Cytokine aberrations in Autism Spectrum Disorder: A systematic review and meta-analysis

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Abstract

The role of non-diagnostic features in the pathophysiology of Autism Spectrum Disorders (ASD) is unclear. Increasing evidence suggests immune system alterations in ASD may be implicated in the severity of behavioral impairment and other developmental outcomes. The primary objective of this meta-analysis was to investigate if there is a characteristic abnormal cytokine profile in ASD compared with healthy controls. We identified relevant studies following a search of MEDLINE, EMBASE, PsycINFO, Web of Knowledge and Scopus. A meta-analysis was performed on studies comparing plasma and serum concentrations of cytokines in unmedicated participants with ASD and healthy controls (HC). Results were reported according to PRISMA statement. Seventeen studies with a total sample size of 743 participants with ASD and 592 HC were included in the analysis. Nineteen cytokines were assessed. Concentrations of interleukin (IL)-1beta ($p < 0.001$), IL-6 ($p = 0.03$), IL-8 ($p = 0.04$), interferon-gamma (IFN-γ) ($p = 0.02$), eotaxin ($p = 0.01$) and monocyte chemotactic protein-1 (MCP-1) ($p < 0.05$) were significantly higher in the participants with ASD compared with the healthy control group, while concentrations of transforming growth factor-beta1 (TGF-β1) were significantly lower ($p < 0.001$). There were no significant differences between ASD participants and controls for the other twelve cytokines analyzed. The findings of our meta-analysis identified significantly altered concentrations of cytokines in ASD compared to healthy controls, strengthening evidence of an abnormal cytokine profile in ASD where inflammatory signals dominate.

Keywords: autism spectrum disorder, cytokine, inflammation, interleukin, meta-analysis, tumor necrosis factor

Introduction

Autism Spectrum Disorder (ASD) is a complex, pervasive neurobiological disorder that is characterized by impairments in the domains of social communication and restricted interests and repetitive behaviors. Despite a variable onset in the manifestation of diagnostic features, the developmental trajectory is usually significantly altered. Heterogeneity in etiology, phenotype, outcome and comorbidities are hallmarks of ASD. The core diagnostic features have long been the focus of research and intervention strategies. However, in recent years, increasing attention has been given to concurrent non-diagnostic features and pathophysiology observed in ASD, including, sleep difficulties [1], immune system abnormalities [2], gastrointestinal disorders [3] and mitochondrial dysfunction [4].

Narrative reviews of immune system abnormalities and cytokine alterations in ASD have increased in recent years [5, 6]. Mechanisms leading to alterations in immune system functioning in ASD have been addressed from numerous perspectives, including genetic [7], maternal immune activation [8, 9], autoimmunity [10] and neuroimmune [11, 12]. Several studies have reported altered cytokine profiles in ASD compared with healthy controls [13-15]. Furthermore, associations between the severity of diagnostic features and elevated cytokines in ASD have been identified [16, 17].

Cytokines are key modulators in the initiation (innate immune system) and maintenance (adaptive immune system) of immune responses, and also facilitate the exchange of information between cells of the immune system and other tissue cells. Cytokines regulate immune responses via pro-inflammatory and anti-inflammatory pathways. The impact of peripheral cytokines most likely extends across the blood-brain-barrier (BBB) into the brain parenchyma via active protein transport mechanisms, and by recruitment of activated immune cells across the vascular

endothelium [18-20]. Cytokine expression has been identified in the brain tissue of patients with ASD, with microglial and astroglial activation in the cerebellum shown to be associated with an upregulation of cytokines indicating an innate immune response [11]. In cerebrospinal fluid, cytokines involved in pro-inflammatory pathways, such as interferon–gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) have been reported at increased levels, supporting the hypothesis of a chronic state of cytokine induction in ASD [11, 21]. Case-control investigations of cytokine concentrations in plasma or serum also suggest several alterations compared with healthy subjects [14, 22-25].

