Maternal Allergic Asthma and the Impacts on Offspring Neurodevelopment

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Abstract:

Autism Spectrum Disorders are neurodevelopmental disorders most commonly characterized by social deficits, such as decreased social interactions and/or communications, as well as stereotyped and/or repetitive behaviors. While the etiology of ASD is not known, environmental factors have been widely studied for their potential contributions to ASD development. Importantly, many of these environmental factors are also suspected contributors to other neuropsychiatric disorders, such as attention deficit/hyperactive disorder (ADHD) and schizophrenia. Several environmental factors have been linked to ASD incidence, including obesity, pollution, infection, and allergy/asthma during pregnancy, with inflammation of the maternal immune system being the common thread. Among these, asthma represents a serious concern due to its prevalence, but also, asthma symptoms become worse during pregnancy. Importantly, ASD risk, and the severity of symptoms, appears to increase with an increase in asthma severity. Here, the current work investigates the impact that maternal allergic asthma (MAA) during pregnancy can have on offspring neurodevelopment.

In the second chapter of this work, we will examine the consequences that maternal allergic asthma (MAA) has on the cytokine concentration of the placenta during gestation as well as the fetal brain. This work expands on our initial studies done in a mouse model of MAA where we demonstrated behavioral changes in offspring consistent with the core behaviors of ASD due to MAA during pregnancy. Specifically, we observed a decrease in social interaction and increased repetitive behaviors in offspring. Using this model of MAA, we collected placenta and fetal brains at gestational day (GD)17.5 and performed Luminex multiplex bead assay on the

tissue homogenates. Our findings demonstrate a decrease in inflammatory cytokine concentrations in the placenta of offspring, but also an increase in many inflammatory cytokines in the offspring fetal brains. The presence of cytokine disruptions in this study provide evidence that MAA disrupts the maternal/fetal compartment which impacts offspring neurodevelopment. These neurodevelopmental changes in offspring suggest a potential driving force behind behaviors previously identified in this model.

In the third chapter of this work, we continue our studies on MAA and investigate whether the previously identified cytokine elevations in fetal offspring persist into adolescence and early adulthood. Additionally, we expand on the MAA model by including particulate matter (PM) exposure following allergic asthma challenge. PM has not only been associated with asthma development and asthma severity, but it has also been identified as a factor with the potential to increase likelihood of ASD in offspring of mothers exposed to PM during pregnancy. Despite the links between allergic asthma during pregnancy with ASD, and the link between PM exposure in pregnancy and ASD incidence, there are no studies investigating the neuroimmune outcome on offspring of these environmental factors in in combination. Here, we provide evidence from this novel model of MAA and PM exposure (using ultrafine-iron soot), that dual exposure results in altered neuroimmunity in both adolescent and young adult offspring. We also show that MAA and PM exposure alters microglial density in the hippocampus, which suggests a deviation from normal functioning of microglia in these offspring.

In the fourth and final chapter of this work, we explore MAA using a human relevant allergen and the impact this has on offspring neurodevelopment. This preliminary study used house dust mite (HDM), a common allergen in human allergic asthma, to explore whether the previously identified changes in our OVA MAA model are mirrored in this more human relevant investigation. Additionally, from human studies, it has been shown that PM exposure during allergen sensitization can increase asthma severity. Here, we investigate how PM exposure during allergen sensitization in preconception might increase asthma severity during pregnancy, and subsequently, how this impacts offspring neuroimmunity. We demonstrate in this novel model, a synergistic impact of PM exposure and allergen sensitization that may worsen neurodevelopmental outcomes in offspring.

Taken together, this body of work begins to uncover the neurobiological changes in offspring that can occur as a result of maternal allergy during pregnancy. We also show, in a first of its kind model, that exposure to asthma and PM during pregnancy increase cytokine concentration in the brains of offspring and impacts microglia function. In addition to this, we show that PM exposure with allergen sensitization, during preconception, can have a synergistic impact on offspring neurodevelopment. The findings from these studies demonstrate a need for further investigation into the links between mothers with asthma during pregnancy and the increased likelihood of birthing a child with ASD.

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Chapter 1: The Influence of Asthma on Neurodevelopment and Neuroinflammation: From Epidemiology to Basic models

Introduction:

Asthma represents one of the leading chronic illnesses among children and affects approximately 24 million people in the United States (CDC, 2021). Prevalence of asthma is on the rise, with rates of asthma estimated at 3.1% in 1980 increasing to 7.8% by 2020 (Gans et al., 2020). Of the populations most impacted, minority groups and those living below the poverty level are disproportionately more susceptible to asthma and asthma-related morbidity (Cardet et al., 2022; Ganti et al., 2022; Matsui et al., 2008; Milligan et al., 2016). Asthma mortality rates are estimated to be over 3000 deaths each year in the US. In addition, those living with asthma face an increased risk of hospitalization and are reported to miss more work and school days than those without asthma (Baek et al., 2022; Jean et al., 2019; Loftus and Wise 2015). As such, asthma represents a serious chronic disease with a substantial impact on the personal lives of asthmatic individuals as well as a heavy economic burden placed on the healthcare system.

Asthma is a heterogeneous disease most often characterized by airway inflammation and bronchial hyperresponsiveness leading to constriction, obstruction, and remodeling of the airways (Gans and Gavrilova, 2020; Mims 2015). A T-helper 2 (T_H2) immune response plays a significant role in the development of asthma through the release of pro-inflammatory cytokines and recruitment of granulocytes (Deo et al., 2010; Leon & Ballesteros, 2021; Yu et al., 2014). Asthma often occurs in response to an allergic trigger which begins with an initial sensitization to an allergen followed by an IgE immunoglobulin response where subsequent exposures to the

allergen, or challenges, lead to inflammation (Sonntag et al., 2019). Allergic asthma is not the only type of asthma, however, and several factors in addition to allergen exposure can lead to the development of asthma.

The exact cause for asthma is unknown, but genetic, epigenetic, and environmental risk factors have been identified. Hundreds of gene variants and epigenetic translational variations have been associated with an increased risk of asthma (Wang et al., 2022; Harb and Renz., 2015). Environmental contributors, such as viral infections, air pollution, and sensitization to allergens have all been associated with development of asthma and disease severity (Castillo et al., 2017; Acevedo et al., 2021; Rosário Filho et al., 2021; Mims 2015). Furthermore, pollutants such as particulate matter (PM), tobacco smoke, diesel exhaust, and ozone can exacerbate disease and symptom expression in individuals already diagnosed with asthma resulting in increased inflammatory response and airway hyperresponsiveness (Castillo et al., 2017; Samoli et al., 2011; Trasande & Thurston 2005; Hansel et al., 2008).

In addition to these contributors to asthma and asthma severity, comorbidities alongside asthma are also highly prevalent and can further increase the burden of the disease. In total, 60% of adults living with asthma have one or more comorbidities which may include other respiratory disorders, cardiac disease, vascular diseases, immune disorders, cancer, and neurological conditions (Boulet & Boulay, 2011). An increase in psychiatric conditions is also seen in both adults and children with asthma. For instance, depression and anxiety disorders are frequently seen in adults with asthma, while children with asthma may show increased symptoms of

behavioral disorders, anxiety, and depression (Cooley et al., 2022; Licari et al., 2022; Fasmer et al., 2011). Furthermore, individuals with asthma have an increased risk of cognitive deficits (Kroll & Ritz, 2023). While asthma can have neurological impacts on the individual patient, asthma during pregnancy can also disrupt the outcome of the pregnancy and disease in the offspring. Maternal asthma is associated with increased complications and risk during pregnancy and delivery (Shaked et al., 2019; Sheiner et al., 2005; Baghlaf et al., 2019). Moreover, children of asthmatic mothers are more likely to develop asthma themselves, especially if the mother's asthma is uncontrolled during pregnancy (Liu et al., 2018; Lim et al., 2010). Another consequence of maternal asthma is an increased risk of neurodevelopmental disease in offspring (Ali & Ulrik, 2013; Croen et al., 2019; Gong et al., 2019; Abdallah et al., 2011; Hisle-Gorman et al., 2018; Lyall et al., 2013; Patel et al., 2020; Fasmer et al., 2011). Together, these impacts of asthma represent not only an important disease that can affect the neuropsychiatric environment of the individuals suffering from asthma themselves, but also pose a potential risk for the neurodevelopment and wellbeing of offspring of mothers suffering from the disease. Here, we will provide a detailed overview of the current literature as it stands with regard to asthma and impacts on behavior and neuroimmunity.

Asthma and the Central Nervous System (CNS)

The phenotype typically associated with asthma is allergic asthma, an atopic disease that occurs due to sensitization to allergens (Schatz and Rosenwasser, 2014). Atopic diseases are conditions in which the immune system is predisposed to becoming sensitized towards allergens and mounting an allergic, IgE mediated response (Vaillant et al., 2022). Non-atopic, or non-allergic, asthma is less common and more severe (Peters, 2014). Development of allergic asthma is characterized by a sensitization phase and a challenge phase (Sonntag et al., 2019). Briefly, sensitization to allergen occurs when dendritic cells (DC) phagocytose an allergen in the lung. The DC then carries the allergen to the draining lymph node where it can present it to naive T cells and elicit a type 2 immune response resulting in the differentiation of T_H2 cells (León, 2017). T_H2 effector cell functions are responsible for the common pathologies and symptoms seen in asthma as T_H2 cells regulate allergen specific IgE production, eosinophil recruitment, inflammatory cytokine secretion, chronic inflammation, and expansion of memory T cells (Holgate et al., 2015; Schatz and Rosenwasser, 2014; León, 2017). Upon subsequent allergen challenge these memory T cells can be reactivated to perform effector functions and cause further inflammation (León, 2017). In both allergic and non-allergic asthma, airway remodeling is common and consists of airway wall thickening, increases in smooth muscle, and increased presence of mucous cells and mucous production which subsequently results in airway hyperresponsiveness, mucus plug formation, and the characteristic symptoms of asthma (Holgate et al., 2015; Boucherat et al., 2013)

The initiation of an allergic asthma response results from an increase in T_H2 cytokines interleukin-4 (IL-4), IL-5, and IL-13, an observation seen in both human patients with asthma

and animal models of the disease (Choy et al., 2015; Barnes 2001). IL-4 drives the differentiation of naive T cells into T_H2 cells after encountering inhaled allergen presented by DC in the lungs (Gans and Gavrilova, 2020; Chapoval et al., 2010). T_H2 cells then produce IL-5, IL-9, and IL-13, leading to eosinophil recruitment, mast cell degranulation, and resulting airway remodeling and obstruction (Hazlewood et al., 2011; Spencer and Weller, 2010; Lambrecht et al., 2000). Lung epithelial cells and fibroblasts will secrete eotaxins in high amounts during allergic airway inflammation, assisting in the recruitment of eosinophils to the lungs (Adar et al., 2014; Conroy & Williams, 2001). Classically, a phenotype of high circulating IgE and eosinophil infiltration in the lungs has defined asthma (Lloyd and Saglani, 2013). However, asthma is a highly heterogeneous disease and some patients do not show evidence of high levels IgE or respond to T_H2 repression as treatment, highlighting the need to look into other mechanisms that may be driving airway hyperresponsiveness (Cosmi et al., 2011; Choy et al., 2015). For example, some cases of asthma are induced by a T_H17 response characterized by neutrophil invasion of the lungs and high levels of IL-17. Neutrophilic asthma is non-atopic and typically non-eosinophilic. Higher levels of IL-17 and neutrophil invasion of the lungs are associated with increased disease severity (Cosmi et al., 2011). In some patients, both a T_H2 and T_H17 response is seen, suggesting that although neutrophilic asthma is non-allergic, the added T_H17 response can exacerbate allergic airway inflammation and lead to an imbalance in T_{regs}/T_H17 cells (Liu et al., 2020; Newcomb & Peebles, 2013).

The underlying inflammatory response in asthma is suspected to play a role in associated neurological comorbidities, including depression, anxiety, and attention hyperactivity disorder (ADHD) (Boulet & Boulay, 2011; King-Dowling et al., 2019; Jiang et al., 2014). Compared to

non-asthmatic individuals, asthmatic patients are twice as likely to develop depressive symptoms. This relationship between asthma and depression is bidirectional; both asthma and depression patients have an increased likelihood of having the other as a comorbidity (Loebrooks et al., 2010; Choi et al., 2019; Liu et al., 2023). In a study of 245,727 individuals spanning 57 countries, it was shown that asthma and depression are comorbid (Loerbrooks et al., 2012). Furthermore, asthmatics with depression have a higher mortality rate than asthmatics without depression (hazard ratio: 1.87) (Walters et al., 2011). There is evidence that immune dysfunction and increased systemic inflammatory molecules can be associated with depression (Blume et al., 2011; Slavich & Irwin, 2014). Specifically, increased levels of IL-6 and tumor necrosis factor alpha (TNF-α) have been observed in individuals with depressive disorders (Slavich & Irwin, 2014). Similarly, IL-6 elevations are seen in patients with anxiety, independent of depressive disorders; however, it is unknown whether this is a cause or a result of anxiety (O'Donovan et al., 2010; Salim et al., 2012). Importantly, elevated IL-6 and TNF-α have been identified in cases of severe asthma and asthma exacerbations (Berry et al., 2007; Dimitrova et al., 2019; Cui et al., 2017). Panic disorders and anxiety are also common among asthma patients, with both general symptoms of anxiety and anxiety disorders appearing at higher rates in asthmatics than non-asthmatic individuals (Ye et al., 2021; Hasler et al., 2005). The prevalence of anxiety is increased even more in individuals with difficult to treat, or treatment-resistant, asthma (Chee Kiang et al., 2015). Similar to depression, there is evidence of a bidirectional relationship between asthma and anxiety (Lee et al., 2016), with asthma increasing risk of associated anxiety, and anxiety disorders leading to increased asthma incidence, deterioration, and severity (Lee et al., 2016). Psychosocial stress is also identified as a risk factor for asthma and increased asthma morbidity,

potentially through immune mechanisms (Slatterly et al., 2012; Kemeny & Schedlowski, 2007; Mathews et al., 2011; Yonas et al., 2012).

