morbidity and mortality associated with MDD (e.g. see McEwen, 2003, Gump et al., 2005, Kiecolt-Glaser et al., 2002, Miller et al., 2002, Wassertheil-Smoller et al., 2004). Cytokines and chemokines are proteins that mediate immune responses to injury, infection and other organismal stress (Charo and Ransohoff, 2006). While methodology and results vary somewhat, there are replicated

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Authors Simon and Wong designed this study, and were involved in data analyses and interpretation and manuscript preparation. Authors Wong, McNamara and Maser were involved in cytokine analyses and interpretation. Authors Fava, Papakostas and Nierenberg designed and were involved in the parent depression study design, clinical data and/or sample collection. Author Chow was involved in data management, analyses and manuscript preparation. Author Pollack was involved in data interpretation and manuscript preparation. All authors contributed to and approved the final manuscript.

reports of elevations of select cytokines (e.g. IL-6, IL-1β and TNF-α: see discussion) in MDD (Schiepers et al., 2005). As technology and medical knowledge of the complex system of cytokines and chemokines develops, there is an opportunity to further elucidate cytokine profiles and specific cytokines perturbed in MDD beyond those previously identified. We provide a detailed examination of 20 cytokines simultaneously assessed in 49 antidepressant-free individuals with MDD compared with 49 healthy controls.

Experimental Procedures

Study procedures were approved by the Massachusetts General Hospital (MGH) Institutional Review Board. We examined previously collected frozen serum from 49 individuals with a current Major Depressive Episode and primary Major Depressive Disorder (MDD), and 49 age (within 3 years) and gender matched healthy control subjects. Subjects with MDD were a random sub-sample recruited from 1997 to 2002 at the MGH site of a two-center prospective fluoxetine study, details of which have been reported (McGrath et al., 2006). Briefly, psychiatric diagnoses were established with the Structured Clinical Interview for DSM-IV. All participants were not pregnant, and were free of significant medical and all substance use disorders as determined by detailed history, physical exam and normal laboratory tests (EKG, CBC, blood chemistry, thyroid function, urinalysis and drug toxicology screen). Participants were free of antidepressants for a minimum of 1 week at entry, and signed written informed consent.

Healthy control subjects were recruited in 2002 through the Healthy Volunteer Specimen Bank at the Harvard Medical School-Partners Healthcare Center for Genetics and Genomics. A medical history and physical exam was performed to exclude significant medical diseases and current prescribed or regular use of over-the-counter medications. Participants were asked whether they had ever been diagnosed with or treated for depression or anxiety, and excluded if affirmative. All participants signed written informed consent for use of their serum and clinical data as control comparators.

In order to obtain as comprehensive a cytokine/chemokine profile as feasible, serum samples were assayed in duplicate for 22 cytokines and chemokines with the Beadlyte® Human 22-Plex Multi-Cytokine Detection System, including appropriate standards, and the Luminex 100 Total System (Austin, Texas). The mean was derived for each duplicate sample. Standard curves were generated with Upstate Beadview software for each analyte using a best-fit 4-parameter or 5-parameter logistic method to calculate sample cytokine concentrations. Standard curves with R-squared ≥ 0.95 were used in analysis; IL-12p40 and RANTES are thus not presented. Samples not within the linear portion of the standard curves were excluded.

Because data were not normally distributed, the association of MDD with cytokine levels was examined with the Wilcoxon Rank Sum test. Means and standard deviations are shown in Table 1 consistent with extant literature. For the primary analyses, the significance level was Bonferroni corrected for multiple testing (alpha=0.05/20=0.0025). Follow-up analyses are considered exploratory.

Results

There were no significant differences in age or gender for the MDD (mean age 41.65 +/- 11.07 years, 40.82% women) and control samples (mean age 41.69 +/- 11.28 years, 42.86 % women). While ethnicity was not matched, the groups were not significantly different (e.g., Caucasian: 42/49 MDD vs. 38/49 control, FET p=n.s.). Lifetime MDD duration was 14.34 +/-13.12 years, with current episode duration 6.22 +/-8.81 years (n=5 and n=2 missing, respectively), and moderate MDD severity at sample colllection (Hamilton Rating Scale for Depression-17 score

= 19.3 +/- 5.3 points; Clinical Global Impression of Severity Scale score = 4.1 +/- 0.8 points). At least one lifetime anxiety disorder was present in 47.8% (22/46): 15% (7/46) panic disorder, 34.8% (16/46) social anxiety disorder, and 17.8% (8/45) PTSD. Further, 34.8% (16/46) had at least one past alcohol or substance abuse disorder.