Associations between the severity of behavioral impairments identified as core diagnostic features of ASD and *ex vivo* cytokine levels have been reported [17, 26, 27]**.** Immune activation in ASD has also been explored, with *in vitro* techniques using various mitogens, and various allergens as stimuli [13, 28-32]. Cytokine profiles have also been investigated in neonatal dried blood samples from a newborn screening biobank, with results suggesting depressed or hypoactive immune cell activity in the neonatal period in ASD [33]. The impact of maternal immune activation has been extensively investigated in animal models, with levels of circulating cytokines influencing fetal brain development [9], CNS development [34] and changes in the balance of cytokines inducing behavioral abnormalities in offspring [35].

Various hypothesis have been proposed addressing the role of cytokines in the pathophysiology of ASD, with the more developed literature in the field of schizophrenia [36 37], bipolar disorder [38] and major depression [39] providing a framework. The objective of this systematic review is to investigate alterations in cytokine levels in the peripheral blood in patients with ASD compared to healthy control subjects. An association between cytokine aberrations and ASD has been reported in several studies [17, 26, 40], however the association is inconsistent between studies and

for individual cytokines [14, 41, 42]. We have used meta-analytical techniques to improve the strength of the accumulating evidence implicating immune system abnormalities in the pathophysiology of autism and to address the inconsistency in published data.

Methods

The systematic review and meta-analysis were undertaken and reported in accordance with the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) [43]

Inclusion and exclusion criteria

Studies comparing cytokines in participants with ASD and healthy control subjects (HC) were included. The inclusion criteria were: 1) an ASD diagnosis according to criteria established in DSM-III or IV; 2) participants aged over two years old; 3) measurement of peripheral blood cytokines in plasma or serum; 4) concentrations of individual cytokines were available in three or more studies, as per prior meta-analyses [38]; and 5) studies which reported cytokine concentrations using a mean and standard deviation, or sample size and p-value. The exclusion criteria were studies which only included: 1) medicated participants; 2) measurement of cytokines in cerebrospinal fluid or brain tissue; 3) *in vitro* analysis of cytokines after mitogen stimulation; 4) measurement of immune markers other than cytokines; and 5) evaluation of cytokine gene expression profiles.

Search strategy

A computerized search of MEDLINE, EMBASE, PsycINFO, Web of Knowledge and Scopus from January 1980 to August 2013 was conducted using the search strategy "(autism) and (cytokine or chemokine or interleukin or interferon or inflammation or tumor necrosis factor)". Duplicates were deleted and the title and abstract of each article were scanned for relevance independently by two researchers. The full text of potentially relevant studies was then retrieved and assessed for eligibility considering established criteria detailed above. The reference lists of relevant studies were also searched for possible studies meeting criteria. Where there was initial disagreement discussion amongst researchers established universal agreement on studies to be included. A flow chart of information pertaining to identification, screening, eligibility and final studies included was constructed according to PRISMA guidelines [43] (See Supplement 1, Figure 1).

Data Extraction

Data for each individual cytokine assessed in eligible studies were extracted into an Excel spreadsheet (sample size, mean, standard deviation and *p*-value). When a *p*-value was reported as an inequality rather than an exact value, the value was rounded down to the nearest 5 thousandth to allow compatibility with meta-analysis software. Data for moderator analysis of gender, age, type of control group and blood fraction were also extracted (Supplement 1, Table 1). One author (A.M) extracted all data. The method used to extract data was independently verified by another author (D.Q). Data was then re-checked for accuracy (A.M).

Data Analysis

Comprehensive Meta-Analysis Version 2 (Biostat, Inc., USA) was used for analysis [44]. Effect sizes were calculated as Hedges' *g* standardized mean difference of cytokine concentrations between ASD and healthy control groups using means and standard deviations or sample size and *p* values. Hedges' *g* offers the same interpretation as Cohen's *d* but incorporates a correction factor addressing the potential biases resulting from a small sample size as Cohen's *d* [45] tends to overestimate the absolute value of the standardized mean difference in small samples. An effect size estimate was calculated for each cytokine analyzed in all eligible studies. Classification of Hedges' *g* effect size can be done in the same way as Cohen's *d*: 0.2 = small; 0.5 = medium; and 0.8 = large [45].