In the context of asthma, ADHD is another common co-occurring neuropsychiatric condition with individuals frequently prescribed medications for both asthma and ADHD (Fasmer et al., 2011). Environmental factors like exposure to smoking and psychosocial stress appear to play a role in this relationship (Holmberg et al., 2015), while those with inflammatory disorders and atopic diseases are more often diagnosed with ADHD (Leffa et al., 2018; Chuang et al., 2022). In a recent meta-analysis that included studies of more than 25,000 individuals, a positive association between atopic diseases, such as asthma, with both ADHD diagnosis and severity of ADHD symptoms was noted (Chuang et al., 2022). In a Swedish study of 1,575,377 individuals, a significant association between asthma and ADHD was observed (odds ratio: 1.60) (Cortese et al., 2018). Taken together, the complex relationships between depression, anxiety, ADHD, and asthma suggests the possibility of shared neuroimmune mechanisms.

The exact mechanisms by which asthma influences neuropsychiatric disorders and the central nervous system (CNS), and vice versa, are not known. However, there is evidence that chronic inflammatory conditions, such as asthma, can lead to a disruption in the homeostasis of the neuroimmune environment. For example, during peripheral inflammation, inflammatory mediators cross the blood-brain barrier (BBB) and subsequently can alter brain chemistry and function (Varatharaj and Galea, 2017; Di Benedetto et al., 2017). Eotaxins present in a T_H2 response can cause neuronal cell death via microglial excitation (Parajuli et al., 2015; Kroll &

Ritz, 2023). In mice, it is seen that the main regions of the brain affected by asthma during periods of difficulty breathing are the hippocampus and frontal cortex. In humans, adults with asthma tend to have decreased volumes of the hippocampus and poor integrity of the hippocampal neurons (Carlson et al., 2017; Kroll et al., 2018). These reductions in the hippocampus are hypothesized to contribute to the cognitive deficits often associated with asthma (Kroll & Ritz, 2023). Moreover, in mouse models of asthma, dendritic spine density is decreased in the hippocampus, a phenomenon often associated with altered behavior and disrupted neurodevelopment (Fiala et al., 2002; Hering and Sheng, 2001; Sala and Segal, 2014). Hypoxia as a result of severe asthma attacks also poses significant risk to brain function leading to depression of the CNS (suppressed brain activity) that could impact behavior and normal brain functioning (Eckert et al., 2004).

Another neuropsychiatric disorder with suspected links to asthma and allergies is autism spectrum disorders (ASD). Autism spectrum disorders are characterized by social impairments, such as a decrease in social interactions and communication deficits, as well as increased restrictive and repetitive behaviors. Similar to asthma, ASD represents a prevalent disorder that appears to be on the rise (Maenner et al., 2021). ASD and asthma are two of the most common childhood afflictions, and altered immune function is suspected to be involved in both ASD and asthma (Hughes et al., 2023; Jónsdóttir et al., 2017; Torpy et al., 2010), with both most often impacting boys (Jónsdóttir et al., 2017). Like asthma, ASD places increased financial, social, and mental health burdens on diagnosed individuals and their surrounding community, especially for those with more severe cases of the disorder (Patel et al., 2018).

It was observed in three cross-sectional population-based studies that parents of individuals with ASD were more likely to report asthma among their children than those without ASD (odds ratios [ORs], 1.35-1.74) (Schieve et al., 2012; Kotey et al., 2014; Chen et al., 2017). Interestingly, branching anomalies of subsegmental airways have been described among ASD individuals (Stewart & Klar 2013), which could impact asthma severity. In addition, children with ASD and asthma were more frequently prescribed asthma controller medications (Jónsdóttir et al., 2017). Lyall et al. found that food allergies were more common in children with ASD and observed a modest association between allergies and higher stereotypy scores in children with ASD (Lyall et al., 2015). Higher frequency of food allergies among individuals with ASD were also reported by Jyonouchi et al. in 2008 (Jyonouchi et al., 2008). This group also found that non-IgE mediated allergies are more frequently found among autistic individuals (Jyonouchi 2010). Of note, non-IgE-mediated asthma, or nonatopic asthma, accounts for approximately 20% of cases, and is mediated by antibodies other than IgE (Quirce 2009).

Some researchers have found T_H2 skewed cytokine levels in individuals with ASD (Molloy et al., 2006; Gupta et al., 1998), and there is also evidence of mast cell activation in ASD children (Theoharides 2009). Two of the main cytokine mediators of an allergic response, IL-5 and IL-13, were found to be elevated in serum samples of high-functioning ASD males (Suzuki et al., 2011). In a case control study, peripheral blood mononuclear cells (PBMC) from children with ASD produced higher IL-4, IL-5, and IL-13 at baseline when compared to PBMC controls (Molloy et al., 2006). Also related to asthma, two chemokines associated with neutrophil and eosinophil recruitment, eotaxin and IL-8, were found to be elevated in neonatal blood spots of children later diagnosed with ASD (Heuer et al., 2019). Additionally, IL-8 and

eotaxin were also found to be significantly elevated in plasma samples of ASD children, with eotaxin elevations being associated with more impaired behaviors (Ashwood et al., 2011a; Ashwood et al., 2011b).

Neutrophils and eosinophils play a significant role in airway and lung inflammation (Pease & Williams 2001; Pease & Sabroe 2002), and increased levels of the chemoattractants eotaxin and IL-8 in ASD suggests the potential for worsening of asthma symptoms in these individuals. Research suggests that individuals with ASD are more prone to pro-inflammatory status, with significant elevations of IL-1β, IL-6, and IL-12p40 in plasma from children ages 2-5 (Ashwood et al., 2011), as well as IL-1β, IL-6, IL-12, IL-23, and TNF-α found in serum samples of ASD individuals ranging from 2-21 years of age (Ricci, 2013). This is on a background of low regulatory cytokines such as transforming growth factor (TGF) beta 1, and IL-35 (Ashwood et al., 2008, Rose at al., 2018). After activation, T_H2 cellular responses of were also associated with more severe impairments in children with ASD (Careaga et al., 2017). Moreover, PBMCs stimulated by gliadin, cow's milk protein or soy, from individuals with ASD (*n*=23) produced twice as much TNF compared to controls (*n*=13) (Jyonouchi et al., 2001). Although not directly linked to asthma, food allergies, and potentially associated gastrointestinal abnormalities, in general are significantly linked to ASD in many epidemiology and animal studies (Rose et al., 2018; Rose et al., 2020; Xu et al., 2018; Nemet et al., 2022; Jyonouchi et al., 2008; Jyonouchi 2010; de Theije et al., 2014). With T_H2 skewed cytokine elevations and cellular responses associated with ASD, and the epidemiology links to allergy and asthma among ASD individuals, the potential for allergy related immune dysfunction in ASD is more likely.

Asthma During Pregnancy and Developmental Impacts to Offspring

Asthma is a common disease during pregnancy impacting an estimated 8-13% of pregnant women worldwide (Murphy 2022). Asthma symptoms worsen during pregnancy for approximately 40% of individuals, and changes in the respiratory system and a shift from T_H1 immune dominance in first trimester, to T_H2 immune dominance during the second and third trimester (Bravo-Solarte et al., 2023). Asthma symptom changes can be unpredictable and are likely related to whether the asthma is IgE mediated or has a cellular basis (Grosso et al., 2018; Kircheret al., 2002). Exacerbations can occur frequently and may require medical intervention for up to 45% of pregnant women (Schatz et al., 2003; Murphy et al., 2010). There also appears to be a link between women with increased risk of having uncontrolled asthma and neuropsychiatric disorders during pregnancy, particularly regarding anxiety and depression (Grzeskowiak et al., 2017). For example, a Danish study of 1,000 pregnant women with asthma, enrolled between 2007-2014, reported that 6.1% suffered with a new onset psychiatric condition (Ali et al., 2016). Asthma can also increase complications in pregnancy such as preeclampsia, gestational diabetes, premature contractions, placenta previa and premature ruptures of membranes (Whalen et al., 2019; Wen et al., 2001; Källén et al., 2000; Dombrowski et al., 2004; Rejnö et al., 2014). Postnatal complications in offspring have been identified as well, with some studies identifying associations between maternal asthma and low birth weight in offspring and/or being small for gestational age (Breton et al., 2009; Rejnö et al., 2014). These associations to low birth weight and small gestational age are stronger in cases of severe asthma and where there is poor asthma control (Namazy et al., 2013).

Maternal asthma during pregnancy can have future consequences on the development of asthma/allergies in offspring. Infant wheeze, preschool wheeze, and childhood asthma are risks associated with mothers with asthma (Litonjua et al., 1998; Mattes et al., 2014; Sherriff et al., 2001; Martel et al., 2009; Liu et al., 2018). In fact, it has been suggested that maternal asthma represents the biggest risk factor for childhood asthma, with at least a 3 times greater risk compared to mothers without asthma (Lim et al., 2010; Litonjua et al., 1998). In a Canadian study including over 26,000 women, the risk of childhood asthma was influenced by asthma severity in mothers. Specifically, moderate to severe uncontrolled asthma increased the chances of a child developing asthma compared to mothers with only mild asthma during pregnancy (Martel et al., 2009). This trend also appeared in a Danish study finding a higher incidence of early-onset persistent asthma in children born to mothers with mild uncontrolled asthma, and moderate to severe asthma that was controlled or uncontrolled, compared to children of mothers with mild but controlled asthma (Liu et al., 2018). While the above findings highlight some of the potential impacts that the asthmatic maternal environment can have on the developing fetus, the potential causes of these trends are not well understood.

There have been recent investigations into the link between maternal asthma and increased risk of neurodevelopmental disorders in offspring. For instance, a Norwegian study of 2,322,657 residents found 50% higher odds of ADHD among children whose mothers had asthma during pregnancy (Instanes et al., 2017). Additionally, a Danish population-based study of 961,202 individuals found associated risk of ADHD among children born from mothers or fathers with asthma, but higher associated risk among those children from mothers with asthma (Hazard ratio: 1.41) (Liu et al., 2019). Leonard et al. reported that asthma was associated with an

increased risk of mild to moderate risk for offspring intellectual disability in a cohort of 2,865 subjects [CI: 1.26–1.83; OD: 1.52] (Leonard et al., 2006). Another neurodevelopmental disorder in offspring with strong links to maternal allergic asthma exposure is ASD. In a population-based study of 407 cases of ASD, Croen et al. found a two-fold elevated risk of ASD in offspring when mothers experienced new onset cases of maternal allergy and asthma during pregnancy (Croen et al., 2005). Data collected from the Study to Explore Development (SEED), which was a study of children born 2003-2006 in the US, reported that history of maternal asthma increased odds of both ASD and developmental disorders by 20%-40%, maternal asthma being associated with increased risk of ASD with intellectual disability and regression, in particular (Croen et al., 2019). This finding of symptom severity is supported by a study done using data from the Western Australian Autism Biological Registry (WAABR), where asthma and allergies during pregnancy were found to be associated with a twofold elevated risk of ASD, and symptom severity found to be worse in these children (Patel et al., 2018). We recently published a study of 363 ASD children from the Autism Phenome Project (APP) and Girls with Autism Imaging of Neurodevelopment (GAIN) study, that found that asthma was the most common immune condition linked to ASD cases (23.95%). Also, maternal asthma during pregnancy was twice as common among male children diagnosed with ASD than with female children diagnosed with ASD. However, the female children with ASD that were born to mothers with maternal asthma during pregnancy showed increased CBCL (Child Behavior Checklist) scores and more impairment (Patel et al., 2020). In addition, maternal asthma was found to increase risk of autism with gastrointestinal disturbances [OR]: 1.39 (CI: 1.17–1.67) relative to ASD with no gastrointestinal issues [OR]: 1.15 (CI: 0.98-1.34) (Carter et al., 2022).

As with asthma itself, there does not appear to be a single etiology associated with ASD, however, inflammation during gestation has been strongly linked with having a child that will go on to develop a neurodevelopmental disorder. Specifically, the elevation of several cytokines has been implicated IN WHAT, and many of these are directly linked to an allergic response. For instance, elevations of three cytokines associated with a human allergic asthma response, interferon-γ (IFN-γ), IL-4 and IL-5, during mid pregnancy were found to be associated with mothers bearing a child with ASD (Goines et al., 2011). In the large prospective study of Early Markers in Autism (EMA), several cytokines were reported to be elevated in mothers during mid-gestation whose children were later diagnosed with ASD and intellectual disability (ID), and these included granulocyte–macrophage colony-stimulating factor (GM-CSF), IFNγ, IL-1α, IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1) (Jones et al., 2017). In the same study, Jones et al. also identified that higher levels of IL-4 were associated with an increased risk of ASD + ID when compared to ASD-noID (Jones et al., 2017).

In the amniotic fluid of individuals with ASD, elevated levels of IL-4, IL-10, TNFα and TNFβ has been noted (Abdallah et al., 2013). Also, in a study investigating chemokines, MCP-1 was found to be significantly elevated in amniotic fluid of ASD cases compared to age and gender matched controls (Abdallah et al., 2012). Moreover, the Childhood Autism Risks from Genetics and the Environment (CHARGE) study found that IL-4 and IL-1β were found to be elevated in newborn blood spots of children later diagnosed with ASD, with IL-4 associated with increased odds of severe ASD [OR]: 1.40 (CI: 1.03-1.91) (Krakowiak et al., 2017). Many of the cytokines implicated in ASD risk during pregnancy or early life/at birth, are innate immune cytokines that are upregulated in asthma and allergy. For instance, innate cytokines IL-6 and IL-

8, along with adaptive cytokines IL-5, IL-13, and IL-17A were found to be elevated in serum of adult patients with moderate and severe bronchial asthma (Dimitrova et al., 2019). IL-6 is not only associated with airway remodeling but can also be found in other biological fluids of individuals with asthma (Lipworth et al., 2020). Of importance, IL-6 is one of the cytokines that is known to cross the placental barrier and may act directly on cells resident to the placenta. This direct passage of IL-6 through the fetal/placenta compartment is one likely mechanism hypothesized to link maternal inflammation to fetal development (Goines et al., 2011).