Examination of median cytokine levels revealed significant elevations in the following cytokines in MDD compared to healthy controls: MCP1, MIP-1α, and IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-15, Eotaxin, GM-CSF, and IFNγ (see Table 1). To assure that our findings were not explained solely by residual effects of antidepressants, we performed follow-up Wilcoxon Rank Sum analyses of cytokines significant in the full sample, limiting analyses to the 19 subjects with MDD documented as naïve to antidepressants lifetime compared to healthy controls. Anxiety and past substance abuse comorbidity did not vary by antidepressant history (both $p>0.75$). The cytokines and chemokines MIP-1α ($p<0.0001$), IL-1α ($p<0.0001$), IL-1β ($p<0.0001$), IL-6 ($P<0.0002$), IL-8 ($p<0.0001$), IL-10 ($p<0.0001$), Eotaxin ($p<0.0001$), GM-CSF ($p<0.0003$), and IFNγ ($p<0.0001$) in the MDD antidepressant-naïve sample remained robustly higher ($p<0.0025$) than healthy controls; however, MCP-1 ($p=0.0072$), IL-2 ($p=0.0436$), IL-13 ($p=0.0476$), and IL-4, IL-7, and IL-12p70, and IL-15 ($p>0.05$) were not significantly different.

In post-hoc exploratory examination of the 9 proinflammatory cytokines (MCP1, MIP-1α, IL-1α, IL-1β, IL-6, IL-8, Eotaxin, GM-CSF, and IFNγ) elevated utilizing $P<0.0025$, 6 or greater were simultaneously elevated for 75% (37/49) with MDD compared with 18% (9/49) of controls (FET $p=0.000$); this proportion was similar (63%: 12/19) for the antidepressant naïve MDD subset. We also explored the presence of IL-4 and IL-10 elevations in the MDD sample, hypothesizing that some anti-inflammatory cytokines may be activated as a compensatory response to a generalized proinflammatory profile; indeed, the vast majority of those with detectable levels of IL-4 or IL-10 concurrently had 6 or more elevated proinflammatory cytokines (100% (13/13) for IL4, 82% (14/17) for IL10), with similar proportions in the antidepressant naïve MDD subset (IL-4 100%, IL-10 78%).

Discussion

We present a large panel of 20 cytokines measured simultaneously in well-characterized individuals with MDD and matched controls. With the exception of TNFα, our findings implicating multiple predominantly proinflammatory cytokines in MDD are in accordance with prior reports utilizing varying methodologies which have somewhat inconsistently shown elevations of one or more of the following cytokines: MCP-1, IL-1α, IL-1β, IL-2, IL-6, IL-8 and IFNγ (e.g., see Schiepers et al, 2005 for recent review) (Marques-Deak et al., 2007, O'Brien S et al., 2007, Tsao et al., 2006, Anisman et al., 1999, Maes et al., 1999, Rajagopalan et al., 2001, Pace et al., 2006, Kaestner et al., 2005, Suarez et al., 2003, Schlatter et al., 2001). We also demonstrate elevations in additional cytokines (the proinflammatory Eotaxin, GM-CSF, MIP-1α, IL-7, IL-12p70, IL-15 and antinflammatory IL-4 and IL-10) to our knowledge not previously implicated in MDD. While any residual antidepressant effects should reduce rather than enhance proinflammatory cytokines (Kenis and Maes, 2002), robust associations with multiple predominantly proinflammatory cytokines and chemokines (IL-6, IL-1β, IL-1α, MIP-1α, Eotaxin, GM-CSF, IFNγ, and IL-8) were present for the subgroup with no lifetime antidepressant exposure, supporting that inflammatory responses occur independent of antidepressant use. We found evidence of a generalized inflammatory state and what may be hypothesized to be a compensatory anti-inflammatory response, with elevations in IL-4 and IL-10 almost exclusively in concert with multiple proinflammatory cytokines regardless of lifetime antidepressant use. Another anti-inflammatory cytokine, IL-13, was somewhat higher in patients ($p<0.0047$) but failed conservative Bonferroni correction.