A positive effect size implied that higher levels of the specified cytokine were reported in the ASD group as compared with the HC group. Effect size estimates were calculated using a random effects model, which estimates a mean of a distribution of effects. Under this model, allocation of study weights is based on the inverse of the total variance, which includes both within study variance and between study variance. This method results in a more conservative approach, as the model yields a wider confidence interval when there is significant heterogeneity among the results of eligible studies [46]. The alternative, a fixed effects model, estimates a common effect and allocates weight based on sample size.

Sensitivity analysis was undertaken using forest plots to initially identify outliers. The impact of any outliers was addressed by removing the study reporting the outlier result and comparing the effect size and *p* value of the subsequent result. Publication bias was assessed using a p value obtained for Egger's test of funnel plot asymmetry [47]**.** Heterogeneity was assessed using Cochrane's *Q* and I^2 was used to evaluate the impact of heterogeneity [48]. The level of

significance for the Cochrane *Q* test was set at 0.10 due to the low power of the test to detect heterogeneity [49]. An I^2 of 0.25, 0.50, and 0.75 denoted small, moderate, and high levels of heterogeneity, respectively [48]. When data permitted, moderator analysis was undertaken in an attempt to identify sources of heterogeneity.

Results

A total of 2,940 studies were identified from the initial search, after excluding duplicates. Titles and abstracts were scanned resulting in identification of 46 articles to be assessed for eligibility. Several studies were excluded as they: analyzed cytokines in cerebrospinal fluid or brain tissue (5 studies); reported stimulated or unstimulated levels of cytokines *in vitro* (17 studies); included participants who were medicated (1 study); the cytokine analyzed was not assessed in at least two other studies (5 studies) and/or insufficient information was provided on the method used to process blood samples (1 study). Seventeen studies, with a total of 1341 participants (746 with ASD and 595 HC), met inclusion and exclusion criteria. There were a total of 1113 (83%) males and 228 (17%) females. In the ASD group, 634 (85%) participants were male, while in the HC group there were 479 (81%) males. Meta-analysis was performed on data for 19 different cytokines, chemokines and cytokine receptors: Interleukin (IL)-4, IL-6, IL-8, IL-10, IL-12p40, IL-17, IL-23, IL-1α, IL-1β, receptor antagonist IL-1RA, interferon-gamma (IFN-γ), granulocyte colony-stimulating factor (G-CSF), transforming growth factor-beta1 (TGF-β1), tumor necrosis factor-alpha (TNF-α), eotaxin (also known as CCL11), monocyte chemotactic protein-1 (MCP-1; CCL2), macrophage inflammatory proteins MIP-1α (CCL3) and MIP-1β (CCL4) and RANTES (CCL5). Fifty five other cytokines were unable to be included in the meta-analysis because of either having only been assessed less than three times, or if assessed three or more times,

inadequate information was provided in one or more papers (Supplement 1 Table 2). Table 1 presents characteristics for the groups of studies measuring each cytokine and the effect size estimates for each individual cytokine. Five studies assessed cytokine concentrations in serum [13, 14, 22, 50, 51], and twelve studies assessed cytokine concentrations in plasma [15, 16, 17, 24-26, 41, 42, 52-55].

Meta-analysis on all cytokines resulted in significantly higher concentrations of cytokines in ASD compared to HC (Hedges' g=0.216, 95% CI: 0.086 to 0.346, p=0.001). Heterogeneity was significant (Q=388, p <0.001). For individual cytokine concentrations in peripheral blood, a large and highly significant effect size estimate was associated with decreased concentrations of TGF-β1, whilst significantly higher concentrations of IL-1β in participants with ASD was associated with a medium effect size (See Table 1). Small to medium effect size estimates were identified for concentrations of IL-6, IL-8, eotaxin and MCP-1. Concentrations of IL-1RA were bordering on significance in participants with ASD, a result associated with a medium effect size. There was a large and highly significant effect size for IFN-γ in participants with ASD compared to HC (See table 1). One outlier study [55], caused significant heterogeneity and resulted in publication bias. Removal of this study from the meta-analysis substantially reduced the effect size, however the results still showed significantly higher concentrations of IFN-γ in participants with ASD compared to HC (Hedges' g=0.489, 95% CI: 0.035 to 0.943, p=0.035), and this finding was associated with a medium effect size. Heterogeneity was reduced by over 20% (I^2 =69). Concentrations of cytokines IL-1α, IL-4, IL-10, IL-12p40, IL-17, IL-23, G-CSF, RANTES, MIP-1α and MIP-1β were all extracted from 3 studies and yielded nonsignificant effect size estimates. Six studies measuring concentrations of TNF-α also yielded nonsignificant effect size estimates.