IFN-γ upregulation is found in severe cases of asthma in mice and humans, which can be mediated by CD8 T cells (Magnan et al., 2000; Raundhal et al., 2015). T cells in asthma also upregulate IL-2 which can induce production of IL-13 (Hashimoto et al., 2006). In another asthma study, mononuclear cells isolated from asthma patients with varying severity of the disease showed increase baseline levels of MIP-1α (Rojas-Dotor et al., 2013). TNFα is also suspected to contribute to asthma through increasing the inflammatory response, recruiting neutrophils and eosinophils, and increasing their cytotoxic effects (Berry et al., 2007). Taken together, these epidemiological studies illustrate the importance of further investigation into the links between maternal allergic inflammation and neuropsychiatric disorders in offspring.

We recently developed a novel model of maternal allergic asthma that displays altered behaviors in offspring exposed to mothers whose immune system was challenged by maternal allergic asthma (MAA). The dams were sensitized to OVA and later challenged with maternal asthma during pregnancy display elevations in the cytokines associate with a typical allergic

response, such as IL-4, IL-5, and IL-13, but also those associated with severe chronic asthma such as IL-6 and IL-17 (Church et al., 2021; Tamayo et al., 2023). The offspring from these MAA mice display the two core characteristic behaviors associated with ASD, specifically a decrease in social interaction and increase in repetitive-like behaviors (Schwartzer et al., 2015; Schwartzer et al., 2017; Church et al., 2021). We have also begun to uncover neurobiological changes in the offspring that echo phenotypic changes associated with human ASD cases. For instances, Ciernia et al. found that adult microglia taken from offspring of MAA dams have DNA methylation differences compared to wild-type microglia, and some of these changes were in immune regulatory genes shared with ASD individuals (Ciernia et al., 2018). Lenz et al., using a model of allergic asthma in rats, showed that maternal allergic asthma resulted in altered mast cells and microglia in early development as well as differences in dendritic spine patterning on neurons in neonates. The authors suspect that these findings may be linked to the social impairments, hyperactivity, and cognitive inflexibility behaviors in their model (Lenz et al., 2019; Breach et al., 2021). Our studies into MAA have also found changes in the neuroimmune environment in offspring fetal brains such as increased proinflammatory cytokines GM-CSF, IFNγ, IL-1α, and IL-6 (Tamayo et al., 2023). Importantly, MAA also appeared to alter the brain neuroimmune environment into adulthood, as cytokine differences were also identified in the hypothalamus in adult offspring (Church et al., 2021). Together, these studies recapitulate findings in human postmortem ASD studies where neuroinflammation and functional differences in microglia have observed (Li et al., 2009; Koyama et al., 2015; Morgan et al., 2010; Morgan et al., 2014).

Asthma Exacerbations and Where to Look Next

As mentioned previously in this review, worsening of asthma symptoms can often occur during pregnancy, and exacerbations can worsen associated comorbidities, including those that occur in offspring of mothers with asthma. As such, it is important to investigate the contributors to asthma and asthma exacerbations. Allergens, respiratory infections, fungus, air pollution, and particulate matter are well known triggers for asthma attacks (Zhuang et al., 2018; Del Giacco et al., 2017; Holgate et al., 2015). Common indoor allergens such as house dust mites, cat and dog dander, and cockroaches also pose a significant risk (Baxi & Phipatanakul 2010; Custovic et al., 1998).

Environmental exposure to air pollution poses a significant health concern for individuals with and without asthma alike. Air pollution can include traffic related air pollution (TRAP), gaseous compounds, and particulate matter (PM), and these pollutants are known to induce oxidative stress, airway hyper-responsiveness, and airway remodeling with or without allergic asthma (Guarnieri & Balmes 2014). Additionally, prenatal exposure to pollutants such as PM have been linked to postnatal allergic asthma manifestations, alerted expression of genes associated with oxidative stress response, and polycyclic aromatic hydrocarbon metabolism (Guarnieri et al., 2014). PM is itself highly heterogenous but is generally classified into coarse, fine, and ultrafine PM. Coarse PM ranges from $PM_{2.5}$ to PM_{10} (2.5μm – 10μm) and can deposit in the bronchi (Wu et al., 2018). Fine PM ranges from 0.1μm to 2.5μm and can pass into the alveoli and terminal bronchioles (Wu et al., 2018). Ultrafine PM (UFP) is less than 0.1μm and

can penetrate deep into tissues and cross through cellular membranes (Wilson et al., 1997; Becker et al., 2003). In addition to size variation, PM also varies in its constituents and can contain organic compounds, soot, metals and metal-oxides, nitrates, and other elements in varying quantities depending on its source (Steiner et al., 2016). Each of these types of PM can pose their own risks for asthmatic individuals, and researchers most often investigate individual components in isolation. As an example, one widely studied type of PM is diesel exhaust which has been strongly implicated in asthma exacerbations and appears to induce a stronger T_H2 inflammatory responses associated with elevations in systemic IL-4 and IL-8 (Chau-Etchepare et al., 2019). Another source of air pollution that poses a particularly increasing threat to parts of the Western USA is wildfire smoke due to its high concentration of $PM_{2.5}$ (Chau-Etchepare et al., 2019). Increased hospitalizations for both pulmonary and cardiac issues during wildfires have been identified, but even after wildfires become controlled, hospital asthma admissions and increased symptoms were reported (Hutchinson et al., 2018; Fann et al., 2018; Künzli et al., 2006). Considering these myriad associations to asthma incidence and exacerbations, many researchers have explored the role of individual environmental contributors to exacerbation of allergic asthma using animal models.

One of the main causes of hospitalization of asthmatic individuals is acute exacerbations (Bahadori et al., 2009; Rodrigo et al., 2004). As such, many investigators have turned to animal models to investigate the mechanisms associated with these exacerbations (Kumar et al., 2016). Animal models of asthma exacerbation most often use allergic asthma models of OVA or HDM sensitization, and most frequently investigate the contribution of viral infections and environmental pollutants to asthma exacerbations. Although exposure to sensitizing allergen can

trigger exacerbations, research suggests that these cases are relatively uncommon (Maltby et al., 2017). Mouse models investigating the link between viral infection and asthma exacerbation are limited, however, due to the inability of HRV to infect mouse cells (Maltby et al., 2017). Due to this limitation, investigators will often use viral analogs, such as double-stranded RNA, or the HRV minor group human rhinovirus 1B (HRV1B), as the major group HRV cannot readily infect mice cells (Maltby et al., 2017). There are far fewer studies investigating fungal related asthma exacerbations but among these, one investigation using Alternaria extract exposure to chronically challenged allergic mice identified increased airway hyperresponsiveness, immune cell infiltration of the lungs, as well as an increased T_H2 response (Snelgrove et al., 2014).

Perhaps more easily modeled in rodents are exposures to environmental pollutants and the impacts of these exposures on allergic asthma. It was demonstrated in a model of allergic asthma that concentrated ambient particle administration and ozone exposure increased AHR (airway hyperresponsiveness), with the latter also increasing allergy-associated cytokine release (North et al., 2011; Kierstein et al., 2008). In a model of chronic asthma via OVA sensitization, PM_{10} exposure following asthma challenge resulted in increased lung inflammation. In another model, diesel exhaust exposure resulted in increased airway inflammation, mucus secretion, and increased inflammatory cytokine production (Maltby et al., 2017). Together, these studies illustrate the utility of animal models in the investigation of mechanisms underlying asthma exacerbation.

An understudied question is how asthma exacerbation via these asthma exacerbating factors impacts pregnancy outcomes and what impacts this may have on offspring neurodevelopment. Research using animal models of prenatal PM exposure have also identified evidence of neurodevelopmental delays associated with prenatal PM exposure (Zheng et al., 2019; Church et al., 2018). Indeed, animal models of asthma and PM exposure identify heightened allergic responses, such as upregulated IL-4, IL-5, IL-6, and IL-13 (Wu et al., 2018). When considering the link between IL-4, IL-5, and IL-6 in humans during mid gestation and the increased incidence in ASD in their offspring, this is indeed important. Furthermore, in our mouse model of MAA, we identify elevations in serum cytokines IL-4, IL-5, IL-6, and IL-13 in the pregnant dams, and offspring from these dams display characteristic ASD behavioral differences, as well as differences in pro-inflammatory brain cytokines (Schwartzer et al., 2015; Schwartzer et al., 2017; Church et al., 2021; Tamayo et al., 2023). The findings between the cytokine increases in dual exposure to PM and asthma, and the evidence that isolated exposure of these environmental factors during pregnancy can negatively impact neurodevelopment raise an important question: how might the dual exposure of PM and asthma during pregnancy impact neurodevelopment? Given the evidence from studies presented in this review, this remains and important, and highly under researched question.

Conclusion:

Asthma represents a pervasive chronic these individuals, and potentially their offspring. The immune mechanisms underlying this heterogenous disease are not well understood, but various types of inflammation play an obvious role. This inflammation and the severity of the asthma response can vary dramatically depending on a number of factors including sensitized allergens, comorbidities, age, genetic susceptibility, infection, and environmental factors. Comorbid neuropsychiatric disorders appear to have a particular importance given their high prevalence among asthmatic individuals. Additionally, the risk factors for asthma exacerbation can confer increased risk for pregnant mothers and their offspring. Pregnancy outcomes can be worsened by asthma but there are also potential long-term impacts on offspring that can result in a significant risk of childhood asthma and neuropsychiatric disorders, such as ASD. Importantly, to our knowledge, there are no studies investigating the combined impact of maternal allergic asthma and PM exposure during pregnancy neurodevelopmental outcomes in the offspring. This is an important data gap, considering that it is rare that individuals are exposed to a single environmental insult. As such, it is necessary for researchers to explore these questions to uncover the impacts that PM exposure and asthma may have on offspring, and if this may be contributing to the rise in neurodevelopmental disorders such as ASD.

References:

Most Recent National Asthma Data | CDC. Published April 7, 2021. Accessed June 15, 2021. https://www.cdc.gov/asthma/most_recent_national_asthma_data.htm

Gans, M. D., & Gavrilova, T. (2020). Understanding the immunology of asthma: Pathophysiology, biomarkers, and treatments for asthma endotypes. Paediatric respiratory reviews, 36, 118–127. https://doi.org/10.1016/j.prrv.2019.08.002

Cardet, J. C., Chang, K. L., Rooks, B. J., Carroll, J. K., Celedón, J. C., Coyne-Beasley, T., Cui, J., Ericson, B., Forth, V. E., Fagan, M., Fuhlbrigge, A. L., Hernandez, P. A., Kruse, J., Louisias, M., Maher, N. E., Manning, B., Pace, W. D., Phipatanakul, W., Rodriguez-Louis, J., Shields, J. B., … Wisnivesky, J. P. (2022). Socioeconomic status associates with worse asthma morbidity among Black and Latinx adults. *The Journal of allergy and clinical immunology*, *150*(4), 841–849.e4. https://doi.org/10.1016/j.jaci.2022.04.030

Ganti, P., Suman, A., Chaudhary, S., Sangha, B., David, L., & Sekhsaria, S. (2022). The effect of the socioeconomic status on the measurement of asthma control. *Allergy and asthma proceedings*, *43*(1), e11–e16. https://doi.org/10.2500/aap.2022.43.210103

Matsui, E. C., Hansel, N. N., McCormack, M. C., Rusher, R., Breysse, P. N., & Diette, G. B. (2008). Asthma in the inner city and the indoor environment. *Immunology and allergy clinics of North America*, *28*(3), 665-686.

Milligan, K. L., Matsui, E., & Sharma, H. (2016). Asthma in urban children: epidemiology, environmental risk factors, and the public health domain. *Current allergy and asthma reports*, *16*, 1-10.

Baek, J., Kash, B. A., Xu, X., Benden, M., Roberts, J., & Carrillo, G. (2022). Pediatric asthma hospitalization: individual and environmental characteristics of high utilizers in South Texas. *The Journal of asthma : official journal of the Association for the Care of Asthma*, *59*(1), 94–104. https://doi.org/10.1080/02770903.2020.1827424

Jean, T., Yang, S. J., Crawford, W. W., Takahashi, S. H., & Sheikh, J. (2019). Development of a pediatric asthma predictive index for hospitalization. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*, *122*(3), 283–288. https://doi.org/10.1016/j.anai.2018.11.021

Loftus, P. A., & Wise, S. K. (2015, September). Epidemiology and economic burden of asthma. In *International forum of allergy & rhinology* (Vol. 5, No. S1, pp. S7-S10).

Mims J. W. (2015). Asthma: definitions and pathophysiology. International forum of allergy & rhinology, 5 Suppl 1, S2–S6. https://doi.org/10.1002/alr.21609

Deo, S. S., Mistry, K. J., Kakade, A. M., & Niphadkar, P. V. (2010). Role played by Th2 type cytokines in IgE mediated allergy and asthma. *Lung India: Official Organ of Indian Chest Society*, *27*(2), 66.

León, B., & Ballesteros-Tato, A. (2021). Modulating Th2 cell immunity for the treatment of asthma. *Frontiers in immunology*, *12*, 637948.

Yu, S., Kim, H. Y., Chang, Y. J., DeKruyff, R. H., & Umetsu, D. T. (2014). Innate lymphoid cells and asthma. *Journal of Allergy and Clinical Immunology*, *133*(4), 943-950.

Sonntag, H. J., Filippi, S., Pipis, S., & Custovic, A. (2019). Blood biomarkers of sensitization and asthma. *Frontiers in pediatrics*, *7*, 251.

