# Autism Spectrum Disorders and Circulating Chemokines

## Abstract

Autism Spectrum Disorders (ASD) refer to a group of neurodevelopmental disorders categorized by qualitative impairments in social interaction, communication and repetitive stereotypic behavior. Besides, several lines of research suggest a pivotal role of immune dysfunction in the pathophysiology of ASD. Chemokines represent a family of cytokines with at least four families identified based on their cysteine motif (CXC [α family], CC [β family], C [γ family] and CX3C [δ family]). They play critical roles in response to viral and bacterial infections as well as in homeostasis. Moreover, they serve as mediators of cellular migration during central nervous system development.

Discrepant levels of chemokines have been previously reported in several neuropsychiatric and neurodegenerative disorders such as schizophrenia, major depressive disorder and multiple sclerosis. However, they have been less extensively examined in ASD. In this chapter, an overview of research findings of circulating chemokine levels in ASD and their potential role in its pathophysiology during perinatal period and later in life is presented.

## Introduction

Autism spectrum disorders (ASD), or pervasive developmental disorders (PDD), as termed in the International Classification of Diseases, 10$^{th}$ version (ICD-10) (WHO 2010), refer to a group of heterogeneous neurodevelopmental disorders characterized by qualitative impairments in social interaction, communication and repetitive stereotypic behavior (APA 2000; Myers, Challman 2010). Despite numerous lines of research aiming to disentangle the etiology of this group of disorders, no definitive biologic tools have been universally accepted and the diagnostic standards remain based on behavioral criteria (APA 2000; WHO 2010).

Immune dysfunction plays a major role in the pathophysiology of ASD (Onore et al. 2011; Abdallah 2012). Inflammatory changes in the central nervous system (CNS) (Pardo-Villamizar 2008) and the peripheral immune system (Ashwood, Van De Water 2004) have been repeatedly reported in different biologic samples of individuals with ASD. Interestingly, such dysfunctional immune profiles have been reported during pregnancy, after birth and post mortem which may indicate an ongoing immune dysfunctional profile in individuals with ASD (Vargas et al. 2005; Abdallah et al. 2011; Onore et al. 2011).

Cytokines are molecules involved in inflammation, growth and neuroplasticity. They participate in innate and the adaptive immune responses and can be broadly classified into four groups: those with pro-inflammatory activity, anti-inflammatory activity, the hematopoietic stimulatory factors and growth factors (Remick 2003). Identifying which specific cytokines play the key role in ASD has not been an easy task and this is mainly due to the overlapping biology and functions of cytokines (Dammann, O'Shea 2008).

Chemokines represent a family of cytokines which has important roles in mediating inflammatory change effects on the neurodevelopmental trajectory in autistic offspring (Deverman, Patterson 2009). In this chapter, a brief overview of this group of cytokines is presented. Besides, current evidence regarding levels of circulating chemokines in individuals with ASD and their potential role in its pathophysiology is summarized.

# Structure of Chemokines

## Composition and classes

Chemokines represent a family of cytokines of 8-to-14-kDa proteins with 20 to 70 percent homology in amino acid sequences (Epstein, Luster 1998). At least four families of chemokines have been identified based on their N terminal cysteine motif (CXC [α family], CC [β family], C [γ family] and CX3C [δ family]). However, two families (α and β families) due to their underlined physiological and pathophysiological functions have been extensively studied. Figure 1A illustrates the structure of the four chemokine families and the loci of their cysteine motifs. A more detailed investigation of the three dimensional structure of chemokines has proved that there are four characteristic domains (three antiparallel β-sheets and an α-helix) joined by loops of peptide chain (30s, 40s, 50s (N) loops) (Figure 1B).