Methodological and Clinical Heterogeneity

Heterogeneity reached significance (p<0.10) for 13 out of the 19 cytokines (Table 1). Data for measurements on the remaining 6 cytokines was extracted from only 3 studies. A possible explanation for this apparent homogeneity is the low power the test for heterogeneity has in meta-analyses with a small numbers of studies [49].

Methodological sources of heterogeneity include: the timing of blood sampling (reflecting circadian variations) and a fasting or non-fasting state, the time interval between blood collection and separation and storage, the number of freeze-thaw cycles of a sample prior to analysis, and the different assay procedures used for analysis. Furthermore, methodological differences in assay procedures and operator reliability are all possible sources of heterogeneity. A substantial lack of information regarding these parameters, and low study numbers for individual cytokines prevented a moderator analysis using any of these variables. A moderator analysis was conducted to assess for potential differences between the level of cytokines in two different blood components, plasma and serum. Subgroups for meta-analysis were formed using data from 73 different cytokine measurements. Eleven of these measurements were performed on sera, whilst the remainder were performed on plasma samples (85%). Overall results remained significant for both components (plasma: Hedges' g=0.148, p=0.02; serum: Hedges' g=0.596, p=0.007).

Possible sources of clinical heterogeneity include age, gender and type of control group (i.e., siblings vs. non-siblings), intercurrent infection or other inflammatory illness, and previous or unreported medication effects. Extensive overlap between age groupings did not allow for evaluation of the impact of age on effect size and heterogeneity. With the exception of one small

study where mean age was approximately 23 years old [22], the age range of participants was between 2 years old and 18 years old (Supplement 1, Table 1). Some studies excluded children older than 10 years of age to avoid potential effects associated with fluctuations in hormonal changes during puberty on cytokine concentrations. Gender subgroups were either classified as all male (52% of individual cytokines analysed) or mixed (48% of individual cytokines analysed), as no study separated out results for female participants. A moderator analysis was performed to investigate the impact of gender on overall results. Results for the male subgroup remained significant (Hedges' g=0.259, p=0.005), however the effect size for the mixed gender group was reduced when compared with the overall results (Hedges' g=0.174, p=0.07). Heterogeneity remained significant for both groups (male: Q=147, p<0.001; mixed: Q=236, p<0.001). Despite the differences between the male and mixed subgroups, effect sizes were similar enough to support collapsing the gender groupings for analysis of effect sizes of individual cytokine concentrations.

Two studies included in the meta-analysis used siblings as healthy controls. One study used related siblings as healthy controls [41], whilst the other study used unrelated siblings as the control group [53]. In this study all of the healthy controls had a sibling with an ASD diagnosis but were unrelated to the children with ASD selected for the study. A meta-analysis was conducted to assess the impact removal of these studies had on effect sizes for individual cytokines and on overall results (Figure 1). Effect sizes for individual cytokines all increased, as did the overall result (Hedges' g=0.370, 95% CI: 0.206 to 0.534, p<0.001).

Discussion

This meta-analysis was undertaken to investigate alterations in peripheral blood cytokine concentrations in ASD compared to healthy control subjects, and to explore if there is a characteristic abnormal cytokine profile in ASD. A combined estimate of effect size indicates that there are peripheral alterations in cytokine levels in ASD, with a small effect size. There were elevations in the pro-inflammatory cytokines, IFN-γ, IL-1β and IL-6, and reduced concentrations of the anti-inflammatory cytokine, TGF-β1. Levels of several chemokines associated with recruitment of inflammatory cells, including eotaxin, IL-8 and MCP-1 were elevated. Effect sizes associated with the results for these individual cytokines varied from small to large. These results provide evidence of a heightened inflammatory state and altered cytokine profile, suggesting immune system dysfunction in ASD.