Wang, S. C., Tsai, C. W., Chang, W. S., Hsia, N. Y., Mong, M. C., Wang, Y. C., Hsia, T. C., Gu, J., & Bau, D. T. (2022). Genetic variants in miR-145 gene are associated with the risk of asthma in Taiwan. *Scientific reports*, *12*(1), 15155. https://doi.org/10.1038/s41598-022-18587-w

Harb, H., & Renz, H. (2015). Update on epigenetics in allergic disease. The Journal of allergy and clinical immunology, 135(1), 15–24. https://doi.org/10.1016/j.jaci.2014.11.009
Castillo, J. R., Peters, S. P., & Busse, W. W. (2017). Asthma Exacerbations: Pathogenesis, Prevention, and Treatment. The journal of allergy and clinical immunology. In practice, 5(4), 918–927. https://doi.org/10.1016/j.jaip.2017.05.001
Acevedo, N., Alashkar Alhamwe, B., Caraballo, L., Ding, M., Ferrante, A., Garn, H., Garssen, J., Hii, C. S., Irvine, J., Llinás-Caballero, K., López, J. F., Miethe, S., Perveen, K., Pogge von Strandmann, E., Sokolowska, M., Potaczek, D. P., & van Esch, B. C. A. M. (2021). Perinatal and Early-Life Nutrition, Epigenetics, and Allergy. *Nutrients*, *13*(3), 724. https://doi.org/10.3390/nu13030724
Rosário Filho, N. A., Urrutia-Pereira, M., D'Amato, G., Cecchi, L., Ansotegui, I. J., Galán, C., Pomés, A., Murrieta-Aguttes, M., Caraballo, L., Rouadi, P., Chong-Neto, H. J., & Peden, D. B. (2021). Air pollution and indoor settings. *The World Allergy Organization journal*, *14*(1), 100499. https://doi.org/10.1016/j.waojou.2020.100499
Samoli, E., Nastos, P. T., Paliatsos, A. G., Katsouyanni, K., & Priftis, K. N. (2011). Acute effects of air pollution on pediatric asthma exacerbation: evidence of association and effect modification. Environmental research, 111(3), 418–424. https://doi.org/10.1016/j.envres.2011.01.014
Trasande, L., & Thurston, G. D. (2005). The role of air pollution in asthma and other pediatric morbidities. The Journal of allergy and clinical immunology, 115(4), 689–699. https://doi.org/10.1016/j.jaci.2005.01.056
Hansel, N. N., Breysse, P. N., McCormack, M. C., Matsui, E. C., Curtin-Brosnan, J., Williams, D. L., Moore, J. L., Cuhran, J. L., & Diette, G. B. (2008). A longitudinal study of indoor nitrogen dioxide levels and respiratory symptoms in inner-city children with asthma. Environmental health perspectives, 116(10), 1428–1432. https://doi.org/10.1289/ehp.11349
Boulet, L. P., & Boulay, M. È. (2011). Asthma-related comorbidities. Expert review of respiratory medicine, 5(3), 377–393. https://doi.org/10.1586/ers.11.34
Cooley, C., Park, Y., Ajilore, O., Leow, A., & Nyenhuis, S. M. (2022). Impact of interventions targeting anxiety and depression in adults with asthma. *The Journal of asthma : official journal of the Association for the Care of Asthma*, *59*(2), 273–287. https://doi.org/10.1080/02770903.2020.1847927
Licari, A., Castagnoli, R., Ciprandi, R., Brambilla, I., Guasti, E., Marseglia, G. L., & Ciprandi, G. (2022). Anxiety and depression in adolescents with asthma: a study in clinical practice. *Acta bio-medica : Atenei Parmensis*, *93*(1), e2022021. https://doi.org/10.23750/abm.v93i1.10731
Fasmer, O. B., Halmøy, A., Eagan, T. M., Oedegaard, K. J., & Haavik, J. (2011). Adult attention deficit hyperactivity disorder is associated with asthma. BMC psychiatry, 11, 128. https://doi.org/10.1186/1471-244X-11-128
Kroll, J. L., & Ritz, T. (2023). Asthma, the central nervous system, and neurocognition: Current findings, potential mechanisms, and treatment implications. Neuroscience and biobehavioral reviews, 146, 105063. https://doi.org/10.1016/j.neubiorev.2023.105063
Shaked, E., Wainstock, T., Sheiner, E., & Walfisch, A. (2019). Maternal asthma: pregnancy course and outcome. *The Journal of Maternal-Fetal & Neonatal Medicine*, *32*(1), 103-108.

Sheiner, E., Mazor, M., Levy, A., Wiznitzer, A., & Bashiri, A. (2005). Pregnancy outcome of asthmatic patients: a population-based study. *The Journal of Maternal-Fetal & Neonatal Medicine*, *18*(4), 237-240.

Baghlaf, H., Spence, A. R., Czuzoj-Shulman, N., & Abenhaim, H. A. (2019). Pregnancy outcomes among women with asthma. *The Journal of Maternal-Fetal & Neonatal Medicine*, *32*(8), 1325-1331.

Liu, X., Agerbo, E., Schlünssen, V., Wright, R. J., Li, J., & Munk-Olsen, T. (2018). Maternal asthma severity and control during pregnancy and risk of offspring asthma. *Journal of Allergy and Clinical Immunology*, *141*(3), 886-892.

Lim, R. H., Kobzik, L., & Dahl, M. (2010). Risk for asthma in offspring of asthmatic mothers versus fathers: a meta-analysis. *PloS one*, *5*(4), e10134.

Ali, Z., & Ulrik, C. S. (2013). Obesity and asthma: a coincidence or a causal relationship? A systematic review. *Respiratory medicine*, *107*(9), 1287-1300.

Croen, L. A., Qian, Y., Ashwood, P., Daniels, J. L., Fallin, D., Schendel, D., Schieve, L. A., Singer, A. B., & Zerbo, O. (2019). Family history of immune conditions and autism spectrum and developmental disorders: Findings from the study to explore early development. Autism research : official journal of the International Society for Autism Research, 12(1), 123–135. https://doi.org/10.1002/aur.1979

Croen, L. A., Qian, Y., Ashwood, P., Zerbo, O., Schendel, D., Pinto-Martin, J., Daniele Fallin, M., Levy, S., Schieve, L. A., Yeargin-Allsopp, M., Sabourin, K. R., & Ames, J. L. (2019). Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. Autism research: official journal of the International Society for Autism Research, 12(10), 1551–1561. https://doi.org/10.1002/aur.2175

Gong, T., Lundholm, C., Rejnö, G., Bölte, S., Larsson, H., D'Onofrio, B. M., ... & Almqvist, C. (2019). Parental asthma and risk of autism spectrum disorder in offspring: A population and family-based case-control study. *Clinical & Experimental Allergy*, *49*(6), 883-891.

Abdallah, M. W., Larsen, N., Grove, J., Nørgaard-Pedersen, B., Thorsen, P., Mortensen, E. L., & Hougaard, D. M. (2012). Amniotic fluid chemokines and autism spectrum disorders: an exploratory study utilizing a Danish Historic Birth Cohort. *Brain, behavior, and immunity*, *26*(1), 170–176. https://doi.org/10.1016/j.bbi.2011.09.003

Hisle-Gorman, E., Susi, A., Stokes, T., Gorman, G., Erdie-Lalena, C., & Nylund, C. M. (2018). Prenatal, perinatal, and neonatal risk factors of autism spectrum disorder. *Pediatric research*, *84*(2), 190-198.

Lyall, K., Van de Water, J., Ashwood, P., & Hertz-Picciotto, I. (2015). Asthma and Allergies in Children With Autism Spectrum Disorders: Results From the CHARGE Study. *Autism research : official journal of the International Society for Autism Research*, *8*(5), 567–574. https://doi.org/10.1002/aur.1471

Patel, S., Dale, R. C., Rose, D., Heath, B., Nordahl, C. W., Rogers, S., Guastella, A. J., & Ashwood, P. (2020). Maternal immune conditions are increased in males with autism spectrum disorders and are associated with behavioural and emotional but not cognitive co-morbidity. Translational psychiatry, 10(1), 286. https://doi.org/10.1038/s41398-020-00976-2

Holgate, S. T., Wenzel, S., Postma, D. S., Weiss, S. T., Renz, H., & Sly, P. D. (2015). Asthma. Nature reviews. Disease primers, 1(1), 15025. https://doi.org/10.1038/nrdp.2015.25

Lange, P., Parner, J., Vestbo, J., Schnohr, P., & Jensen, G. (1998). A 15-year follow-up study of ventilatory function in adults with asthma. *New England Journal of Medicine*, *339*(17), 1194-1200.

Kumar, A., & Ghosh, B. (2009). Genetics of asthma: a molecular biologist perspective. Clinical and molecular allergy : CMA, 7, 7.

Schatz, M., & Rosenwasser, L. (2014). The allergic asthma phenotype. *The Journal of Allergy and Clinical Immunology: In Practice*, *2*(6), 645-648.

Vaillant, A. A. J., Modi, P., & Jan, A. (2022). Atopy. In *StatPearls [Internet]*. StatPearls Publishing.

Peters, S. P. (2014). Asthma phenotypes: nonallergic (intrinsic) asthma. *The Journal of Allergy and Clinical Immunology: In Practice*, *2*(6), 650-652.

León, B. T Cells in Allergic Asthma: Key Players Beyond the Th2 Pathway. *Curr Allergy Asthma Rep* 17, 43 (2017). https://doi.org/10.1007/s11882-017-0714-1

Boucherat, O., Boczkowski, J., Jeannotte, L. & Delacourt, C. Cellular and molecular mechanisms of goblet cell metaplasia in the respiratory airways. *Exp. Lung Res.* 39, 207–216 (2013).

Choy, D. F., Hart, K. M., Borthwick, L. A., Shikotra, A., Nagarkar, D. R., Siddiqui, S., ... & Bradding, P. (2015). TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. *Science translational medicine*, *7*(301), 301ra129-301ra129.

Barnes, P. J. (2001). Th2 cytokines and asthma: an introduction. *Respiratory research*, *2*, 1-2.

Hazlewood, L. C., Wood, L. G., Hansbro, P. M., & Foster, P. S. (2011). Dietary lycopene supplementation suppresses Th2 responses and lung eosinophilia in a mouse model of allergic asthma. *The Journal of nutritional biochemistry*, *22*(1), 95-100.

Spencer, L. A., & Weller, P. F. (2010). Eosinophils and Th2 immunity: contemporary insights. *Immunology and cell biology*, *88*(3), 250-256.

Lambrecht, B. N., De Veerman, M., Coyle, A. J., Gutierrez-Ramos, J. C., Thielemans, K., & Pauwels, R. A. (2000). Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *The Journal of clinical investigation*, *106*(4), 551-559.

Chapoval, S., Dasgupta, P., Dorsey, N. J., & Keegan, A. D. (2010). Regulation of the T helper cell type 2 (Th2)/T regulatory cell (Treg) balance by IL-4 and STAT6. *Journal of leukocyte biology*, *87*(6), 1011-1018.

Adar, T., Shteingart, S., Ya'acov, A. B., Shitrit, A. B. G., & Goldin, E. (2014). From airway inflammation to inflammatory bowel disease: eotaxin-1, a key regulator of intestinal inflammation. *Clinical immunology*, *153*(1), 199-208.

Conroy, D. M., & Williams, T. J. (2001). Eotaxin and the attraction of eosinophils to the asthmatic lung. *Respiratory research*, *2*(3), 1-7.

Lloyd, C. M., & Saglani, S. (2013). T cells in asthma: influences of genetics, environment, and T-cell plasticity. *Journal of allergy and clinical immunology*, *131*(5), 1267-1274.

Cosmi, L., Liotta, F., Maggi, E., Romagnani, S., & Annunziato, F. (2011). Th17 cells: new players in asthma pathogenesis. *Allergy*, *66*(8), 989-998.

Liu, D., Tan, Y., Bajinka, O., Wang, L., & Tang, Z. (2020). Th17/IL-17 axis regulated by airway microbes get involved in the development of asthma. *Current allergy and asthma reports*, *20*, 1-9.

Newcomb, D. C., & Peebles Jr, R. S. (2013). Th17-mediated inflammation in asthma. *Current opinion in immunology*, *25*(6), 755-760.
King-Dowling, S., Proudfoot, N. A., & Obeid, J. (2019). Comorbidity among chronic physical health conditions and neurodevelopmental disorders in childhood. *Current Developmental Disorders Reports*, *6*, 248-258.
Loerbroks, A., Apfelbacher, C. J., Bosch, J. A., & Stürmer, T. (2010). Depressive symptoms, social support, and risk of adult asthma in a population-based cohort study. *Psychosomatic medicine*, *72*(3), 309-315.
Choi, H. G., Kim, J. H., Park, J. Y., Hwang, Y. I., Jang, S. H., & Jung, K. S. (2019). Association between asthma and depression: a national cohort study. *The Journal of Allergy and Clinical Immunology: In Practice*, *7*(4), 1239-1245.
Liu, X., Plana-Ripoll, O., McGrath, J. J., Petersen, L. V., Dharmage, S. C., & Momen, N. C. (2023). Bidirectional associations between asthma and types of mental disorders. *The Journal of Allergy and Clinical Immunology: In Practice*, *11*(3), 799-808.
Jiang, M., Qin, P., & Yang, X. (2014). Comorbidity between depression and asthma via immune-inflammatory pathways: a meta-analysis. *Journal of affective disorders*, *166*, 22-29.
Loerbroks, A., Herr, R. M., Subramanian, S. V., & Bosch, J. A. (2012). The association of asthma and wheezing with major depressive episodes: an analysis of 245 727 women and men from 57 countries. *International journal of epidemiology*, *41*(5), 1436-1444.
Walters, P., Schofield, P., Howard, L., Ashworth, M., & Tylee, A. (2011). The relationship between asthma and depression in primary care patients: a historical cohort and nested case control study. *PloS one*, *6*(6), e20750.
Blume, J., Douglas, S. D., & Evans, D. L. (2011). Immune suppression and immune activation in depression. *Brain, behavior, and immunity*, *25*(2), 221-229.
Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychological bulletin*, *140*(3), 774.
Berry, M., Brightling, C., Pavord, I., & Wardlaw, A. (2007). TNF-alpha in asthma. *Current opinion in pharmacology*, *7*(3), 279–282. https://doi.org/10.1016/j.coph.2007.03.001
Dimitrova, D., Youroukova, V., Ivanova-Todorova, E., Tumangelova-Yuzeir, K., & Velikova, T. (2019). Serum levels of IL-5, IL-6, IL-8, IL-13 and IL-17A in pre-defined groups of adult patients with moderate and severe bronchial asthma. *Respiratory medicine*, *154*, 144–154. https://doi.org/10.1016/j.rmed.2019.06.024
Lipworth, B., Chan, R., & Kuo, C. (2020). Systemic IL-6 and Severe Asthma. American journal of respiratory and critical care medicine, 202(9), 1324–1325. https://doi.org/10.1164/rccm.202006-2354LE
Cui, A. H., Zhao, J., Liu, S. X., & Hao, Y. S. (2017). Associations of IL-4, IL-6, and IL-12 levels in peripheral blood with lung function, cellular immune function, and quality of life in children with moderate-to-severe asthma. Medicine, 96(12), e6265. https://doi.org/10.1097/MD.0000000000006265
Ye, G., Baldwin, D. S., & Hou, R. (2021). Anxiety in asthma: a systematic review and meta-analysis. *Psychological Medicine*, *51*(1), 11-20
Hasler, G., Gergen, P. J., Kleinbaum, D. G., Ajdacic, V., Gamma, A., Eich, D., Rössler, W., & Angst, J. (2005). Asthma and panic in young adults: a 20-year prospective community study. *American journal of respiratory and critical care medicine*, *171*(11), 1224–1230. https://doi.org/10.1164/rccm.200412-1669OC

Chee Kiang, T., Anthony, Y., Kwok Wai Adrian, C., Therese Sophie, L., & Mariko Siyue, K. (2015). Anxiety, depression and hyperventilation symptoms in treatment-resistant severe asthma. *Clinical and translational allergy*, *5*(2), 1-1.