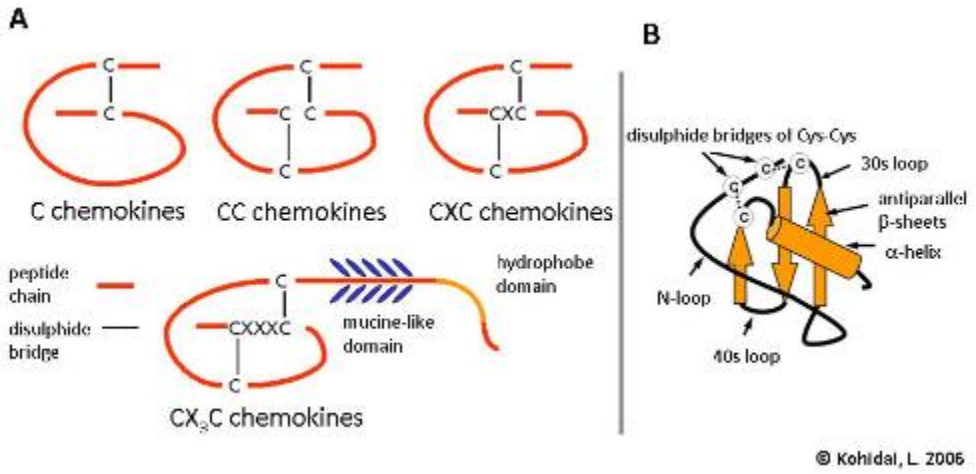


Fig. 1 Structural organization of chemokines: A – main classes of chemokines; B – three-dimensional structure and main domains in general chemokine structure.

## Nomenclature

Nomenclature of chemokines is rather complex: while the actually most frequently used acronyms apply the names of the four chemokine classes in numbered form, the descriptive traditional names and their acronyms are still used as they represent the source or target cells of the molecules or their special activity (Zlotnik, Yoshie 2000), (Table 1).

Table 1 Chemokine classes in human, acronyms and names used in literature

| Chemokine family | Acronym | Traditional name | Traditional acronym |
|---|---|---|---|
| CXC | CXCL1 | growth-related oncogene-1 | GRO1 |
| | CXCL2 | growth-related oncogene-2 | GRO2 |
| | CXCL3 | growth-related oncogene-3 | GRO3 |
| | CXCL4 | platelet factor 4 | PF-4 |
| | CXCL5 | epithelial neutrophil-activating protein 78 | ENA-78 |
| | CXCL6 | granulocyte chemotactic protein 2 | GCP-2 |
| | CXCL7 | neutrophil activating protein 2 | NAP-2 |
| | CXCL8 | interleukin-8 | IL-8 |
| | CXCL9 | monokine induced by γ-interferon | MIG |
| | CXCL10 | γ-interferon-inducible protein | IP-10 |
| | CXCL11 | interferon-inducible T-cell chemoattractant | I-TAC |
| | CXCL12 | stromal cell-derived factor | SDF-1 |
| | CXCL13 | B-cell activating chemokine | BCA-1 |