Effect sizes for IFN-γ and TGF-β1 were the largest of the nineteen cytokines analyzed, with concentrations in ASD significantly elevated for IFN-γ, and concentrations of TGF-β1 significantly reduced. The roles of IFN-γ are diverse and multi-faceted. IFN-γ amplifies immune system sensitivity and response to pathogens, and coordinates the transition from innate immunity to adaptive immunity [56]. IFN-γ is the prototypic T cell derived cytokine [57], and through promotion of macrophage activation and cytotoxicity skews the immune response towards a so-called T-helper-1 (Th1) phenotype generally intended to foster immune responses against intracellular pathogens, but also recognized in many auto-immune conditions. IFN-γ also inhibits proliferation of Th2 cells and subsequent production of anti-inflammatory cytokines, including IL-4, IL-5 and IL-10 - all of which yielded non-significant results in this study. TGF-β1 also performs many cellular functions, including promoting cell growth, proliferation and

differentiation, and is a potent immunosuppressant essential for immune homeostasis [22, 26, 58]. IL-1β, a potent pro-inflammatory cytokine, found in many settings of acute and chronic inflammation, and known to affect the hypothalamic-pituitary-adrenal axis [59]. IL-6, another pro-inflammatory cytokine, also induces production of acute phase proteins and stimulates B cell differentiation and antibody production [60]. Apart from direct actions on immune functions, IL-6 influences many hematologic, hepatic, endocrine and metabolic functions [61]. Secretion of IL-6 has previously been reported to be enhanced in those with disruption of the sleep-wake cycle and changes in circulation IL-6 can cause changes in sleep quality [62, 63]. IL-6 is an important facilitator of CNS-immune communication [62]. Specifically in ASD, elevated brain levels of IL-6 have been shown to impair neural cell adhesion and migration and is thought to impact synapse formation [12]. The three chemokines that were elevated relative to HC, IL-8, eotaxin and MCP-1, are involved in the recruitment of neutrophils, eosinophils, monocytes and T cells from the circulation into settings of tissue inflammation.

This meta-analysis combined results from studies assessing single measures of cytokines in plasma and serum samples. Whilst the meta-analysis technique attempts to adjust for methodological confounders, the procedure is limited by the number of available studies, the number of participants in each study and the information that can be extracted from the included studies, therefore cautious interpretation of the results of this meta-analysis is warranted. There are numerous methodological issues associated with specimen collection, handling and cytokine measurement [64-67], as well as clinical confounders that should be considered in future research. The former technical concerns include assay reliability, particularly at the low end of the detection range, and variability in results using kits from different assay manufacturers [68]. Issues

following venipuncture procedure also require consideration, including artifactual *ex vivo* increases in cytokine levels by release from leukocytes [69], and *ex vivo* degradation by plasma proteases [70]. The effects of anti-coagulants [71], freeze-thaw effects [71] and potential for degradation following long term storage [66] all may potentially confound results. Given the clinical heterogeneity inherent in ASD, differences in patient characteristics, including the degree of severity of diagnostic features, should be considered to ensure methodological issues are not confounding results. Biological issues such as circadian rhythm [72] and the menstrual cycle, and the effects of high or low body mass index, medication, smoking and alcohol can all influence cytokine concentrations [73]. Data relating to these factors should be collected, controlled for and considered when establishing protocols investigating markers of inflammation such as cytokine concentrations.