Lee, Y. C., Lee, C. T. C., Lai, Y. R., Chen, V. C. H., & Stewart, R. (2016). Association of asthma and anxiety: A nationwide population-based study in Taiwan. *Journal of affective disorders*, *189*, 98-105.

O'Donovan, A., Hughes, B. M., Slavich, G. M., Lynch, L., Cronin, M. T., O'Farrelly, C., & Malone, K. M. (2010). Clinical anxiety, cortisol and interleukin-6: Evidence for specificity in emotion–biology relationships. *Brain, behavior, and immunity*, *24*(7), 1074-1077.

Salim, S., Chugh, G., & Asghar, M. (2012). Inflammation in anxiety. *Advances in protein chemistry and structural biology*, *88*, 1-25.

Slattery, D. A., Uschold, N., Magoni, M., Bär, J., Popoli, M., Neumann, I. D., & Reber, S. O. (2012). Behavioural consequences of two chronic psychosocial stress paradigms: anxiety without depression. *Psychoneuroendocrinology*, *37*(5), 702-714.

Kemeny, M. E., & Schedlowski, M. (2007). Understanding the interaction between psychosocial stress and immune-related diseases: a stepwise progression. *Brain, behavior, and immunity*, *21*(8), 1009-1018.

Mathews, H. L., Konley, T., Kosik, K. L., Krukowski, K., Eddy, J., Albuquerque, K., & Janusek, L. W. (2011). Epigenetic patterns associated with the immune dysregulation that accompanies psychosocial distress. *Brain, behavior, and immunity*, *25*(5), 830-839.

Yonas, M. A., Lange, N. E., & Celedón, J. C. (2012). Psychosocial stress and asthma morbidity. *Current opinion in allergy and clinical immunology*, *12*(2), 202–210. https://doi.org/10.1097/ACI.0b013e32835090c9

Holmberg, K., Lundholm, C., Anckarsäter, H., Larsson, H., & Almqvist, C. (2015). Impact of asthma medication and familial factors on the association between childhood asthma and attention-deficit/hyperactivity disorder: a combined twin-and register-based study: epidemiology of allergic disease. *Clinical & Experimental Allergy*, *45*(5), 964-973.

Leffa, D. T., Torres, I. L., & Rohde, L. A. (2018). A review on the role of inflammation in attention-deficit/hyperactivity disorder. *Neuroimmunomodulation*, *25*(5-6), 328-333.

Chuang, Y. C., Wang, C. Y., Huang, W. L., Wang, L. J., Kuo, H. C., Chen, Y. C., & Huang, Y. J. (2022). Two meta-analyses of the association between atopic diseases and core symptoms of attention deficit hyperactivity disorder. *Scientific reports*, *12*(1), 3377.

Cortese, S., Sun, S., Zhang, J., Sharma, E., Chang, Z., Kuja-Halkola, R., ... & Faraone, S. V. (2018). Association between attention deficit hyperactivity disorder and asthma: a systematic review and meta-analysis and a Swedish population-based study. *The Lancet Psychiatry*, *5*(9), 717-726.

Varatharaj, A., & Galea, I. (2017). The blood-brain barrier in systemic inflammation. *Brain, behavior, and immunity*, *60*, 1-12.

Di Benedetto, S., Müller, L., Wenger, E., Düzel, S., & Pawelec, G. (2017). Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. *Neuroscience & Biobehavioral Reviews*, *75*, 114-128.

Parajuli, B., Horiuchi, H., Mizuno, T., Takeuchi, H., & Suzumura, A. (2015). CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. *Glia*, *63*(12), 2274-2284.

Carlson, S. M., Kim, J., Khan, D. A., King, K., Lucarelli, R. T., McColl, R., ... & Brown, E. S. (2017). Hippocampal volume in patients with asthma: results from the Dallas Heart Study. *Journal of Asthma*, *54*(1), 9-16.

Kroll, J. L., Steele, A. M., Pinkham, A. E., Choi, C., Khan, D. A., Patel, S. V., ... & Ritz, T. (2018). Hippocampal metabolites in asthma and their implications for cognitive function. *NeuroImage: Clinical*, *19*, 213-221.

Fiala, J. C., Spacek, J., & Harris, K. M. (2002). Dendritic spine pathology: cause or consequence of neurological disorders?. *Brain research reviews*, *39*(1), 29-54.

Hering, H., & Sheng, M. (2001). Dentritic spines: structure, dynamics and regulation. *Nature Reviews Neuroscience*, *2*(12), 880-888.

Sala, C., & Segal, M. (2014). Dendritic spines: the locus of structural and functional plasticity. *Physiological reviews*, *94*(1), 141-188.

Eckert, D. J., Catcheside, P. G., Smith, J. H., Frith, P. A., & McEvoy, R. D. (2004). Hypoxia suppresses symptom perception in asthma. *American journal of respiratory and critical care medicine*, *169*(11), 1224-1230.

Maenner,M.J.; Shaw, K.A.; Bakian, A.V.; Bilder, D.A.; Durkin,M.S.; Esler, A.; Furnier, S.M.; Hallas, L.; Hall-Lande, J.; Hudson, A.; et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018. Morb. Mortal. Wkly. Rep. Surveill. Summ. 2021, 70, 1–16.

Torpy, J. M., Campbell, A., & Glass, R. M. (2010). JAMA patient page. Chronic diseases of children. *JAMA*, *303*(7), 682. https://doi.org/10.1001/jama.303.7.682

Hughes, H. K., R J Moreno, & Ashwood, P. (2023). Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain, behavior, and immunity*, *108*, 245–254. https://doi.org/10.1016/j.bbi.2022.12.001

Jónsdóttir, U., & Lang, J. E. (2017). How does autism spectrum disorder affect the risk and severity of childhood asthma?. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*, *118*(5), 570–576. https://doi.org/10.1016/j.anai.2017.02.020

Patel, S., Masi, A., Dale, R. C., Whitehouse, A. J. O., Pokorski, I., Alvares, G. A., Hickie, I. B., Breen, E., & Guastella, A. J. (2018). Social impairments in autism spectrum disorder are related to maternal immune history profile. Molecular psychiatry, 23(8), 1794–1797. https://doi.org/10.1038/mp.2017.201

Schieve, L. A., Gonzalez, V., Boulet, S. L., Visser, S. N., Rice, C. E., Van Naarden Braun, K., & Boyle, C. A. (2012). Concurrent medical conditions and health care use and needs among children with learning and behavioral developmental disabilities, National Health Interview Survey, 2006-2010. *Research in developmental disabilities*, *33*(2), 467–476. https://doi.org/10.1016/j.ridd.2011.10.008

Kotey, S., Ertel, K., & Whitcomb, B. (2014). Co-occurrence of autism and asthma in a nationally-representative sample of children in the United States. *Journal of autism and developmental disorders*, *44*(12), 3083–3088. https://doi.org/10.1007/s10803-014-2174-y

Chen, M. H., Su, T. P., Chen, Y. S., Hsu, J. W., Huang, K. L., Chang, W. H., Chen, T. J., & Bai, Y. M. (2017). Comorbidity of Allergic and Autoimmune Diseases Among Patients With ADHD. *Journal of attention disorders*, *21*(3), 219–227. https://doi.org/10.1177/1087054712474686

Stewart, B. A., & Klar, A. J. (2013). Can bronchoscopic airway anatomy be an indicator of autism?. *Journal of autism and developmental disorders*, *43*(4), 911–916. https://doi.org/10.1007/s10803-012-1635-4

Jyonouchi, H., Geng, L., Cushing-Ruby, A., & Quraishi, H. (2008). Impact of innate immunity in a subset of children with autism spectrum disorders: a case control study. *Journal of neuroinflammation*, *5*, 52. https://doi.org/10.1186/1742-2094-5-52

Jyonouchi H. (2010). Autism spectrum disorders and allergy: observation from a pediatric allergy/immunology clinic. *Expert review of clinical immunology*, *6*(3), 397–411. https://doi.org/10.1586/eci.10.18

Quirce S. (2009). Asthma in Alergológica-2005. *Journal of investigational allergology & clinical immunology*, *19 Suppl 2*, 14–20.

Molloy, C. A., Morrow, A. L., Meinzen-Derr, J., Schleifer, K., Dienger, K., Manning-Courtney, P., Altaye, M., & Wills-Karp, M. (2006). Elevated cytokine levels in children with autism spectrum disorder. *Journal of neuroimmunology*, *172*(1-2), 198–205. https://doi.org/10.1016/j.jneuroim.2005.11.007

Gupta, S., Aggarwal, S., Rashanravan, B., & Lee, T. (1998). Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. *Journal of neuroimmunology*, *85*(1), 106–109. https://doi.org/10.1016/s0165-5728(98)00021-6

Theoharides T. C. (2009). Autism spectrum disorders and mastocytosis. *International journal of immunopathology and pharmacology*, *22*(4), 859–865. https://doi.org/10.1177/039463200902200401

Suzuki, K., Matsuzaki, H., Iwata, K., Kameno, Y., Shimmura, C., Kawai, S., Yoshihara, Y., Wakuda, T., Takebayashi, K., Takagai, S., Matsumoto, K., Tsuchiya, K. J., Iwata, Y., Nakamura, K., Tsujii, M., Sugiyama, T., & Mori, N. (2011). Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. *PloS one*, *6*(5), e20470. https://doi.org/10.1371/journal.pone.0020470

Heuer, L. S., Croen, L. A., Jones, K. L., Yoshida, C. K., Hansen, R. L., Yolken, R., Zerbo, O., DeLorenze, G., Kharrazi, M., Ashwood, P., & Van de Water, J. (2019). An Exploratory Examination of Neonatal Cytokines and Chemokines as Predictors of Autism Risk: The Early Markers for Autism Study. *Biological psychiatry*, *86*(4), 255–264. https://doi.org/10.1016/j.biopsych.2019.04.037

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., & Van de Water, J. (2011). Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain, behavior, and immunity*, *25*(1), 40–45. https://doi.org/10.1016/j.bbi.2010.08.003

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I. N., & Van de Water, J. (2011). Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *Journal of neuroimmunology*, *232*(1-2), 196–199. https://doi.org/10.1016/j.jneuroim.2010.10.025

Pease, J. E., & Williams, T. J. (2001). Eotaxin and asthma. *Current opinion in pharmacology*, *1*(3), 248–253. https://doi.org/10.1016/s1471-4892(01)00044-3

Pease, J. E., & Sabroe, I. (2002). The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. *American journal of respiratory medicine : drugs, devices, and other interventions*, *1*(1), 19–25. https://doi.org/10.1007/BF03257159

Ricci, S., Businaro, R., Ippoliti, F., Lo Vasco, V. R., Massoni, F., Onofri, E., Troili, G. M., Pontecorvi, V., Morelli, M., Rapp Ricciardi, M., & Archer, T. (2013). Altered cytokine and BDNF levels in autism spectrum disorder. Neurotoxicity research, 24(4), 491–501. https://doi.org/10.1007/s12640-013-9393-4

Ashwood, P., Enstrom, A., Krakowiak, P., Hertz-Picciotto, I., Hansen, R. L., Croen, L. A., Ozonoff, S., Pessah, I. N., & Van de Water, J. (2008). Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. *Journal of neuroimmunology*, *204*(1-2), 149–153. https://doi.org/10.1016/j.jneuroim.2008.07.006

Careaga, M., Rogers, S., Hansen, R. L., Amaral, D. G., Van de Water, J., & Ashwood, P. (2017). Immune Endophenotypes in Children With Autism Spectrum Disorder. *Biological psychiatry*, *81*(5), 434–441. https://doi.org/10.1016/j.biopsych.2015.08.036

Jyonouchi, H., Sun, S., & Le, H. (2001). Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *Journal of neuroimmunology*, *120*(1-2), 170–179. https://doi.org/10.1016/s0165-5728(01)00421-0

Rose, D. R., Yang, H., Serena, G., Sturgeon, C., Ma, B., Careaga, M., Hughes, H. K., Angkustsiri, K., Rose, M., Hertz-Picciotto, I., Van de Water, J., Hansen, R. L., Ravel, J., Fasano, A., & Ashwood, P. (2018). Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. *Brain, behavior, and immunity*, *70*, 354–368. https://doi.org/10.1016/j.bbi.2018.03.025