| | | | |
|---|---|---|---|
| | CXCL14 | breast and kidney expressed chemokine | BRAK |
| | CXCL15 | lungkine | Lungkine |
| | CXCL16 | scavenger receptor for phosphatidylserine and oxidized lipoprotein | SR-PSOX |
| | CXCL17 | VEGF co regulated chemokine 1 | VCC1 |
| CC | CCL1 | | I-309 |
| | CCL2 | monocyte chemoattractant protein-1 | MCP-1 |
| | CCL3 | macrophage inflammatory protein-1α | MIP-1α |
| | CCL4 | macrophage inflammatory protein-1β | MIP-1β |
| | CCL5 | egulated on activation normal T-cell expressed and secreted | RANTES |
| | CCL6 | chemokine-10 | C10 |
| | CCL7 | monocyte chemoattractant protein-3 | MCP-3 |
| | CCL8 | monocyte chemoattractant protein-2 | MCP-2 |
| | CCL9 | macrophage inflammatory protein-1γ | MIP-1γ |
| | CCL10 | not in use, identical to CCL9 | |
| | CCL11 | eotaxin | Eotaxin |
| | CCL12 | monocyte chemoattractant protein-5 | MCP-5 |
| | CCL13 | monocyte chemoattractant protein-4 | MCP-4 |
| | CCL14 | hemofiltrate cc chemokine-1 | HCC-1 |
| | CCL15 | macrophage inflammatory protein-5 | MIP-5 |
| | CCL16 | liver-expressed chemokine | LEC |
| | CCL17 | thymus and activation related chemokine | TARC |
| | CCL18 | macrophage inflammatory protein-4 | MIP-4 |
| | CCL19 | Epstein-Barr virus-induced receptor ligand chemokine | ELC |
| | CCL20 | liver- and activation-induced chemokine | LARC |
| | CCL21 | secondary lymphoid tissue chemokine | SLC |
| | CCL22 | macrophage-derived chemokine | MDC |
| | CCL23 | macrophage inflammatory protein-3 | MIP-3 |
| | CCL24 | eotaxin-2 | Eotaxin-2 |
| | CCL25 | thymus-expressed chemokine | TECK |
| | CCL26 | eotaxin-3 | Eotaxin-3 |
| | CCL27 | cutaneous T-cell attracting chemokine | CTACK |
| | CCL28 | mucosae associated epithelia chemokine | MEC |
| C (or XC) | CL1 | lymphotactin α | Lymphotactin α |
| | CL2 | lymphotactin β | Lymphotactin β |
| $CX_3C$ | $CX_3CL$ | fractalkine | Fractalkine |

## Functions of chemokines

Based on their functions, chemokines can be classified into two groups; Homeostatic and inflammatory. Homeostatic (constitutive) chemokines have a decisive role in embryogenesis and organogenesis (e.g. CXCL12, CXCL13 and CCL12), they are tissue or cell specific (CCL5 – thymus) and continuously expressed, however, they are produced regularly in small quantities. These chemokines have also a significant role even in the immune surveillance of adults.
Synthesis of inflammatory (inducible) chemokines (IL-8, IP-10, RANTES, MCPs, and MIPs) is induced by proinflammatory cytokines and their production is a transient, accompanying phenomenon of immune responses (e.g. inflammation). These chemokines are synthesized typically in immune responder cells; their expression level is rather high in this functionally well defined group of cells. A special set of chemokines is expressed in tumors and other clinical disorders which has an increasing importance even in clinical laboratory diagnostics (Epstein, Luster 1998)

Production of chemokines shows class dependency. For example, majority of CXC chemokines are synthesized in

monocytes, lymphocytes or in endothelial cells. A more wide range of cells are responsible for production of CC chemokines such as monocytes, fibroblasts, epithelial and endothelial cells, smooth muscle and glioma or melanoma cells as representatives of tumors. On the other hand, both endothelial cells and microglia are able to release the single CX3C chemokine, while the two C chemokines are synthesized by CD8+ T lymphocytes. (Table 2)

Investigations of chemokines revealed specific target cells of each chemokine classes, however, phenomena of redundancy (more chemokines acting on one receptor) and pleiotropy (more receptors for one chemokine) are both characteristic to this group of signal molecules (Rostène et al. 2011). The main target cells of CXC chemokines are neutrophils and T or NK lymphocytes; CC chemokines, however, are acting primarily on monocytes and dendritic cells; C chemokines are activators of T lymphocytes while the only CX3C chemokine (fractalkine) induces the chemotaxis of monocytes and T lymphocytes. (Table 2)

Due to the above mentioned redundancy of chemokines, a complex network of chemokine producer cells and chemokines target cells was described. Such networks provide crucial plastic moiety to the immune system and are responsible for the rapid, tissue specific tunings of target cells. Furthermore, the high level homology (65%) of the clinically most significant CC chemokines results in having a wide range of actions. The highly related three CC chemokines MCPs, MIPs and eotaxin are cross-reacting in monocytes and eosinophils which target cell specificity depending on the integrity of the N-terminal region of the molecules.