Future research protocols investigating the relationship between behavioral factors in ASD and inflammation should assess and control for the impact of bio-behavioral factors on peripheral markers of inflammation, in order to facilitate a more robust interpretation of results [73]. Longitudinal and concurrent measurement of peripheral blood and cerebrospinal fluid inflammatory markers could also assist with elucidation of the relationship between cytokine levels and pathological effect, and the identification of a characteristic cytokine profile in ASD. Further, larger studies are needed to identify the impact of gender [74]. The impact of two studies using siblings as healthy controls had on overall effect size and results for individual cytokines is of particular interest [41, 53]. Excluding results for cytokines analyzed in these studies lead to an increase in effect size overall and for individual cytokines, skewing an imbalanced immune system even more towards an inflammatory state. This corroborates with previous findings that

immune profiles of children with ASD are more similar to siblings without a diagnosis of ASD than to healthy controls [31, 41]. These results suggest that siblings should not be employed as healthy controls, or additional unrelated healthy control subjects should also be included. Instead, evaluation of immune function in unaffected siblings may provide information that guides investigation in to the causal pathway leading to immune system perturbation in ASD [75].

To our knowledge, no previous studies have synthesized data on cytokine alterations in ASD. Effect size estimates for seven cytokines were significant (TGF-β1, IFN-γ, IL-1β, IL-8, IL-6, eotaxin and MCP-1). Based on these results we propose there is a pathophysiology in ASD that either manifests as an inflammatory disease process, similar to that of autoimmunity, or as a neuro-immune epiphenomenon that changes susceptibility to factors, such as pathogens and allergens, that trigger inflammatory responses and subsequent abnormalities in the immune system. Evidence of immune system perturbations in ASD, specifically a pro-inflammatory state, has been strengthened by this meta-analysis. Associations between immune system abnormalities and the severity of aberrant behavior in ASD [52], however, could not be investigated in this meta-analysis due to insufficient data. Interestingly, efficacious use of a nonsteroidal anti-inflammatory drug as an adjunctive intervention in reducing the severity of diagnostic features [76] supports our findings and potential role of the immune system in the manifestation of diagnostic features in ASD. Important considerations to ensure biological and methodological issues do not interfere with the strength of future findings are highlighted by this meta-analysis. A better understanding of the inflammatory biology of ASD and possible associations with behavioral impairments and non-diagnostic features warrants further investigation and may have significant therapeutic implications.

Supplementary information is available at *Molecular Psychiatry's* website

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Conflict of Interest

Ian B. Hickie is a member of the Medical Advisory Panel for BUPA Health Insurance (Australia) and also a Board Member of Psychosis Australia Trust. From 2012, he is a Commissioner in Australia's new National Mental Health Commission. He was until January 2012 a director of headspace: the national youth mental health foundation. Professor Hickie was previously the chief executive officer (till 2003) and clinical adviser (till 2006) of beyondblue, an Australian National Depression Initiative. He is supported principally for clinical research in depression and health services and population health initiatives related to anxiety and depression by an NHMRC Australian Medical Research Fellowship (2007–2012). He has led projects for health professionals and the community supported by governmental, community agency and pharmaceutical industry partners (Wyeth, Eli Lily, Servier, Pfizer, AstraZeneca) for the identification and management of depression and anxiety. He has received honoraria for presentations of his own work at educational seminars supported by a number of non-government organisations and the pharmaceutical industry (including Pfizer, Servier and Astra Zeneca). He has served on advisory boards convened by the pharmaceutical industry in relation to specific antidepressants, including nefazodone, duloxetine and desvenlafaxine. He leads a new investigator-initiated study of the effects of agomelatine on circadian parameters (supported in part by Servier but also by other NHMRC funding) and has participated in a multicentre clinical

trial of the effects of agomelatine on sleep architecture in depression and a Servier-supported study of major depression and sleep disturbance in primary care settings. In addition to national and international government-based grant bodies, investigator-initiated mental health research at the BMRI, he has been supported by various pharmaceutical manufacturers (including Servier and Pfizer) and not-for-profit entities (including the Heart Foundation, beyondblue and the BUPA Foundation).