Rose, D. R., Yang, H., Careaga, M., Angkustsiri, K., Van de Water, J., & Ashwood, P. (2020). T cell populations in children with autism spectrum disorder and co-morbid gastrointestinal symptoms. *Brain, behavior, & immunity - health*, *2*, 100042. https://doi.org/10.1016/j.bbih.2020.100042

Xu, G., Snetselaar, L. G., Jing, J., Liu, B., Strathearn, L., & Bao, W. (2018). Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder in Children. *JAMA network open*, *1*(2), e180279. https://doi.org/10.1001/jamanetworkopen.2018.0279

Nemet, S., Asher, I., Yoles, I., Baevsky, T., & Sthoeger, Z. (2022). Early childhood allergy linked with development of attention deficit hyperactivity disorder and autism spectrum disorder. *Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology*, *33*(6), 10.1111/pai.13819. https://doi.org/10.1111/pai.13819

de Theije, C. G., Wu, J., Koelink, P. J., Korte-Bouws, G. A., Borre, Y., Kas, M. J., Lopes da Silva, S., Korte, S. M., Olivier, B., Garssen, J., & Kraneveld, A. D. (2014). Autistic-like behavioural and neurochemical changes in a mouse model of food allergy. *Behavioural brain research*, *261*, 265–274. https://doi.org/10.1016/j.bbr.2013.12.008

Murphy V. E. (2022). Asthma in pregnancy - Management, maternal co-morbidities, and long-term health. Best practice & research. Clinical obstetrics & gynaecology, 85(Pt A), 45–56. https://doi.org/10.1016/j.bpobgyn.2022.06.005

Bravo-Solarte, D. C., Garcia-Guaqueta, D. P., & Chiarella, S. E. (2023). Asthma in pregnancy. *Allergy and asthma proceedings*, *44*(1), 24–34. https://doi.org/10.2500/aap.2023.44.220077

Grosso, A., Locatelli, F., Gini, E., Albicini, F., Tirelli, C., Cerveri, I., & Corsico, A. G. (2018). The course of asthma during pregnancy in a recent, multicase-control study on respiratory health. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology, 14, 16. https://doi.org/10.1186/s13223-018-0242-0

Kircher, S., Schatz, M., & Long, L. (2002). Variables affecting asthma course during pregnancy. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, 89(5), 463–466. https://doi.org/10.1016/S1081-1206(10)62082-0

Schatz, M., Dombrowski, M. P., Wise, R., Thom, E. A., Landon, M., Mabie, W., Newman, R. B., Hauth, J. C., Lindheimer, M., Caritis, S. N., Leveno, K. J., Meis, P., Miodovnik, M., Wapner, R. J., Paul, R. H., Varner, M. W., O'sullivan, M. J., Thurnau, G. R., Conway, D., & McNellis, D. (2003). Asthma morbidity during pregnancy can be predicted by severity classification. The Journal of allergy and clinical immunology, 112(2), 283–288. https://doi.org/10.1067/mai.2003.1516

Murphy, V. E., Clifton, V. L., & Gibson, P. G. (2010). The effect of cigarette smoking on asthma control during exacerbations in pregnant women. Thorax, 65(8), 739–744. https://doi.org/10.1136/thx.2009.124941

Ali, Z., Nilas, L., & Ulrik, C. S. (2016). Low risk of adverse obstetrical and perinatal outcome in pregnancies complicated by asthma: A case control study. Respiratory medicine, 120, 124–130. https://doi.org/10.1016/j.rmed.2016.10.004

Grzeskowiak, L. E., Smith, B., Roy, A., Schubert, K. O., Baune, B. T., Dekker, G. A., & Clifton, V. L. (2017). Impact of a history of maternal depression and anxiety on asthma control during pregnancy. The Journal of asthma : official journal of the Association for the Care of Asthma, 54(7), 706–713. https://doi.org/10.1080/02770903.2016.1258080

Whalen, O. M., Karayanidis, F., Murphy, V. E., Lane, A. E., Mallise, C. A., & Campbell, L. E. (2019). The effects of maternal asthma during pregnancy on child cognitive and behavioral development: A systematic review. The Journal of asthma : official journal of the Association for the Care of Asthma, 56(2), 130–141. https://doi.org/10.1080/02770903.2018.1437174

Wen, S. W., Demissie, K., & Liu, S. (2001). Adverse outcomes in pregnancies of asthmatic women: results from a Canadian population. Annals of epidemiology, 11(1), 7–12. https://doi.org/10.1016/s1047-2797(00)00077-6

Källén, B., Rydhstroem, H., & Aberg, A. (2000). Asthma during pregnancy--a population based study. European journal of epidemiology, 16(2), 167–171. https://doi.org/10.1023/a:1007678404911

Dombrowski, M. P., Schatz, M., Wise, R., Momirova, V., Landon, M., Mabie, W., Newman, R. B., McNellis, D., Hauth, J. C., Lindheimer, M., Caritis, S. N., Leveno, K. J., Meis, P., Miodovnik, M., Wapner, R. J., Paul, R. H., Varner, M. W., O'Sullivan, M. J., Thurnau, G. R., Conway, D. L., … National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network and the National Heart, Lung, and Blood Institute (2004). Asthma during pregnancy. Obstetrics and gynecology, 103(1), 5–12. https://doi.org/10.1097/01.AOG.0000103994.75162.16

Rejnö, G., Lundholm, C., Gong, T., Larsson, K., Saltvedt, S., & Almqvist, C. (2014). Asthma during pregnancy in a population-based study--pregnancy complications and adverse perinatal outcomes. PloS one, 9(8), e104755. https://doi.org/10.1371/journal.pone.0104755

Breton, M. C., Beauchesne, M. F., Lemière, C., Rey, E., Forget, A., & Blais, L. (2009). Risk of perinatal mortality associated with asthma during pregnancy. Thorax, 64(2), 101–106. https://doi.org/10.1136/thx.2008.102970

Namazy, J. A., Murphy, V. E., Powell, H., Gibson, P. G., Chambers, C., & Schatz, M. (2013). Effects of asthma severity, exacerbations and oral corticosteroids on perinatal outcomes. The European respiratory journal, 41(5), 1082–1090. https://doi.org/10.1183/09031936.00195111

Litonjua, A. A., Carey, V. J., Burge, H. A., Weiss, S. T., & Gold, D. R. (1998). Parental history and the risk for childhood asthma. Does mother confer more risk than father?. American journal of respiratory and critical care medicine, 158(1), 176–181. https://doi.org/10.1164/ajrccm.158.1.9710014

Mattes, J., Murphy, V. E., Powell, H., & Gibson, P. G. (2014). Prenatal origins of bronchiolitis: protective effect of optimised asthma management during pregnancy. Thorax, 69(4), 383–384. https://doi.org/10.1136/thoraxjnl-2013-203388

Sherriff, A., Peters, T. J., Henderson, J., Strachan, D., & ALSPAC Study Team. Avon Longitudinal Study of Parents and Children (2001). Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3(1/2) years. International journal of epidemiology, 30(6), 1473–1484. https://doi.org/10.1093/ije/30.6.1473

Martel, M. J., Rey, E., Beauchesne, M. F., Malo, J. L., Perreault, S., Forget, A., & Blais, L. (2009). Control and severity of asthma during pregnancy are associated with asthma incidence in offspring: two-stage case-control study. The European respiratory journal, 34(3), 579–587. https://doi.org/10.1183/09031936.00074608

Instanes, J. T., Halmøy, A., Engeland, A., Haavik, J., Furu, K., & Klungsøyr, K. (2017). Attention-Deficit/Hyperactivity Disorder in Offspring of Mothers With Inflammatory and Immune System Diseases. Biological psychiatry, 81(5), 452–459. https://doi.org/10.1016/j.biopsych.2015.11.024

Kim, Y. S., Leventhal, B. L., Koh, Y. J., Fombonne, E., Laska, E., Lim, E. C., Cheon, K. A., Kim, S. J., Kim, Y. K., Lee, H., Song, D. H., & Grinker, R. R. (2011). Prevalence of autism spectrum disorders in a total population sample. *The American journal of psychiatry*, *168*(9), 904–912. https://doi.org/10.1176/appi.ajp.2011.10101532

Werling, D. M., Parikshak, N. N., & Geschwind, D. H. (2016). Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders. *Nature communications*, *7*, 10717. https://doi.org/10.1038/ncomms10717

Croen, L. A., Grether, J. K., Yoshida, C. K., Odouli, R., & Van de Water, J. (2005). Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Archives of pediatrics & adolescent medicine, 159(2), 151–157. https://doi.org/10.1001/archpedi.159.2.151

Patel, S., Dale, R. C., Rose, D., Heath, B., Nordahl, C. W., Rogers, S., Guastella, A. J., & Ashwood, P. (2020). Maternal immune conditions are increased in males with autism spectrum disorders and are associated with behavioural and emotional but not cognitive co-morbidity. Translational psychiatry, 10(1), 286. https://doi.org/10.1038/s41398-020-00976-2

Carter, S. A., Lin, J. C., Chow, T., Yu, X., Rahman, M. M., Martinez, M. P., ... & Xiang, A. H. (2022). Maternal obesity, diabetes, preeclampsia, and asthma during pregnancy and likelihood of autism spectrum disorder with gastrointestinal disturbances in offspring. *Autism*, 13623613221118430.

Leonard, H., de Klerk, N., Bourke, J., & Bower, C. (2006). Maternal health in pregnancy and intellectual disability in the offspring: a population-based study. *Annals of epidemiology*, *16*(6), 448–454. https://doi.org/10.1016/j.annepidem.2005.05.002

Goines, P. E., Croen, L. A., Braunschweig, D., Yoshida, C. K., Grether, J., Hansen, R., Kharrazi, M., Ashwood, P., & Van de Water, J. (2011). Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Molecular autism, 2, 13. https://doi.org/10.1186/2040-2392-2-13

Krakowiak, P., Goines, P. E., Tancredi, D. J., Ashwood, P., Hansen, R. L., Hertz-Picciotto, I., & Van de Water, J. (2017). Neonatal Cytokine Profiles Associated With Autism Spectrum Disorder. *Biological psychiatry*, *81*(5), 442–451. https://doi.org/10.1016/j.biopsych.2015.08.007

Jones, K. L., Croen, L. A., Yoshida, C. K., Heuer, L., Hansen, R., Zerbo, O., DeLorenze, G. N., Kharrazi, M., Yolken, R., Ashwood, P., & Van de Water, J. (2017). Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. *Molecular psychiatry*, *22*(2), 273–279. https://doi.org/10.1038/mp.2016.77

Abdallah, M. W., Larsen, N., Grove, J., Nørgaard-Pedersen, B., Thorsen, P., Mortensen, E. L., & Hougaard, D. M. (2013). Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. *The World Journal of Biological Psychiatry*, *14*(7), 528-538.

Magnan, A. O., Mely, L. G., Camilla, C. A., Badier, M. M., Montero-Julian, F. A., Guillot, C. M., ... & Vervloet, D. (2000). Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma: increased IFN-γ–producing CD8+ T cells in asthma. *American journal of respiratory and critical care medicine*, *161*(6), 1790-1796.

Raundhal, M., Morse, C., Khare, A., Oriss, T. B., Milosevic, J., Trudeau, J., Huff, R., Pilewski, J., Holguin, F., Kolls, J., Wenzel, S., Ray, P., & Ray, A. (2015). High IFN-γ and low SLPI mark severe asthma in mice and humans. *The Journal of clinical investigation*, *125*(8), 3037–3050. https://doi.org/10.1172/JCI80911

Rojas-Dotor, S., Segura-Méndez, N. H., Miyagui-Namikawa, K., & Mondragón-González, R. (2013). Expression of resistin, CXCR3, IP-10, CCR5 and MIP-1α in obese patients with different severity of asthma. Biological research, 46(1), 13-20.

Hashimoto, T., Kobayashi, N., Kajiyama, Y., Kaminuma, O., Suko, M., & Mori, A. (2006). IL-2-induced IL-13 production by allergen-specific human helper T cell clones. *International archives of allergy and immunology*, *140 Suppl 1*, 51–54. https://doi.org/10.1159/000092711

Church, J. S., Tamayo, J. M., Ashwood, P., & Schwartzer, J. J. (2021). Repeated allergic asthma in early versus late pregnancy differentially impacts offspring brain and behavior development. Brain, behavior, and immunity, 93, 66–79. https://doi.org/10.1016/j.bbi.2020.12.014

Tamayo, J. M., Rose, D., Church, J. S., Schwartzer, J. J., & Ashwood, P. (2022). Maternal Allergic Asthma Induces Prenatal Neuroinflammation. Brain sciences, 12(8), 1041. https://doi.org/10.3390/brainsci12081041

Schwartzer, J. J., Careaga, M., Chang, C., Onore, C. E., & Ashwood, P. (2015). Allergic fetal priming leads to developmental, behavioral and neurobiological changes in mice. Translational psychiatry, 5(4), e543. https://doi.org/10.1038/tp.2015.40

Schwartzer, J. J., Careaga, M., Coburn, M. A., Rose, D. R., Hughes, H. K., & Ashwood, P. (2017). Behavioral impact of maternal allergic-asthma in two genetically distinct mouse strains. Brain, behavior, and immunity, 63, 99–107. https://doi.org/10.1016/j.bbi.2016.09.007

Vogel Ciernia, A., Careaga, M., LaSalle, J. M., & Ashwood, P. (2018). Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. Glia, 66(3), 505–521. https://doi.org/10.1002/glia.23261

Lenz, K. M., Pickett, L. A., Wright, C. L., Galan, A., & McCarthy, M. M. (2019). Prenatal Allergen Exposure Perturbs Sexual Differentiation and Programs Lifelong Changes in Adult Social and Sexual Behavior. Scientific reports, 9(1), 4837. https://doi.org/10.1038/s41598-019-41258-2

Breach, M. R., Dye, C. N., Joshi, A., Platko, S., Gilfarb, R. A., Krug, A. R., Franceschelli, D. V., Galan, A., Dodson, C. M., & Lenz, K. M. (2021). Maternal allergic inflammation in rats impacts the offspring perinatal neuroimmune milieu and the development of social play, locomotor behavior, and cognitive flexibility. Brain, behavior, and immunity, 95, 269–286. https://doi.org/10.1016/j.bbi.2021.03.025

Li, X., Chauhan, A., Sheikh, A. M., Patil, S., Chauhan, V., Li, X. M., Ji, L., Brown, T., & Malik, M. (2009). Elevated immune response in the brain of autistic patients. Journal of neuroimmunology, 207(1-2), 111–116. https://doi.org/10.1016/j.jneuroim.2008.12.002

Koyama, R., & Ikegaya, Y. (2015). Microglia in the pathogenesis of autism spectrum disorders. Neuroscience research, 100, 1-5.