Table 2 Comparative charts of organ/tissue/cell specificity of chemokine synthesis and their target cells with focus on chemokine groups of reviewed in the chapter

| Chemokine family | Acronym | Traditional acronym | Source organ /tissue | Produced by | Target cell |
|---|---|---|---|---|---|
| | CXCL1 | GRO1 | respiratory organs | macrophage, neutrophil grc., epithel, melanoma | neutrophil grc. endothel |
| | CXCL8 | IL-8 | | macrophage, epithel, endothel | neutrophil grc., endothel, macrophage, mast cell, keratinocyte |
| | CXCL10 | IP-10 | | monocyte, endothel, fobroblast | monocyte, macrophage, T and NK lymphocytes, dendritic cell, endothel |
| | CCL2 | MCP-1 | | monocyte, macrophage, dendritic cell, osteoblast, osteclast, glial cell | monocyte, T lymphocyte, mast cell, basophil grc., stem cells |
| | CCL3 | MIP-1α | | monocyte, macrophage, dendritic cell, T and B lymphocyte | monocyte, T, B, and NK lyphocytes, mast cell, basophil grc., dendritic cell, stem cell |
| | CCL4 | MIP-1β | | monocyte, macrophage, dendritic cell, T and B lymphocyte | monocyte, T and NK lymphocyte, stem cell |
| | CCL5 | RANTES | | T lymphocyte | monocyte, T and NK lymphocytes, eosinophil grc., dendritic cell |
| | CCL7 | MCP-3 | tumors | macrophage | monocyte, T lymphocyte, mast cell, eosinophil grc., dendritic cell |
| | CCL8 | MCP-2 | | B lymphocyte | monocyte, T and NK lymphocyte, mast cell, basophil grc. eosinophil grc. |
| CXC | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | CCL9 | MIP-1γ | Peyers patches | epithel | dendritic cell<br>osteoclasts |
| | CCL11 | Eotaxin-1 | | monocyte,<br>macrophage,<br>dendritic cell,<br>T lymphocyte | eosinophil grc. |
| | CCL12 | MCP-5 | lymph node<br>thymus | macrophage | monocyte, lymphocyte, eosinophil grc. |
| | CCL13 | MCP-4 | small intestine,<br>thymus,<br>colon,<br>lung,<br>trachea, stomach,<br>lymph node | epithel,<br>endothel | monocyte,<br>T lymphocyte,<br>basophil grc.,<br>eosinophil grc. |
| | CCL15 | MIP-5 | liver,<br>small intestine,<br>colon,<br>lung | macrophage | neutrophil grc., monocyte, lymphocyte |
| | CCL18 | MIP-4 | lung,<br>lymph nodes,<br>placenta,<br>bone marrow | macrophage,<br>dendritic cell | T lymphocyte |
| | CCL23 | MIP-3 | lung,<br>liver,<br>bone marrow,<br>placenta | myeloid cell lines | monocyte,<br>T lymphocyte, neutrophil grc. |
| | CCL24 | Eotaxin-2 | | monocyte,<br>T lymphocyte | eosinophil grc.,<br>T lymphocyte, neutrophil grc. |
| | CCL26 | Eotaxin-3 | heart,<br>lung,<br>ovary, endothel | umbilical vein endothel | eosinophil and basophil grc. |
| C<br>(or XC) | CL1 | Lymphotactin α | spleen, intestine,<br>thymus,<br>blood leukocytes | CD8+ T lymphocyte | T lymphocyte |
| | CL2 | Lymphotactin β | | T lymphocyte | T lymphocyte |
| $CX_3C$ | $CX_3CL$ | Fractalkine | small intestine,<br>colon,<br>testis,<br>prostate,<br>heart,<br>brain,<br>lung,<br>skeletal muscle,<br>kidney, pancreas | microglia<br>neuron | monocyte,<br>T and NK lymphocytes |

## Levels of chemokines and ASD

Altered levels of chemokines have been previously reported in several neuropsychiatric and neurodegenerative disorders such as schizophrenia, major depressive disorder, Multiple Sclerosis and Alzheimer's disease (Brietzke et al. 2009). For example, elevated levels of CCL2/MCP-1 in serum samples of patients with schizophrenia (reviewed in Drexhage et al. 2009) and major depressive disorders (Sutcigil et al. 2007) were reported. Conversely, reduced levels of CCL2 were reported in neurodegenerative disorders such as Alzheimer's disease (Reale et al. 2008) and multiple sclerosis (Moreira et al. 2006).