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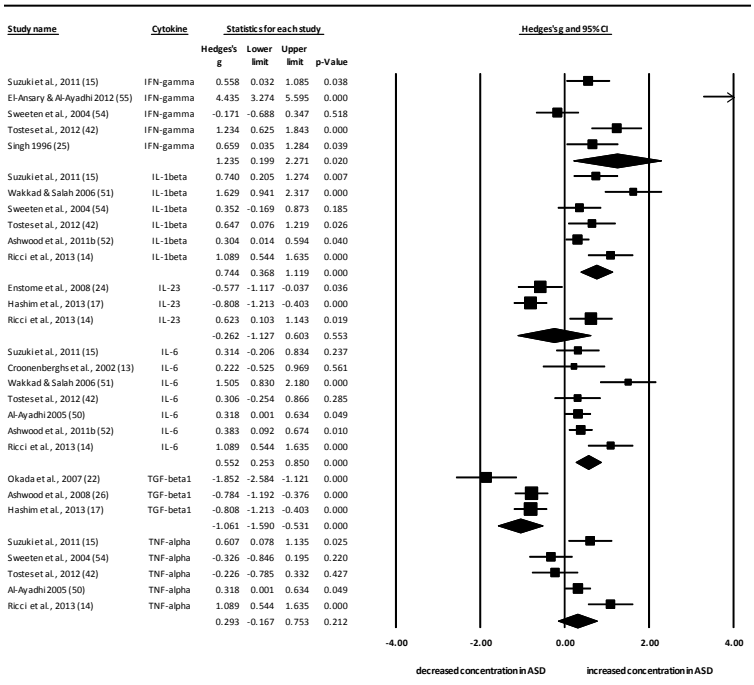
Table 1. Characteristics and Effect Size Estimates for Cytokine Concentrations in Autism Spectrum Disorder compared to Healthy Controls

Cytokine	Studies (n)	Participants (ASD)	Participants (HC)	% Male (ASD/HC)	Hedges' *g*	*p* value	95% CI	Heterogeneity χ^2	Heterogeneity *p* value	I^2	*Eggers*	Eggers p value	References
IL-1α	3	152	93	84/87	-0.128	0.49	-0.493 to 0.237	3.60	0.17	44.5	4.438	0.46	(15,41,53)
IL-1β	7	253	241	88/85	0.652	< 0.001	0.304 to 0.999	19.87	< 0.01	69.8	3.64	0.13	(14,15,41,42,51,52,54)
IL-4	3	74	74	96/96	0.137	0.64	-0.430 to 0.704	6.48	0.04	69.1	-54.152	0.42	(15,41,42)
IL-6	9	452	383	82/81	0.381	0.03	0.039 to 0.722	38.97	< 0.01	79.5	2.277	0.36	(13-15,41,42,50-53)
IL-8	3	150	140	91/89	0.455	0.04	0.024 to 0.885	5.42	0.07	63.1	-0.762	0.99	(15,41,52)
IL-10	3	77	77	92/92	0.026	0.87	-0.286 to 0.337	1.08	0.58	0	-18.07	0.21	(15,41,42)
IL-12p40	3	150	140	91/89	0.251	0.10	-0.052 to 0.553	2.93	0.23	31.9	-0.997	0.8	(15,41,52)
IL-17	3	103	103	78/81	0.193	0.67	-0.700 to 1.087	19.50	< 0.01	89.7	10.59	0.5	(15,17,41)
IL-23	3	119	99	76/74	-0.262	0.55	-1.127 to 0.603	19.03	< 0.01	89.5	11.431	0.58	(14,17,24)
IL-1RA	3	66	66	100/100	0.558	0.05	-0.005 to 1.121	5.11	0.08	60.8	-1.55	0.89	(13,15,41)
IFN-□	6	171	164	77/77	1.044	0.02	0.202 to 1.885	56.22	< 0.01	91.1	13.12	0.004	(15,25,41,42,54,55)
TGF-β1	3	144	107	79/70	-1.061	< 0.001	-1.590 to -0.531	6.95	0.03	71.2	-6.329	0.03	(17,22,26)
TNF-α	6	212	210	92/91	0.227	0.27	-0.175 to 0.629	20.29	< 0.01	75.4	-0.937	0.82	(14,15,41,42 50,54)
G-CSF	3	152	93	84/87	-0.031	0.93	-0.694 to 0.632	11.53	< 0.01	82.7	7.754	0.47	(15,41,53)
Eotaxin	3	133	111	90/83	0.317	0.01	0.065 to 0.570	1.00	0.61	0	-0.652	0.83	(15,16, 41)
MCP-1	3	133	111	90/83	0.257	0.05	0.005 to 0.508	0.57	0.75	0	-1.747	0.26	(15,16, 41)
RANTES	3	133	111	90/83	-0.008	0.97	-0.439 to 0.423	5.2	0.07	61.5	-5.719	0.11	(15,16, 41)
MIP-1α	3	152	93	84/87	-0.153	0.31	-0.445 to 0.140	2.40	0.30	16.8	4.702	0.15	(15,41,53)
MIP-1β	3	152	93	84/87	0.082	0.82	-0.618 to 0.783	12.85	< 0.01	84.4	9.839	0.29	(15,41,53)

