Cytokine profile in a cohort of Egyptian patients with autism spectrum disorders

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Background
Autism spectrum disorders (ASD) are considered one of the wide-ranging spectrums of neurodevelopmental disorders identified as 'pervasive developmental disorders,' which happen during the childhood. Evidence has been accumulating which suggests that immune system dysregulation is involved in the pathophysiology of autism. This study aims at investigating the serum levels of 16 cytokines in addition to $CD4^+$ T cell count in patients with ASD.

Patients and methods
A multiplex assay for a panel of 16 cytokines and chemokines was applied to serum samples from 39 autistic patients and matched controls (n = 23) using Luminex technology. $CD4^+$ T cell was estimated for all patients and controls using flow cytometry.

Results
Among a total of 16 analytes examined, the serum concentrations of monocyte chemoattractant protein-1/CCL2, receptor for advanced glycation end products, and growth-regulated oncogene-α (P = 0.03, 0.01, and 0.03, respectively) were significantly higher in ASD patients as compared with matched controls. However, the macrophage-inhibitory factor result showed significant downregulation (P = 0.008) in autistic patients compared with controls. In addition, $CD4^+$ T cell count was significantly increased (P = 0.0002) in ASD patients in comparison with apparently healthy controls.

Conclusion
Children with ADS have significantly increased levels of monocyte chemoattractant protein-1/CCL2, growth-regulated oncogene-α, receptor for advanced glycation end products, and $CD4^+$ T cell count, while macrophage-inhibitory factor was significantly decreased in comparison to controls, which suggests an underlying defect in the immune function and abnormal immune responses. These results may have intense implications for the treatment and diagnosis of ASD.

Keywords:
autism, chemokines, cytokines, immune system

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Introduction

Autism spectrum disorders (ASDs) are described as multifarious, pervasive neurodevelopmental conditions. Etiology is generally unknown and there is a male predilection. ASDs are known by defects concerning social interaction and communication in addition to limited, repetitive patterns of behavior, interests, or activities (APA, 2013). The onset usually occurs in the first 36 months of childhood (APA, 1994). ASD prevalence has increased intensely, which cannot be fully attributed to increased awareness and improved diagnostic techniques (Fombonne, 2003).

Various neurological abnormalities have been detected in ASD, which proposes that during a critical window of development, standard neurodevelopment was disturbed (Rice and Barone, 2000). However, the interface between the cellular immune system and the nervous system is remarkably complex. There is broad communication taking place between them both in health as well as in disease (Jarskog *et al*., 1997). The immune system and the nervous system are complexly connected. Numerous biological processes are affected by the functional status of the immune system. This includes brain function and brain development that however may be affected by the deregulation of immune responses whether innate or adaptive (Filiano *et al*., 2015).

Bauer *et al*. (2007) demonstrated that cytokines are considered secreted proteins that control duration, intensity, and the immune response pattern. Moreover; they interact with neural systems as well, in addition to being involved in neural development and maintenance. Immune system abnormalities, including transformed cytokine profiles, are thought

to play a role in ASD (Goines and Van de Water, 2010; Masi *et al*., 2015). Immune-mediated mechanisms are assumed to reflect a chronic condition of cytokine activation (Vargas *et al*., 2005). Likewise, a raised dominance of immune-related comorbidities, comprising allergies, autoimmune diseases, and psoriasis, has been encountered in children with ASD compared with healthy controls (Zerbo *et al*., 2015).

Patterns by which plasma cytokines levels vary has been studied by Suzuki *et al*. (2011), who suggested that these patterns might act as a convenient ASD biological marker. However, these results seem to be unreliable. This may have resulted from differences in the diagnostic criteria, experimental designs, and patients' age ranges. Another explanation may be that such discrepancy reveals heterogeneity of the ASD themselves. Moreover, multiplex technologies have been developed which allowed simultaneous measurement of various analytes. Multiplex techniques offer data on a broad profile of analytes, even in the presence of an inadequate sample volume (Di Nisio *et al*., 2005; Hart *et al*., 2005). In this study, Luminex multiplex assay was used to measure a sequence of 16 cytokines in the serum and plasma of children with ASD as compared with matched healthy normally developing controls to provide further clarification of the relationship between immune responses and ASD.