In ASD, levels of chemokines have been less extensively examined (Ashwood et al. 2011). Current body of evidence suggests discrepant levels of number of chemokines in patients with ASD compared to controls. While several research groups examined different chemokine levels in diverse biologic samples including serum, cerebrospinal fluid (CSF), and amniotic fluid, a convergence toward a common chemokine profile has not been reached yet.

To examine the current evidence regarding chemokine levels and ASD a search of PubMed using the Medical Subject Headings of the National Library of Medicine (MeSH) terms presented in Table 3 was performed. We limited our search to studies reporting human measurements of chemokines from January 1, 2002, through June 15, 2012 published in English language.

Table 3: MeSH Terms used in Literature Search

| Exposure MeSH terms | Outcome MesH terms |
|---|---|
| Chemokines | Autistic Disorder |
| Chemokines, CC | Child Development Disorders, Pervasive |
| Chemokines, CXC | Rett Syndrome |
| Chemokines, C | |
| Chemokines, CX3C | |
| Chemokine CCL2 | |
| Interleukin-8 | |
| Chemokine CCL1 | |

The literature search yielded twelve articles. After reviewing all the articles, only eight articles were original research based on human subjects. Furthermore, a recently published relevant Danish doctoral dissertation was included in the review. Table 4 lists the studies included, the number of subjects as well as method of measurement and the biologic materials analyzed.

Table 4: List of the studies included in the literature review performed, the examined chemokines, the number of subjects and the biologic material analyzed

| No. | Reference | Subjects included | Biologic Material | Method of Measurement |
|---|---|---|---|---|
| 1. | (Abdallah 2012) | ASD, n=359<br>Controls, n= 741 | nDBSS | multiplex assay |
| 2. | (Abdallah et al. 2012) | ASD, n=331<br>Controls, n= 698 | Amniotic Fluid | multiplex assay |
| 3. | (Suzuki et al. 2011) | ASD, n=28<br>Controls, n= 28 | plasma | multiplex assay |
| 4. | (Ashwood et al. 2011) | ASD, n= 80<br>Typically developing controls, n = 58<br>Developmental disability other than ASD, n=37 | plasma | multiplex assay |
| 5. | (Ashwood et al. 2010)* | FXS + ASD, n = 40<br>Only FXS, n = 64<br>Typically developing controls, n= 19 | plasma | multiplex assay |
| 6. | (Li et al. 2009) | ASD, n=8<br>Controls, n= 8 | Frozen human brain tissue | multiplex assay |
| 7. | (Nelson et al. 2006) | Autistic disorder, n=47<br>Down Syndrome, n= 46<br>Controls, n= 90 | nDBSS | multiplex assay |
| 8. | (Vargas et al. 2005) | Autistic disorder, n=11<br>Controls, n= 12<br>Autistic disorder, n=6<br>Controls, n= 9 | Fixed brain tissues from MFG, ACG and CBL<br>CSF | multiplex assay and ELISA |
| 9. | (DeFelice et al. 2003) | ASD, n=6<br>Controls, n= 9 | Intestinal endoscopic biopsies | ELISA |

Abbreviations: ASD: Autism Spectrum Disorders; nDBSS: neonatal dried blood spot samples; FXS: Fragile X Syndrome; MFG: middle frontal gyrus; ACG: anterior cingulate gyrus; CBL: cerebellar hemisphere

* Only male subjects were included.

Based on research findings, discrepant chemokine levels were reported in central nervous system (CNS) as well as peripheral immune system. In CNS, MCP-1, a chemokine involved in innate immune reactions, was significantly elevated in brain tissues (cingulate gyrus and cerebellum) of subjects with autism compared to controls (Vargas et al. 2005). In cerebral cortex, however, elevated levels of IL-8 which has powerful chemotactic effects on T cells and neutrophils were reported. One study examined levels of chemokines in cerebrospinal fluid and reported significantly elevated levels of MCP-1, IL-8 and IP-10 by respectively 12.2, 6 and 18.2 folds (Vargas et al. 2005) in subjects diagnosed with ASD compared to controls.