Morgan, J. T., Chana, G., Pardo, C. A., Achim, C., Semendeferi, K., Buckwalter, J., ... & Everall, I. P. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biological psychiatry, 68(4), 368-376.

Morgan, J. T., Barger, N., Amaral, D. G., & Schumann, C. M. (2014). Stereological study of amygdala glial populations in adolescents and adults with autism spectrum disorder. PloS one, 9(10), e110356.

Zhuang, T. T., Pan, C., Chen, J. J., Han, F., Zhu, X. L., Xu, H., & Lu, Y. P. (2018). Chronic asthma-induced behavioral and hippocampal neuronal morphological changes are concurrent with BDNF, cofilin1 and Cdc42/RhoA alterations in immature mice. Brain research bulletin, 143, 194–206. https://doi.org/10.1016/j.brainresbull.2018.09.006

Del Giacco, S. R., Bakirtas, A., Bel, E., Custovic, A., Diamant, Z., Hamelmann, E., Heffler, E., Kalayci, Ö., Saglani, S., Sergejeva, S., Seys, S., Simpson, A., & Bjermer, L. (2017). Allergy in severe asthma. Allergy, 72(2), 207–220. https://doi.org/10.1111/all.13072

Baxi, S. N., & Phipatanakul, W. (2010). The role of allergen exposure and avoidance in asthma. Adolescent medicine: state of the art reviews, 21(1), 57–ix.

Custovic, A., Simpson, A., & Woodcock, A. (1998). Importance of indoor allergens in the induction of allergy and elicitation of allergic disease. Allergy, 53(48 Suppl), 115–120. https://doi.org/10.1111/j.1398-9995.1998.tb05011.x

Guarnieri, M., & Balmes, J. R. (2014). Outdoor air pollution and asthma. Lancet (London, England), 383(9928), 1581–1592. https://doi.org/10.1016/S0140-6736(14)60617-6

Wu, J. Z., Ge, D. D., Zhou, L. F., Hou, L. Y., Zhou, Y., & Li, Q. Y. (2018). Effects of particulate matter on allergic respiratory diseases. *Chronic diseases and translational medicine*, *4*(2), 95–102. https://doi.org/10.1016/j.cdtm.2018.04.001

Wilson, W. E., & Suh, H. H. (1997). Fine particles and coarse particles: concentration relationships relevant to epidemiologic studies. Journal of the Air & Waste Management Association (1995), 47(12), 1238–1249. https://doi.org/10.1080/10473289.1997.10464074

Becker, S., Soukup, J. M., Sioutas, C., & Cassee, F. R. (2003). Response of human alveolar macrophages to ultrafine, fine, and coarse urban air pollution particles. *Experimental lung research*, *29*(1), 29–44. https://doi.org/10.1080/01902140303762

Steiner, S., Bisig, C., Petri-Fink, A., & Rothen-Rutishauser, B. (2016). Diesel exhaust: current knowledge of adverse effects and underlying cellular mechanisms. *Archives of toxicology*, *90*(7), 1541–1553. https://doi.org/10.1007/s00204-016-1736-5

Chau-Etchepare, F., Hoerger, J. L., Kuhn, B. T., Zeki, A. A., Haczku, A., Louie, S., Kenyon, N. J., Davis, C. E., & Schivo, M. (2019). Viruses and non-allergen environmental triggers in asthma. Journal of investigative medicine : the official publication of the American Federation for Clinical Research, 67(7), 1029–1041. https://doi.org/10.1136/jim-2019-001000

Hutchinson, J. A., Vargo, J., Milet, M., French, N. H. F., Billmire, M., Johnson, J., & Hoshiko, S. (2018). The San Diego 2007 wildfires and Medi-Cal emergency department presentations, inpatient hospitalizations, and outpatient visits: An observational study of smoke exposure periods and a bidirectional case-crossover analysis. PLoS medicine, 15(7), e1002601. https://doi.org/10.1371/journal.pmed.1002601

Fann, N., Alman, B., Broome, R. A., Morgan, G. G., Johnston, F. H., Pouliot, G., & Rappold, A. G. (2018). The health impacts and economic value of wildland fire episodes in the U.S.: 2008-2012. The Science of the total environment, 610-611, 802–809. https://doi.org/10.1016/j.scitotenv.2017.08.024

Künzli, N., Avol, E., Wu, J., Gauderman, W. J., Rappaport, E., Millstein, J., Bennion, J., McConnell, R., Gilliland, F. D., Berhane, K., Lurmann, F., Winer, A., & Peters, J. M. (2006). Health effects of the 2003 Southern California wildfires on children. American journal of respiratory and critical care medicine, 174(11), 1221–1228. https://doi.org/10.1164/rccm.200604-519OC

Kumar, R. K., Herbert, C., & Foster, P. S. (2016). Mouse models of acute exacerbations of allergic asthma. Respirology (Carlton, Vic.), 21(5), 842–849. https://doi.org/10.1111/resp.12760

Bahadori, K., Doyle-Waters, M. M., Marra, C., Lynd, L., Alasaly, K., Swiston, J., & FitzGerald, J. M. (2009). Economic burden of asthma: a systematic review. *BMC pulmonary medicine*, *9*, 24. https://doi.org/10.1186/1471-2466-9-24

Rodrigo, G. J., Rodrigo, C., & Hall, J. B. (2004). Acute asthma in adults: a review. *Chest*, *125*(3), 1081–1102. https://doi.org/10.1378/chest.125.3.1081

Maltby, S., Tay, H. L., Yang, M., & Foster, P. S. (2017). Mouse models of severe asthma: Understanding the mechanisms of steroid resistance, tissue remodelling and disease exacerbation. *Respirology (Carlton, Vic.)*, *22*(5), 874–885. https://doi.org/10.1111/resp.13052

Snelgrove, R. J., Gregory, L. G., Peiró, T., Akthar, S., Campbell, G. A., Walker, S. A., & Lloyd, C. M. (2014). Alternaria-derived serine protease activity drives IL-33-mediated asthma exacerbations. *The Journal of allergy and clinical immunology*, *134*(3), 583–592.e6. https://doi.org/10.1016/j.jaci.2014.02.002

North, M. L., Amatullah, H., Khanna, N., Urch, B., Grasemann, H., Silverman, F., & Scott, J. A. (2011). Augmentation of arginase 1 expression by exposure to air pollution exacerbates the airways hyperresponsiveness in murine models of asthma. *Respiratory research*, *12*(1), 19. https://doi.org/10.1186/1465-9921-12-19

Kierstein, S., Krytska, K., Sharma, S., Amrani, Y., Salmon, M., Panettieri, R. A., Jr, Zangrilli, J., & Haczku, A. (2008). Ozone inhalation induces exacerbation of eosinophilic airway inflammation and hyperresponsiveness in allergen-sensitized mice. *Allergy*, *63*(4), 438–446. https://doi.org/10.1111/j.1398-9995.2007.01587.x

Zheng, X., Wang, X., Wang, T., Zhang, H., Wu, H., Zhang, C., Yu, L., & Guan, Y. (2019). Gestational Exposure to Particulate Matter 2.5 (PM2.5) Leads to Spatial Memory Dysfunction and Neurodevelopmental Impairment in Hippocampus of Mice Offspring. Frontiers in neuroscience, 12, 1000. https://doi.org/10.3389/fnins.2018.01000

Church, J. S., Tijerina, P. B., Emerson, F. J., Coburn, M. A., Blum, J. L., Zelikoff, J. T., & Schwartzer, J. J. (2018). Perinatal exposure to concentrated ambient particulates results in autism-like behavioral deficits in adult mice. Neurotoxicology, 65, 231–240. https://doi.org/10.1016/j.neuro.2017.10.007

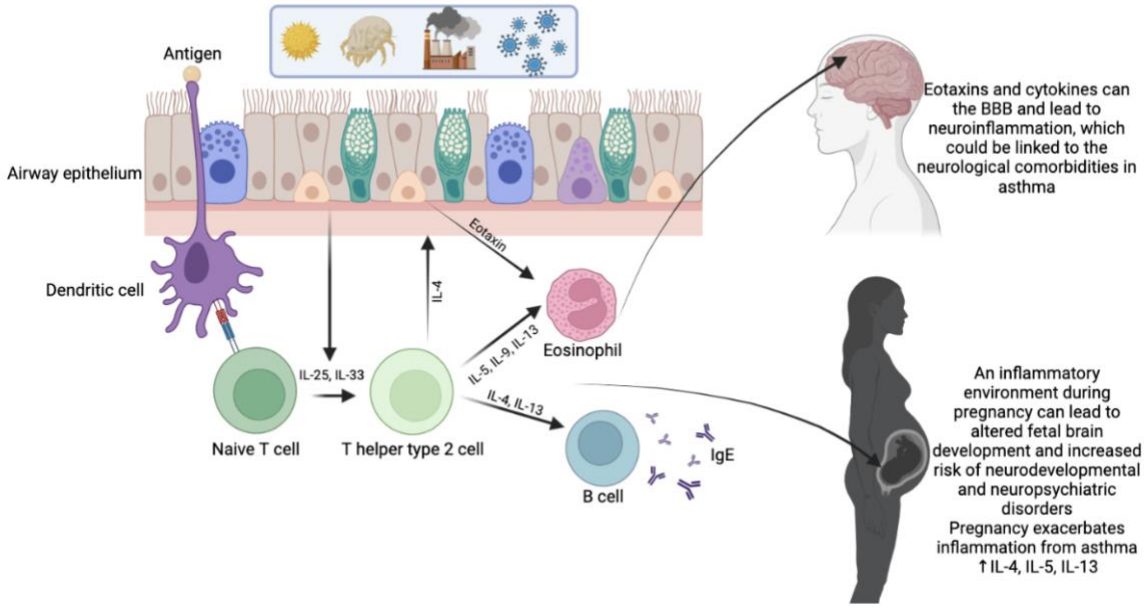

Figure 1. The airway responds to stimuli from allergens, pollutants, or infection. Dendritic cells (DCs) are recruited by chemokines secreted by the airway epithelial cells and migrate to the airways to take up and process antigen. DCs then present the antigen to naive T cells. Epithelial cell secretion of cytokines IL-33 and IL-25, along with antigen presentation via DCs, stimulate the development of a naive T cell to a T_H2 cell. T_H2 cells secrete cytokines IL-4, IL-5, IL-9, and IL-13. IL-4 stimulates airway epithelial cells to produce eotaxin and promote eosinophil recruitment. IL-4 also promotes IgE production by B cells, and IL-13 promotes IgE class switching. IL-5 is crucial for promoting eosinophil maturation, activation, and survival. IL-9 promotes T cell and mast cell proliferation. Pro-inflammatory cytokines and eotaxins can cross the BBB, resulting in neuroinflammation. Inflammation in the brain is noted in several neurological disorders comorbid with asthma. Inflammation during pregnancy is associated with neurodevelopmental disorders in the offspring, including ASD and ADHD. Pregnancy can also exacerbate asthma and cause increases in pro-inflammatory cytokines IL-4, IL-5, and IL-13.

Comorbidities of depression, anxiety, and ADHD are common in asthmatics. Maternal allergic asthma is associated with an increased risk of neurodevelopmental disorders in the offspring.

Chapter 2: Maternal Allergic Asthma Induces Prenatal Neuroinflammation

Abstract:

Autism spectrum disorder (ASD) is a class of neurodevelopmental disorders characterized by impaired social interactions and communication skills, and repetitive or stereotyped behaviors. Rates of ASD diagnosis continue to rise, with current estimates at 1 in 44 children in the US (Maenner 2021). Epidemiological studies have suggested a link between maternal allergic asthma and an increased likelihood of having a child diagnosed with ASD. However, a lack of robust laboratory models prevents mechanistic research from being carried out. We developed a novel mouse model of maternal asthma-allergy (MAA) and previously reported that offspring from these mothers exhibit behavioral deficits compared to controls. In addition, it was shown that epigenetic regulation of gene expression in microglia was altered in these offspring, including several autism candidate genes. To further elucidate if there is neuroinflammation in the fetus following MAA, we investigated how allergic asthma impacts the maternal environment and inflammatory markers in the placenta and fetal brain during gestation. Female C57Bl/6 mice were primed with ovalbumin prior to allergic asthma induction during pregnancy by administering aerosolized ovalbumin, or PBS control, to pregnant dams at gestational days 9.5, 12.5, and 17.5. Four hours after the final induction, placenta and fetal brains were collected and measured for changes in cytokines using Luminex bead-based multiplex assay. Placental MAA tissue showed a decrease in interleukin (IL)-17 in male and female offspring. There was a sex-dependent decrease in female monocyte chemoattractant protein 1 (MCP-1). In male placentas, IL-4, C–X–C motif chemokine 10 (CXCL10) also known as interferon γ-induced protein 10 kDa (IP-10), and chemokine (C-C motif) ligand 5 (RANTES) were decreased. In fetal brains, elevated inflammatory cytokines were found in MAA offspring

when compared to controls. Specifically, interferon-gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 1α (IL-1α), IL-6, and tumor necrosis factor α (TNFα) were elevated in both males and females. In contrast, a decrease in the cytokine IL-9 was also observed. There were slight sex differences after OVA exposures. Male fetal brains showed elevated levels of macrophage inflammatory protein-2 (MIP-2), whereas female brains showed increased keratinocytes-derived chemokine (KC). In addition, IL-1β and IP-10 in male fetal brains were decreased. Together, these data indicate that repeated exposure to allergic asthma during pregnancy alters cytokine expression in the fetal environment, in a sex-dependent way, resulting in homeostatic and neuroinflammatory alterations in the fetal brain.