Patients and methods

This study included 39 autistic patients; 11 males and 28 females, aged from 13 months to 26 years representing all patients with ASD, who were referred to the Genetics Clinics at the Medical Research Centre of Excellence, National Research Centre (NRC), Egypt during a period of 3 years. They were subjected to detailed history (personal, pregnancy, delivery). Detailed milestones of development, onset of symptoms, vaccination history, nutritional history, history of sleep disorders, gastrointestinal tract manifestations, and history of repeated infections were all recorded. Pedigree construction up to three generations with particular emphasis on similarly affected family members and other psychiatric disorders was performed. All ASD patients met the diagnostic criteria of ASDs according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V). Severity rating of autism using Childhood Autism Rating Scale (CARS) was done. All patients were subjected to electroencephalography. A written consent was taken from each child parent or legal guardian. Approval was taken to conduct this research from the ethics committee of the NRC in Egypt. Twenty-three age-matched and sex-matched apparently healthy individuals served as controls, who had no history of delay in developmental milestones or any neurological or psychiatric disorders.

Blood samples

A volume of 5 ml of fresh peripheral blood samples from all participants was drawn in sterile EDTA-containing tubes for the procedure of flow cytometry, which is performed according to the procedure mentioned below. Another 5 ml blood was centrifuged at a rate of 3000 rounds per minute for 10 min to separate the serum, which was collected and immediately frozen in 0.5 ml aliquots at −80°C until assayed for cytokine and chemokine levels by Luminex immunoassay.

Luminex immunoassay technology

Interleukin 2 (IL-2), intercellular adhesion molecule-1, E-selectin, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, IL-6, IL-1α, monocyte chemoattractant protein-1 (MCP-1)/CCL2, macrophage-inhibitory factor (MIF), receptor for advanced glycation end products (RAGE), regulated upon activation, normal T cell expressed and secreted/CCL5, tissue inhibitor matrix metalloproteinase-1, growth-regulated oncogene-α (GRO-α), IL-15, MIP-1α/CCL3, and Tie-2 levels were estimated using a fluorescent bead-based Luminex multiplex immunoassay kit (California, USA). Luminex is a flow analyzer; the LABScan 100 uses a labeled microsphere or beads, allowing for simultaneous capture of multiple analytes from a single reaction; it identifies the fluorescent intensity of phycoerythrin on each microsphere. Antigen–antibody reactions between the serum of patients and antibodies attached on the microspheres is the principle of this technology. The assignment of cytokines and its concentration are based on the reaction pattern which applies Luminex technology recommended by the Luminex Corporation (R&D Systems Inc., Minneapolis, CA, USA) .

Flow cytometry

$CD4^+$ T cells were detected and counted using flow cytometry. The monoclonal antibody was purchased from Dako (Denmark). Isotopic control was used to provide a baseline for determining the minimum fluorescence above which positive cells were identified. Data analysis was done to determine the percentage positivity for the antibody (Carleton and Sigrid, 2004).

Statistical analysis

Data were analyzed using SPSS, version 19.0 software (SPSS Inc., Chicago, Illinois, USA). Mann–Whitney *U* test was used for comparing the nonparametric results among groups, while correlations were done using Spearman's rank correlation test. Data were presented as median. All analyses were two-tailed, and *P* value less than 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of all autistic patients are represented in Table 1. This study involved 11 (28.2%) autistic males in addition to 28 (71.8%) autistic females with ages that ranged from 1.1 to 26 years. Moreover, 23 age-matched and sex-matched apparently healthy individuals were included as controls.

Table 2 sums up the laboratory findings of IL-2 and intercellular adhesion molecule-1 in addition to E-selectin, IFN-γ, TNF-α, IL-6, IL-1α, MCP-1/CCL2, MIF, RAGE, regulated upon activation, normal T cell expressed and secreted/CCL5, tissue inhibitor matrix metalloproteinase-1, GRO-α, IL-15, MIP-1α/CCL3, and Tie-2 levels and total leukocytic count and CD4 absolute count in ASD patients and the control group. Serum levels of MCP-1/CCL2, RAGE, and GRO-α (*P* = 0.03, 0.01, and 0.03, respectively) were significantly increased in ASD patients compared with the corresponding values of matched controls, while MIF results showed significant downregulation (*P* = 0.008) in autistic patients compared with controls. In addition, $CD4^+$ T cell count was significantly increased (*P* = 0.0002) in ASD patients in comparison with healthy normally developing controls.

The percentage of the T helper cells (CD4) in children with autism and age-matched healthy controls is presented in Fig. 1. Autistic children had elevated percentage and number of $CD4^+$ cells compared with controls.

Correlation analysis of our results showed that there are no significant correlations between MCP-1/CCL2, RAGE, GRO-α, or MIF with CARS score in autistic patients (Table 3).