Discrepant levels of chemokines were reported in plasma, amniotic fluid and neonatal dried blood spot samples (nDBSS). However, a single study investigating levels of chemokines in intestinal endoscopic biopsies failed to report differential chemokine profile of IL-8. Two studies examining levels of chemokines in plasma reported elevated levels of IL-8 and GRO-α (Suzuki et al. 2011) and MCP-1, RANTES and eotaxin (Ashwood et al. 2011) in ASD subjects compared to controls or to subjects with developmental disability other than ASD. Interestingly, in the latter study, authors found significant associations between increased MCP-1, RANTES and eotaxin levels and more aberrant behaviors or impairments in cognitive and adaptive function. Conversely, a third study which measured chemokine and other cytokine levels in plasma comparing male subjects with ASD and Fragile X syndrome (FXS) to those with only FXS and typically developing controls found decreased levels of eotaxin, MCP-1α, RANTES and IP-10 in subjects with ASD+FXS compared to typically developing controls (Ashwood et al. 2010).

Despite evidence suggesting that the critical period of the pathogenesis of ASD occurs during fetal brain development and the first year of life. (Pardo et al. 2005), studies examining levels of chemokines during this period are limited. Based on neonatal samples retrieved from a neonatal screening program for metabolic disorders in the state of California, USA, Nelson et al. reported no significant difference in n-DBSS levels of IL-8 retrieved from later diagnosed ASD subjects compared to controls (Nelson et al. 2006).

To date, the largest study that examined levels of chemokines during fetal and neonatal period, was based on a Danish historic birth cohort (HBC) and the Danish Newborn Screening Biobank (DNSB) (Abdallah 2012). The HBC is based on a collection of antenatal biological samples of amniotic fluid and maternal serum samples obtained during screening/diagnostic procedures and kept Statens Serum Institute (SSI) in Copenhagen, Denmark. On the other hand, the DNSB has been a collection of n-DBSS in SSI since 1982 (Nørgaard-Pedersen, Simonsen 1999). It enjoys almost universal coverage with more than 65,000 samples are stored every year (Nørgaard-Pedersen, Simonsen 1999).

In their study, Abdallah et al. analyzed levels of three β chemokines (MCP-1, MIP-1α and RANTES) and IL-8 (αchemokine) in amniotic fluid and n-DBSS samples belonging to the same subjects. (Abdallah 2012; Abdallah et al. 2012) Their analyses based on samples of amniotic fluid yielded significantly elevated levels of MCP-1 in ASD cases compared to controls (Abdallah et al. 2012). In postnatal n-DBSS, authors reported significantly decreased levels of RANTES and increased levels of IL-8 in subjects with ASD compared to frequency matched controls based on year of birth and gender (Abdallah 2012).

## Discussion

Chemokines are a group of cytokines, structurally similar and important in the development of lymphocytes, including their recruitment and trafficking to specific tissue compartments. Besides, they play an important role in normal CNS development and in directing the trafficking and movement of mononuclear cells in the CNS (Ashdown et al. 2006).

Several studies have indicated a potential role of chemokines in the etiopathology of different psychiatric and neurodevelopmental disorders (Meyer et al. 2009). While altered chemokine profiles have been reported in ASD individuals in different biologic samples, a unified chemokine profile has not been identified yet.

It is possible that differences in study population characteristics (especially age), analytic platforms and methodology may explain some of the inconsistencies. In addition, the biology of the chemokines themselves, where there is a high degree of overlap in their functions, their receptors and sources of secretion, adds to the complexity of identifying a unique pattern. Given that chemokines can play role in both acute systemic reactions as well as neuroinflammatory processes (Pardo et al. 2005), their specific role may also vary depending on the level, the characteristics, and the timing of the trigger (Meyer et al. 2006; Dammann, O'Shea 2008).