Limitations of this cross-sectional study preclude conclusions about causality. Limitations of our data also prevented matching or statistical adjustment for potentially important variables such as smoking, lifetime use of anti-inflammatory agents, and body mass index. Stable or minor medical illnesses, and over-the-counter analgesic use were not specifically assessed or matched. Further, while there is no reason to suspect systematic differences, time of day was not controlled. Healthy controls did not receive a structured psychiatric interview; however, the presence of undetected mood or anxiety disorders should dilute rather then enhance control-MDD differences.

Although prospective studies replicating our findings under more tightly controlled conditions are needed prior to definitive conclusions, these data add to the growing literature supporting a generalized chronic inflammatory state in MDD, and suggest that additional research may elucidate a unique cytokine signature with therapeutic and/or prognostic value. Of particular interest, we found significant elevations in chemokines (MCP-1, MIP-1α, Eotaxin) and IL-6 which have been directly implicated in cardiovascular disease (Martinovic et al., 2005, Pai et al., 2004). While the etiology of cytokine elevations in MDD remains unknown and significantly more work is needed, the growing understanding of cytokines in individuals with MDD suggests the intriguing possibility that early intervention, perhaps with anti-inflammatory agents and/or antioxidants, might reduce or prevent downstream medical consequences of MDD.

Acknowledgements

The authors with to thank the *Harvard Medical School-Partners Healthcare Center for Genetics and Genomics* for access to the resources of the Healthy Volunteer Specimen Bank (HVS).

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Eur Neuropsychopharmacol. Author manuscript; available in PMC 2009 March 1.

Table 1

Cytokines and Chemokine Profiles in Major Depressive Disorder (n=49) Compared with Age and Gender Matched Healthy Controls (n=49)

	MDD mean ± SD	Control mean ± SD	Rank sum Z	Rank sum p
MIP-1α	463.8 ± 706.88	60.33 ± 95.91	-5.929	0.0000*
MCP-1	191.00 ± 381.69	56.66 ± 106.19	-3.420	0.0006*
IL-1α	223.75 ± 258.50	2.06 ± 8.45	-8.585	0.0000*
IL-1β	42.53 ± 105.19	1.29 ± 4.07	-6.060	0.0000*
IL-2	65.19 ± 316.57	10.58 ± 43.43	-4.316	0.0000*
IL-3	40.53 ± 261.34	1.14 ± 4.53	-0.903	0.3667
IL-4	13.72 ± 50.31	2.90 ± 11.45	-3.269	0.0011*
IL –5	24.03 ± 100.63	3.78 ± 12.69	-1.046	0.2954
IL-6	5.98 ± 14.22	1.23 ± 6.16	-3.291	0.0010*
IL-7	1.70 ± 3.10	.17 ± 1.16	-3.280	0.0010*
IL-8	231.19 ± 754.78	1.09 ± 3.50	-7.556	0.0000*
IL-10	8.68 ± 36.76	.70 ± 3.39	-4.192	0.0000*
IL12p70	17.40 ± 84.09	.39 ± 1.91	-3.425	0.0006*
IL-13	21.45 ± 98.85	4.13 ± 14.51	-2.825	0.0047
IL-15	.96 ± 5.06	.24 ± 1.62	-4.005	0.0001*
Eotaxin	167.08 ± 365.27	23.57 ± 62.61	-4.861	0.0000*
GM-CSF	141.30 ± 606.87	8.52 ± 24.81	-4.330	0.0000*
IFNγ	24.46 ± 25.43	6.67 ± 11.90	-5.057	0.0000*
IP-10	163.01 ± 171.24	130.71 ± 120.09	-1.456	0.1453
TNFα	7.84 ± 45.34	3.13 ± 11.73	0.415	0.6784

Starred P values significant after Bonferroni correction for 20 tests (P< 0.0025).

MDD= Major Depressive Disorder, SD= standard deviation.