Table 1 Demographic and clinical characteristics

Characteristic	ASD (*n*=39)
Age (years) [mean (SD)] [range]	5.3 (2.8) [1.1-26]
Sex [*n* (%)]	
Male	11 (28.2)
Female	28 (71.8)
Severity [*n* (%)]	
Mild	16 (41)
Moderate	15 (38.5)
Severe	8 (20.5)
CARS score [mean (SD)] [range]	38.46 (5.99) [31-51]
Consanguinity [*n* (%)]	11 (28.2)
Family history of psychological diseases [*n* (%)]	13 (33.33)

ASD, autism spectrum disorder; CARS, Childhood Autism Rating Scale.

Discussion

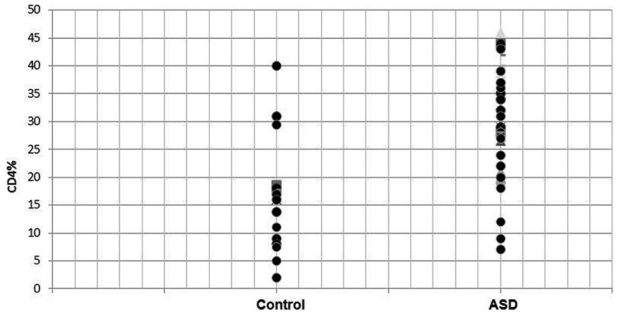
The present study included 39 patients in the age range of 1.1–26 years. They were 28 males and 11 females. Twenty-three age-matched and sex-matched normal individuals were included in this study as a normal control group. They were attending the Clinical Genetics Clinic at NRC for follow-up during the period from June 2016 to January 2019. They were subjected to full clinical examination, family history, and laboratory investigations including estimation of 16 different cytokines using the multiplex technique by Luminex and cellular expression of CD4. In this study, there were statistically significant increase in MCP-1/CCL2, RAGE, GRO-α, and CD4, while there was a decrease in MIF as compared with the control group.

Evidence has been accumulating concerning changes in the functioning of central and peripheral immune systems suggesting that a subgroup of ASD shows evidence of immune system deregulation (Mead and Ashwood, 2015).

Cytokines and chemokines are cell-signaling molecules used by the immune system to coordinate appropriate immune response (Heuer *et al.*, 2019). The early identification of biomarkers for ASD may improve the determination of risk, leading to earlier diagnosis and intervention and improves outcomes (Heuer *et al.*, 2019).

MCP-1/CCL2 is considered one of the main chemokines that control both infiltration and

Figure 1



Comparison of CD4% in children with autism spectrum disorders (*n* = 39) and age-matched typically developing controls (*n* = 23).

Table 2 Laboratory findings of autism spectrum disorder patients and healthy controls

	Control (*n*=23)	ASD (*n*=39)	*P*
IL-2 (pg/ml)	92.9 (81.3-100.6)	85.1 (46.4-119.9)	0.66
ICAM-1 (ng/ml)	1168.2 (1024.8-1450.4)	1005.6 (107.8-1643.5)	0.31
E-selectin (ng/ml)	43.5 (38.0-52.9)	39 (24.5-53.5)	0.28
IFN-γ (pg/ml)	4.7 (1.2-7.1)	4.7 (2.4-7.1)	0.56
TNF-α (pg/ml)	1.1 (0.4-4.9)	1.1 (0.4-3.0)	0.44
IL-6 (pg/ml)	0.8 (0.6-2.8)	1.1 (0.6-1.7).	0.71
IL-1α (pg/ml)	0.8 (0.4-1.2)	0.8 (0.6-1.6)	0.8
MCP-1/CCL2 (pg/ml)	239.9 (128.8-303.7)	509.8 (203.4-1494.0)	0.03*
MIF (ng/ml)	111.4 (103.3-113.8)	23.6 (1.3-87.1)	0.008*
RAGE (pg/ml)	239.6 (134.6-476.0)	418.5 (338.2-566)	0.01*
RANTES/CCL5 (ng/ml)	12.3 (11.7-12.7)	10.9 (.08-12.1)	0.16
TIMP-1 (ng/ml)	21.2 (19.9-22.8)	21.1 (15.2-23.0)	0.48
GRO-α (pg/ml)	30.2 (9.1-334.9)	111.2 (9.1-268.2)	0.03*
IL-15 (pg/ml)	4.1 (3.5-5.0)	3.9 (2.7-4.9)	0.07
MIP-1α/CCL3 (pg/ml)	25.2 (8.4-39.9)	25.2 (4.1-37.8)	0.94
Tie-2 (pg/ml)	82.6 (66.1-1474.2)	198.2 (99.1-944.9)	0.14
TLC (cell/mm^6)	7.9 (3.5-9.8)	7.7 (4.3-19.3)	0.53
CD4 (cell/mm^3)	389 (48.9-972.4)	829.9 (450.2-1986.2)	0.0002*