For example, elevated levels of RANTES were reported in ASD later in life compared to decreased levels during neonatal period. This finding draws parallel with Carlo et al. RANTES measurements in cerebral palsy, where authors reported

significantly decreased RANTES levels measured on day three and day seven after birth in low birth weight offsprings who later develop cerebral palsy compared to controls (Carlo et al. 2011). However, they failed to document such differences at age two or three weeks of life in the same subjects.

The decreased neonatal chemokine levels during neonatal period reported by Abdallah et al. could suggest a hypoactive immune cell pattern during the early neonatal period in ASD. Such pattern of dysfunctional cell-mediated immunity was suggested earlier by Gupta et al. (1998), and given that “too little immune activity” can also lead to impairments of neurogenesis, (Ziv, Schwartz 2008) it is plausible that such a scenario is existing during early neonatal period in ASD.

Interestingly, RANTES acts as a potent chemoattractant for many cell types such as monocytes, Natural Killer (NK) cells and memory T cells. It also regulates protective immunity to several infections (Crawford et al. 2011). Therefore, its decreased neonatal levels can also explain increased rates of infection reported in children with ASD (Atladottir et al. 2010; Abdallah et al. 2012).

Elevated levels of chemokines during pregnancy in amniotic fluid samples (mainly MCP-1 and IL-8) may decipher either an etiologic immunologic dysfunction or play rather an indirect role in the pathophysiology of ASD. For example, the role of MCP-1 in neuroinflammation has been well-established through use of the animal model of Experimental Autoimmune Encephalomyelitis (EAE) (Mahad, Ransohoff 2003). MCP-1 is also postulated to play an important role in the maturation of cerebellar Purkinje cells and may serve as a useful marker of abnormal neuronal development (Zhen Meng et al. 1999). Consequently, a genetic predisposition and/or an environmental insult during pregnancy may result in an overexpression of MCP-1 in the fetal brain. This overexpression may intervene with normal neurodevelopment and ultimately lead to autism. (Hesselgesser, Horuk 1999)

The ability of MCP-1 to induce a blood-brain barrier (BBB) breakdown (Stamatovic et al. 2005) and the localization of MCP-1 in nerve terminals in the posterior pituitary gland may explain its presence in the fetal circulation (Rostène et al. 2007) and eventually in the amniotic fluid (Chow et al. 2008).

Moreover, elevated levels of IL-8 were also associated with neurodevelopmental impairment in preterm infants (Carlo et al. 2011). Animal studies have shown that transcripts of CXCR1 (an IL-8 receptor) are expressed almost ubiquitously in several regions of the brain, what may suggest that a potential role of IL-8 in neurodevelopment is not related to inflammatory effects, (Nelson et al. 2001; Danik et al. 2003) drawing a parallel with the hypoactive immune response seen in neonatal period in ASD.

As an epiphenomenon, elevated levels chemokines can be approached in concert with other obstetric complications. Elevated levels of MCP-1 have been repeatedly reported in preterm labor and premature rupture of membranes (PROM) (Holst et al. 2007; Gomez-Lopez et al. 2010). Interestingly, the role of MCP-1 in preterm labor was proposed to take place regardless of the presence of intra-amniotic infection (IAI) (Esplin et al. 2005). Given the consistently reported associations between preterm delivery and autism in epidemiological studies, (Newschaffer et al. 2007) it is also possible that the connection between MCP-1 and autism is a rather indirect one.

Additionally, given the important role of chemokines in response to different viral and bacterial infections, (Epstein, Luster 1998) and the fact that maternal infections have been etiologically linked to autism, (Nahmias et al. 2006; Atladottir et al. 2010) one can postulate that elevated intrauterine chemokine levels may be induced by a maternal viral or bacterial infection or , in more broader terms, a maternal immune activation that eventually leads to the clinical phenotype later in life (Ashdown et al. 2006).