G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon-γ; IL, interleukin; IL-1RA, IL-1 receptor antagonist; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α; RANTES, regulated on activation, normal t cell expressed and secreted; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α


Study name
Cytokine
Statistics for each study
Hedges's g
Lower limit
Upper limit
p-Value
Hedges's g and 95% CI
Suzuki et al., 2011 (15) IFN-gamma 0.558 0.032 1.085 0.038
El-Ansary & Al-Ayadhi 2012 (55) IFN-gamma 4.435 3.274 5.595 0.000
Sweeten et al., 2004 (54) IFN-gamma -0.171 -0.688 0.347 0.518
Tostes et al., 2012 (42) IFN-gamma 1.234 0.625 1.843 0.000
Singh 1996 (25) IFN-gamma 0.659 0.035 1.284 0.039
1.235 0.199 2.271 0.020
Suzuki et al., 2011 (15) IL-1beta 0.740 0.205 1.274 0.007
Wakkad & Salah 2006 (51) IL-1beta 1.629 0.941 2.317 0.000
Sweeten et al., 2004 (54) IL-1beta 0.352 -0.169 0.873 0.185
Tostes et al., 2012 (42) IL-1beta 0.647 0.076 1.219 0.026
Ashwood et al., 2011b (52) IL-1beta 0.304 0.014 0.594 0.040
Ricci et al., 2013 (14) IL-1beta 1.089 0.544 1.635 0.000
0.744 0.368 1.119 0.000
Enstome et al., 2008 (24) IL-23 -0.577 -1.117 -0.037 0.036
Hashim et al., 2013 (17) IL-23 -0.808 -1.213 -0.403 0.000
Ricci et al., 2013 (14) IL-23 0.623 0.103 1.143 0.019
-0.262 -1.127 0.603 0.553
Suzuki et al., 2011 (15) IL-6 0.314 -0.206 0.834 0.237
Croonenberghs et al., 2002 (13) IL-6 0.222 -0.525 0.969 0.561
Wakkad & Salah 2006 (51) IL-6 1.505 0.830 2.180 0.000
Tostes et al., 2012 (42) IL-6 0.306 -0.254 0.866 0.285
Al-Ayadhi 2005 (50) IL-6 0.318 0.001 0.634 0.049
Ashwood et al., 2011b (52) IL-6 0.383 0.092 0.674 0.010
Ricci et al., 2013 (14) IL-6 1.089 0.544 1.635 0.000
0.552 0.253 0.850 0.000
Okada et al., 2007 (22) TGF-beta1 -1.852 -2.584 -1.121 0.000
Ashwood et al., 2008 (26) TGF-beta1 -0.784 -1.192 -0.376 0.000
Hashim et al., 2013 (17) TGF-beta1 -0.808 -1.213 -0.403 0.000
-1.061 -1.590 -0.531 0.000
Suzuki et al., 2011 (15) TNF-alpha 0.607 0.078 1.135 0.025
Sweeten et al., 2004 (54) TNF-alpha -0.326 -0.846 0.195 0.220
Tostes et al., 2012 (42) TNF-alpha -0.226 -0.785 0.332 0.427
Al-Ayadhi 2005 (50) TNF-alpha 0.318 0.001 0.634 0.049
Ricci et al., 2013 (14) TNF-alpha 1.089 0.544 1.635 0.000
0.293 -0.167 0.753 0.212
-4.00
-2.00
0.00
2.00
4.00
decreased concentration in ASD
increased concentration in ASD