ASD, autism spectrum disorder; GRO-α, growth-regulated oncogene-α; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage-inhibitory factor; MIP-1α, macrophage inflammatory protein; RAGE, receptor for advanced glycation end products; RANTES, regulated upon activation, normal T cell expressed and secreted; Tie-2, tyrosine kinase receptor highly expressed on endothelial cells; TIMP, tissue inhibitor matrix metalloproteinase; TLC, total leukocytic count; TNF, tumor necrosis factor. Results were expressed as median (minimum–maximum). *P* values were determined with two-tailed Mann–Whitney *U* test. *Significant compared with controls.

Table 3 Spearman's rho correlation between monocyte chemoattractant protein-1/CCL2, receptor for advanced glycation end products, growth-regulated oncogene-α, macrophage-inhibitory factor, and Childhood Autism Rating Scale

Spearman's rho	MCP-1	RAGE	MIF	GRO-α	CARS
MCP-1					
Correlation coefficient		0.14	0.28	0.33	0.45
P value (two-tailed)		0.66	0.38	0.27	0.22
RAGE					
Correlation coefficient	NS		−0.41	0.2	0.25
P value (two-tailed)			0.18	0.51	0.48
MIF					
Correlation coefficient	NS	NS		−0.21	0.5
P value (two-tailed)				0.51	0.21
GRO-α					
Correlation coefficient	NS	NS	NS		0.24
P value (two-tailed)					0.39
CARS					
Correlation Coefficient	NS	NS	NS	NS	
P value (two-tailed)					

CARS, Childhood Autism Rating Scale; GRO-α, growth-regulated oncogene-α; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage-inhibitory factor; NS, not significant; RAGE, receptor for advanced glycation end products.

migration of monocytes/macrophages (Zaki *et al*., 2016). In this study, it was increased in autistic patients when comparing them with the normal control group. This was in agreement with Vargas *et al*. (2005) who stated that MCP-1 is pro-inflammatory and is raised in brain specimens from patients with ASD.

Another cytokine recently linked to ASD is MIF (Grigorenko *et al*., 2008). This conserved cytokine found as a homotrimer protein is considered a pro-inflammatory immune regulator, which is mainly expressed within the brain tissues (Bacher *et al*., 1998) mediates both acute and chronic inflammatory responses, (Leyton-Jaimes *et al*., 2018) and has important influences on neural and endocrine systems (Fingerle-Rowson and Bucala, 2001). Grigorenko *et al*. (2008) demonstrated that genotyping studies on more than 1000 families revealed two polymorphisms. These were within the promoter region of MIF which interrelated with autism. Furthermore, MIF plasma levels were found higher in autism cases compared with controls. Where Han *et al*. (2017) did not find any significant differences in the concentrations of MIF in children with ASD compared with those observed for typically developing children. Also Tomoum and Aly (2009) showed that results of MIF were not considerably altered in autistic patients and control groups. Yet, they reported that patients with severe disorders had significantly lower levels of the MIF compared with those with milder disease, which is in line with our results.

GRO-α is a chemokine released by macrophages in addition to other cell types as epithelial and endothelial cells. This chemokine has a chemotactic activity on neutrophils and has significant roles in innate immune response. Preceding studies (Ashwood *et al*., 2011) have examined GRO-α level in serum, and found no significant difference as compared with controls, but in the present study GRO-α was significantly increased in ASD patients compared with the corresponding

values of matched controls. In another study by Suzuki *et al*. (2011) a multiplex assay for cytokines and chemokines was applied to plasma samples from 28 males with ASD and 28 controls; they found significant increase in the plasma level of GRO-α in ASD patients in comparison with the controls, which is in agreement with our results. However, why this chemokine increased in patients with ASD is not clear. In the same context, IL-17 is recognized as a strong mediator of GRO-α production from epithelial cells (Eyerich *et al*., 2010). That is to say that activated Th17 cells secrete IL-17, which leads to the elevation of GRO-α levels in autistic patients.