Another explanation of intrauterine/postnatal chemokine levels discrepancy could be due to different sites of secretion. Hence, elevated amniotic fluid MCP-1 levels reported earlier could be of maternal or placental rather than fetal origin. For example, maternal leukocytes production of MCP-1 and other chemokines were reported earlier in placentas infected with parasites. (Suguitan Jr et al. 2003) Furthermore, ex-vivo analyses of placental tissue showed its ability to produce chemokines such as MCP-1 in response to inflammatory triggers. (Rajesh et al. 2010) Even though evidence regarding the ability of cytokines/chemokines to traverse placental barrier to the fetus is inconclusive,(Zaretsky et al. 2004; Aaltonen et al. 2005) a non-placental maternal/fetal transfer of cytokines through paracellular diffusion through fetal membranes is also possible.(Aaltonen et al. 2005)

Finally, specificity of findings of chemokine levels to ASD remains another venue to be investigated in the future. The heterogeneity within ASD diagnoses themselves (Jones, Klin 2009) and the potential pathophysiological overlap with other disorders such as schizophrenia (Meyer et al. 2011) leave several questions regarding the specificity of reported chemokine profiles to ASD unanswered and makes the task to identify “pathognomonic” findings for ASD a difficult one to achieve.

## Definitions of Key Terms:

Cytokines: A group of molecules which are mainly involved in inflammation and participate in innate and the adaptive immune responses. Other functions of cytokines are their role in growth and neuroplasticity. Cytokines can be roughly classified into two groups: pro-inflammatory and anti-inflammatory cytokines.
Chemokines: A group of cytokines smaller in size and mainly involved in the attraction of leukocytes to different tissue targets. At least four families of chemokines have been identified based on their cysteine motif: α, β, γ, and δ families. Current evidence suggests a crucial role of chemokines in brain development, especially as mediators of cellular migration during embryogenesis.
Neonatal dried blood spot samples (n-DBSS):Blood samples retrieved from neonates usually after piercing the skin of the heel and blotting the blood onto a filter paper. The blood spots are allowed to dry on the filter paper for immediate testing or later storage. Several neonatal screening tests for congenital and inherited disorders such as phenylketonuria, congenital hypothyroidism can be performed utilizing the blood samples stored on the filter papers.
The Danish Historic Birth Cohort (HBC): A collection of antenatal biological samples obtained during screening and diagnostic procedures performed mainly in three Danish regions and kept later at minus 20ºC at Statens Serum Institute (SSI) in Copenhagen, Denmark. The collection of samples goes from the late 1970's until 2004 and includes more than 100,000 samples of amniotic fluid, first and second trimester maternal serum samples.
Blood-brain barrier (BBB): A series of tight junctions between endothelial cells of capillaries in the central nervous system as well as astrocytes with processes that terminate in overlapping fashion on capillary walls. Lipophilic molecules with a molecular mass less than 400 to 600 Da are transported readily through the BBB. However, many drugs and other small molecules cannot cross this barrier system.

## Key Facts

- Immune dysfunction plays important role in the pathophysiology of ASD.
- Converging evidence sheds light on the important role of cytokines in mediating inflammatory effects on the neurodevelopmental trajectory in subjects with ASD.
- Chemokines represent a family of cytokines comprised of four subgroups based on their cysteine motif.
- Discrepant levels of chemokines were reported in subjects with ASD during pregnancy, after birth and post mortem.
- Chemokines may serve as intermediate players in the well-established linkage between neuroinflammation and autism.

## Summary Points

- Inflammatory changes in the central nervous system and the peripheral immune system have been repeatedly reported in different biologic samples of individuals with ASD.
- At least four families of chemokines have been identified based on their cysteine motif: α, β, γ, and δ families.
- Research examining the role of chemokines in the pathophysiology of ASD is limited and further research is needed.
- Despite the potential role of chemokines in ASD suggested by current evidence, identifying which specific chemokine play the key role in ASD has not been an easy task due to the overlapping biology and functions of chemokines and the possible differences in study population characteristics (especially age), analytic platforms and methodology.

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