In this study, RAGE levels were upregulated in autistic patients. This result came in line with Boso *et al*. (2006), who indicated that a pro-inflammatory advanced glycation end product level was elevated in people with autism and the expression of its receptor RAGE was hampered.

T and B lymphocytes mediate adaptive immunity. Nevertheless, T helper cells are those vital for nearly all adaptive immune responses (Depino and de los Angeles Robinson-Agramonte, 2015). They participate in the activation of B cells to secrete antibodies and macrophages to eradicate pathogens. On the other hand, cytokine pattern of production and function differentiate naïve T helper cells into different types, namely, type 1 T helper (Th1), type 2 T helper (Th2), T-regulator, and Th17 cells (Zhu *et al*., 2010). Our study showed that children with autism had diminished cell-mediated immunity as demonstrated by increased numbers of $CD4^+$ T cells compared with controls. This result was in agreement with López-Cacho *et al*. (2016), who reported that B and T lymphocyte numbers were found to be increased in 59 adult ASD patients compared with 26 control adults using flow cytometry. On the other hand, Bradstreet *et al*. (2014) found that $CD19^+$ B lymphocytes are increased and $CD4^+$ T helper cells were found decreased in 45 ASD children and teens (3–15-year olds).

Regarding IFN-γ there was no significant difference, which was in agreement with Basheer *et al*. (2018). These finding are also consistent with the study by Suzuki *et al*. (2011), who demonstrated that plasma levels of IFN-γ and IL-2 were similar between patients with ASD and controls. Also, we encountered no major variations of peripheral pro-inflammatory cytokine levels including IL-1α, IL-2, IL-15, and TNF-α in patients with ASD as compared with the control group. This was in agreement with Saghazadeh *et al*. (2019), where elevated TNF-α in ASD compared with healthy controls was identified by Ricci *et al*. (2013) suggesting a dysregulated immune response.

Finally, no analyte serum level was linked to the brutality of autism symptoms as measured with CARS. These results are in line with Suzuki *et al*. (2011), who proposed that the elevation of cytokines might signify an atypical state of steady immune response in ASD, and cytokine multiplex analysis may present one of the biological trait markers for the disease.

Conclusion

This study demonstrated the role of the immune system in ASDs through evaluating the cytokines levels in autistic patients, which may have profound effects in the diagnosis as biological markers and may lead to therapeutic interventions for this disease.

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Conflicts of interest

There are no conflicts of interest.

References

APA (1994) *Diagnostic and statistical manual of mental disorders*. 4th edition Washington, DC: American Psychiatric Association Publishing Inc.

APA (2013). *Diagnostic and statistical manual of mental disorders*. 5th edition. Washington, DC: American Psychiatric Association. Publishing Inc.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J (2011). Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *J Neuroimmunol* **232**:196–199.

Bacher M, Meinhardt A, Lan HY, Dhabhar FS, Mu W, Metz CN, *et al*. (1998). MIF expression in the rat brain: implications for neuronal function. *Mol Med* 4:**217**–230.

Basheer S, Venkataswamy MM, Christopher R, Van Amelsvoort T, Srinath S, Girimaji SC, Ravi V (2018). Immune aberrations in children with Autism Spectrum Disorder: a case-control study from a tertiary care neuropsychiatric hospital in India. *Psychoneuroendocrinology* **94**:162–167.

Bauer S, Kerr BJ, Patterson PH (2007). The neuropoietic cytokine family in development, plasticity, disease and injury. *Nat Rev Neurosci* **8**:221–232.

Boso M, Emanuele E, Minoretti P, Arra M, Politi P, Ucelli di Nemi S, Barale F (2006). Alterations of circulating endogenous secretory RAGE and S100A9 levels indicating dysfunction of the AGE-RAGE axis in autism. *Neurosci* Lett **410**:169–173.

Bradstreet JJ, Sych N, Antonucci N, Klunnik M, Ivankova O, Matyashchuk I, *et al*. (2014). Efficacy of fetal stem cell transplantation in autism spectrum disorders: an open-labeled pilot study. *Cell Transplant* 23(Suppl. 1):S105–S112.

Carleton CS, Sigrid JS (2004). Multiparameter data acuisition and analysis of leukocytes. In: Hawley TS, Hawley RG, editors. *Flow cytometry protocols*. New York: Springer; 45–65.

Depino MA, de los Angeles Robinson-Agramonte M (2015). *Understanding on neuroimmunology in autism spectrum disorder. Translational approaches to autism spectrum disorder*. Havana, Cuba: Springer International Publishing: 155–180.

Di Nisio M, Niers TM, Reitsma PH, Buller HR (2005). Plasma cytokine and Pselectin levels in advanced malignancy: prognostic value and impact of low molecular weight heparin administration. *Cancer* **104**:2275–2281.

Eyerich S, Eyerich K, Cavani A, Schmidt-Weber C (2010). IL-17 and IL-22: siblings, not twins. *Trends Immunol* **31**:354–361.

Filiano AJ, Gadani SP, Kipnis J (2015). Interactions of innate and adaptive immunity in brain development and function. *Brain Res* **1617**:18–27.

Fingerle-Rowson GR, Bucala R (2001). Neuroendocrine properties of macrophage migration inhibitory factor (MIF). *Immunol Cell* Biol **79**:368–375.

Fombonne E (2003). The prevalence of autism. *JAMA* **289**:87–89.

Geneva: World Health Organization WHO (1992). Clinical description and diagnostic guidelines. Available at: https://goo.gl/1zs5yk.

Goines P, Van de Water J (2010). The immune system's role in the biology of autism. *Curr Opin Neurol* **23**:111–117.

Grigorenko EL, Han SS, Yrigollen CM, Leng L, Mizue Y, Anderson GM, *et al*. (2008) Macrophage migration inhibitory factor and autism spectrum disorders. *Pediatrics* **122**:e438–e445.

Han YMY, Cheung WKY, Wong CK, Sze SL, Cheng TWS, Yeung MK and Chan AS (2017). Distinct cytokine and chemokine profiles in autism spectrum disorders. *Front Immunol* **8**:1–12.

Hart JP, Broadwater G, Rabbani Z, Moeller BJ, Clough R, Huang D, *et al*. (2005). Cytokine profiling for prediction of symptomatic radiation-induced lung injury. *Int J Radiat Oncol Biol Phys* **63**:1448–1454.

Heuer LS, Croen LA, Jones KL, Yoshida CK, Hansen RL, Yolken R, *et al*. (2019). An exploratory examination of neonatal cytokines and chemokines as predictors of autism risk: the early markers for autism study. Biol Psychiatry **86**:255–264.

Jarskog LF, Xiao H, Wilkie MB, Lauder JM, Gilmore JH (1997). Cytokine regulation of embryonic rat dopamine and serotonin neuronal survival in vitro. *Int J Dev Neurosci* **15**:711–716.

Leyton-Jaimes MF, Kahn J, Israelson A (2018). Macrophage migration inhibitory factor: a multifaceted cytokine implicated in multiple neurological diseases. *Exp Neurol* **301**:83–91.

López-Cacho JM, Gallardo S, Posada M, Aguerri M, Calzada D, Mayayo T, *et al*. (2016). Characterization of immune cell phenotypes in adults with autism spectrum disorders. *J Investig Med* **64**:1179–1185.

Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ (2015). Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Mol Psychiatry* **20**:440–446.

Mead J, Ashwood P (2015). Evidence supporting an altered immune response in ASD. *Immunol Lett* **163**:49–55.

Ricci S, Businaro R, Ippoliti F, Lo Vasco VR, Massoni F, Onofri E, *et al*. (2013). Altered cytokine and BDNF levels in autism spectrum disorder. *Neurotox Res* **24**:491–501.

Rice D, Barone Jr S (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect* **108**(Suppl. 3):511–533.

Saghazadeh A, Ataeinia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N (2019). Anti-inflammatory cytokines in autism spectrum disorders. A systematic review and meta-analysis 4. *Cytokine* **123**:154740.

Suzuki K, Matsuzaki H, Iwata K, Kameno Y, Shimmura C, Kawai S, *et al*. (2011). Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. *PLoS ONE* **6**:e20470.

Tomoum HY, Aly IM (2009). SERUM levels of macrophage migration inhibitory factor in children and adolescents with autistic disorders. *Egypt J Pediatr Allergy Immunol* **7**:79–86.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol* **57**:67–81.

Zaki M, Kholoussi S, Abdel Raouf H, Helwa I (2016). Association between serum monocyte chemoattractant protein 1 concentration and lipid parameters in obese women. *Res J Pharma Biol Chem Sci* **7**:894–898.

Zerbo O, Leong A, Barcellos L, Bernal P, Fireman B, Croen LA (2015). Immune mediated conditions in autism spectrum disorders. *Brain Behav Immun* **46**:232–236.

Zhu J, Yamane H, Paul WE (2010). Differentiation of effector CD4 T cell populations. *Annu Rev Immunol* **28**:445–489.