1.0 Introduction:

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interactions and communications skills, and repetitive or stereotyped behaviors. Rates of ASD diagnosis continue to rise, with current estimates at 1 in 44 children in the US (Maenner 2021). ASD appears to impact males more frequently than females with a 4:1 ratio, respectively (Kim et al., 2011; Werling et al., 2016). While the etiology of ASD is unknown, research has identified both genetic and environmental contributing factors. Many of these suspected contributors are shared among other neuropsychiatric disorders, such as attention-deficit/hyperactive disorder (ADHD) and schizophrenia. Some reports put the genetic risk for ASD at over 50%, although presence of specific genes associated with ASD does not guarantee that an individual will be later diagnosed with ASD. That is, many of the risk genes associated with ASD represent common genetic variants in the population that carry low risk (Gaugler et al., 2014). This moderate contribution of genetics to the development of ASD underscores the importance of various environmental factors, particularly during fetal development, that likely contribute to increased risk and severity of neurodevelopmental disorders.

Epidemiology studies have begun to uncover some of the underlying environmental etiologies with a focus on the immune system. Environmental factors, such as maternal exposure to stress, obesity, pollution, infection, and allergy/asthma during pregnancy can increase the likelihood of having a child later diagnosed with ASD. Animal models of these environmental risk factors suggest behavioral and transcriptional changes as a result of cytokine signaling mediated by the placenta (as reviewed by Han et al., 2021). A common theme among these models is the activation of the maternal immune system. For example, one of the most widely

studied environmental factors contributing to an increased likelihood of neurodevelopmental disorders (NDD) in mice is the activation of the maternal immune system during pregnancy using bacterial or viral mimics (Han et al., 2021). Though infectious agents have been widely studied, recent meta-analyses of viral infections during pregnancy as a risk for humans with ASD was not supported (Jiang et al., 2016). Rates of asthma, however, are currently on the rise, and multiple studies have shown that mothers with asthma were more likely to have asthma exacerbations during pregnancy, and also a child with an ASD diagnosis (Ali and Ulrik 2013; Murphy et al., 2005, 2006; Croen, Grether, Yoshida, Odouli, & Van de Water, 2005; Croen et al., 2019; Gong et al., 2019; Hisle-Gorman et al., 2018; Lyall et al., 2014; Patel et al., 2020). Moreover, increased asthma severity was linked to increased ASD risk and independent of medication usage (Croen et al., 2019). In early reports, Croen et al., showed that childhood ASD was associated with mothers' asthma diagnosis in the first and second trimester (Croen, Grether, Yoshida, Odouli, & Van de Water, 2005). In addition, maternal asthma has also been linked with other NDD such as ADHD (Fasmer, 2011; Instanes, 2017; Liu et al., 2019). Although these studies suggest a link between maternal asthma and later diagnosis of ASD, there are very few studies that have directly investigated the impact that asthma has on the developing fetal brain and its environment during gestation.

Recently, our laboratory developed a model of maternal allergic asthma (MAA) in which offspring mimic behavioral outcomes relevant to the core features of ASD including decreased social interaction and repetitive-like behaviors (Schwartzer et al., 2015, 2017; Church et al., 2021). Within this model, MAA exposure results in changes in gene expression in microglia of adult offspring, highlighting the lasting epigenetic impact of MAA to the offspring's neuro-

immune environment (Ciernia et al., 2018). These behavioral and neuroimmune changes in offspring occur in response to allergic asthma-associated elevations in inflammatory cytokine in the dams, namely IL-4, IL-5, IL-6, and IL-17 (Schwartzer et al 2015), and closely mirror observations reported in clinical settings. Specifically, in a case control study by Goines et al., elevated levels of IL-4 and IL-5 during mid-pregnancy were shown to be associated with mothers who had children with autism (Goines et al., 2011). In further studies, high levels of IL-4 were detected in the amniotic fluid and newborn blood spots of children who were later diagnosed with ASD (Abdallah et al., 2013; Krakowiak et al., 2017). These observations in humans demonstrate an important role for the maternal inflammatory environment in shaping risk for ASD in the offspring and support the validity of our mouse model of MAA in identifying potential mechanistic links.

Maternal systemic inflammation can alter the fetal environment and have both short and long-term consequences on the developing brain (Hughes et al., 2018). For example, Zaretsky et al. reported that IL-6 can cross the human placenta in both the maternal-fetal and fetal-maternal directions (Zaretsky et al., 2004). This phenomenon has also been demonstrated in rat models showing that IL-6 administration during mid or late pregnancy can transfer across the placental barrier to reach the fetus (Dahlgren et al., 2006). In addition, mouse fetal brain cytokine expression of IL-1b, IL-6, IL-17, IL-13, MCP-1, and monocyte inflammatory protein-1 alpha (MIP-1α) are altered hours after an inflammatory viral insult to the dam (Fatemi et al., 2008; Meyer et al., 2006b; Meyer et al., 2008). While these studies show a link between viral/bacterial maternal infection and changes in the fetal brain, it has yet to be established in a model of MAA.

Given the links in humans, and the observed behavioral and neuroimmunological impacts of MAA in mice, it can be reasoned that MAA-induced changes in the offspring brain may begin at the fetal stages of development in response to elevated allergic-asthma associated cytokines. In these studies, we hypothesized that MAA results in sex-dependent changes in fetal brain and placenta cytokine expression following allergic asthma episodes. To test this hypothesis, our laboratory collected placental and fetal brain tissue following MAA exposure to investigate whether the inflammatory environment in these tissue are altered in response to this maternal insult.

2.0 Methods:

2.1 Animals

Male and female C57BL/6J mice generated from breeding pairs purchased from Jackson Laboratory (Bar Harbor, MA, USA) were bred and maintained at Mount Holyoke College, South Hadley, MA. Mice were raised on a 12 h light/dark cycle (lights on at 0800 h) and group housed in individually ventilated cages with same-sex littermates until breeding at 8-weeks of age. Cages were maintained in a temperature-controlled (23°C) vivarium with food and water provided *ad libitum*. All procedures were performed with approval by the Mount Holyoke College Institutional Animal Care and Use Committee and in accordance with the guidelines provided by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Maternal allergic asthma induction

Allergic asthma inductions were carried out using procedures previously described (Schwartzer et al., 2015, 2017). Briefly, sexually naïve female mice were sensitized with 10µg ovalbumin (OVA, Sigma, St Louis, MO, USA) and 1mg $(Al)OH_3$ (Invitrogen, San Diego, CA, USA) dissolved in 200µl of phosphate buffered saline (PBS) injected intraperitoneally at 6 and 7 weeks of age. Beginning at 8 weeks of age, females were mated overnight, presence of seminal plug was checked daily, noted as gestational day (GD)0.5, and single-housed. Pregnant mice were randomly assigned to receive either an aerosolized solution of 1% (wt/vl) OVA in PBS (n=10) or PBS alone (n=8) for three 45-minute induction sessions throughout gestation. Specifically, these induction sessions occurred at gestational days 9.5, 12.5, and 17.5, to

correspond with early, middle, and late gestation as previously described (Schwartzer et al., 2015).

2.3 Serum, Placenta, and Fetal Brain Collection

Four hours after the final induction, mice were anesthetized with isoflurane (2-4% inhalation) and 500ul of whole blood was collected from dams via cardiac puncture. Blood was allowed to clot at room temperature for 30 min, centrifuged at 10000 x g for 10 min at 4°C, and then serum collected and stored at -80°C. In addition, each placenta and fetal brain was extracted, flash frozen in liquid nitrogen, and individually stored at -80°C until further processing. To determine sex of the specimens, placenta and brain were genotyped for the presence or absence of Sry using polymerase chain reaction (PCR). A total of 74 brains and placenta were extracted from the 18 experimental litters resulting in the following groups: PBS male (n=15), PBS female (n=18), MAA male (n=23), MAA female (n=18).

2.4 Placenta and Fetal Brain Tissue Processing

Placenta and brain tissue were lysed using cell lysis buffer (Cell Signaling Technologies, Danvers, MA) containing protease and phosphatase inhibitors. The tissue was incubated in lysis buffer with agitation for 20 min on ice followed by sonication for 30 seconds. Cell lysate was then vortexed at top speed for 30 seconds and centrifuged at 20,000 x g for 10 minutes at 4°C. Protein concentrations were measured using Bio-Rad Benchmark Plus Spectrophotometer system and all samples were standardized to 70mg/ml for subsequent immunoassays.

2.6 Multiplex Bead-based Cytokine Analysis

Analysis of serum cytokines was performed using multiplex mouse 25-plex bead immunoassay (Milliplex mouse cytokine/chemokine magnetic bead panel #MCYTMAG70PMX25BK). The following cytokines were quantified: G-CSF, GM-CSF, IFN-g, IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, KC, MCP-1, MIP-1a, MIP-1b, MIP-2, RANTES, TNF-a. Standards and reagents were all prepared according to manufacturer's recommendations. Each serum, brain, and placenta samples were diluted to a standardized concentration and run in duplicate. Twenty-five microliters of sample, standards, and budder blanks were loaded to the 96-well plate with appropriate amounts of assay buffer and matrix solution. The plate was then incubated overnight with antibody-coupled magnetic beads. The following day, after a series of washes, the plate was incubated with a biotinylates detection antibody on a shaker for 1 h. Streptavidin-phycoerythrin was added and incubated while shaking continued for 30 min. Washes were done using Bio-Plex handheld magnet (Bio-Rad Laboratories, Hercules, CA, USA). After the final wash, the plate was analyzed using plate reader Bio-Rad Bio-Plex 200 (Bio-Rad Laboratories, Hercules, CA, USA) and analyzed using Bio-Plex Manager software (Bio-Rad Laboratories). The following were the minimal amounts of detectable cytokine concentrations: G-CSF: 1.7 pg/mL; GM-CSF: 10.9 pg/mL; IFNγ: 1.1 pg/mL; IL-1α: 10.3 pg/mL; IL-1β: 5.4 pg/mL; IL-2: 1.0 pg/mL; IL-4: 0.4 pg/mL; IL-5: 1.0 pg/mL; IL-6: 1.1 pg/mL; IL-7: 1.4 pg/mL; IL-9: 17.3 pg/mL; IL-10: 2.0 pg/mL; IL-12 (p40): 3.9 pg/mL; IL-12 (p70): 4.8 pg/mL; IL-13: 7.8 pg/mL; IL-15: 7.4 pg/mL; IL-17: 0.5 pg/mL; IP-10: 0.8 pg/mL; KC: 2.3 pg/mL; MCP-1: 6.7 pg/mL; MIP-1α: 7.7 pg/mL; MIP-1β: 11.9 pg/mL; MIP-2: 30.6 pg/mL; RANTES: 2.7 pg/ mL; TNF-α: 2.3 pg/mL. Sample

concentration that fell below minimal detection value were given a proxy value of half the limit of detection for statistical comparisons.

2.7 Statistical Analysis

Data were analyzed using GraphPad Prism Version 9.3.1 and RStudio version 1.4.1106 (2021) using the "nlme" package. Maternal serum cytokine concentrations between MAA and PBS dams were assessed using non-parametric Mann-Whitney U analyses. Similarly,

placental cytokine measures were pooled to increase signal detection and concentrations were evaluated between MAA and PBS dams using Mann-Whitney U. To control for pseudoreplications and litter-to-litter variations, offspring brain cytokine measures were evaluated separately for male and female mice using multilevel modeling to control for type I error (Lazic and Essioux, 2013; Lazic et al., 2018) with offspring as the level 1 measure nested in dams as the level 2 two variable. Treatment (MAA or PBS) was set as the fixed effect and litter as the random effect. Model estimates with a p-value less than 0.05 were considered significant.

3.0 Results:

3.1 Maternal Serum cytokines

Following the final induction at E17.5, the maternal sera was collected from dams of both treatment groups. Maternal serum in the MAA dams showed elevated levels of the T-helper type 2 allergic asthma-associated cytokines including IL-4 (p=0.019), IL-5 (p=0.0004), IL-13 (p=0.003). Moreover, MAA dams had elevated levels of IL-6 (p=0.006), IL-12 (p=0.008), IL-17 (p=0.030), and MIP-1a (p=0.045) compared to PBS-treated control dams (Figure 1). While there were also trends for increased levels of GM-CSF and IL-10, these increases did not reach statistical significance.

3.2 Offspring Placenta Cytokines

Placentas were collected following the final induction and cytokine analysis was performed on placenta homogenates. Placental levels of IL-17 levels were decreased in offspring from MAA offspring that were either female (p= 0.025) or male (p=0.016) (Figure 2 & 3, respectively), compared to PBS controls. In addition, in placentas from female offspring (Figure 2), we observed a decrease in MCP-1 (p=0.003), which was not found in male counterparts. Conversely, in male placentas only (Figure 3), there were significant decreases in IL-4 (p=0.026), IP-10 (p=0.025) and RANTES (*p*=0.010).

3.3 Whole Brain Fetal Cytokines

Following the removal of the fetus from the placenta, whole fetal brains were collected, homogenized, and analyzed for cytokine concentration. Multilevel mixed-effects modeling

revealed elevated levels of several cytokines in both sexes of MAA offspring, most of which are generally considered inflammatory in nature, namely GM-CSF, IFNγ, IL-1α, IL-6, and TNFα. Specifically, MAA exposure resulted in an average of 21-22pg/ml increase in GM-CSF concentrations in both male (Figure 4) and female (Figure 5) offspring brains compared to sex-matched offspring of PBS dams (Male, b= 22.32, CI: 6.66 – 37.98, t(16) = 2.94, p = 0.010; Female, b=21.36, CI: 2.88 – 39.84, t(16) = 2.38, p = 0.030). Similar increases were also observed in both male and female offspring for IFNγ (Male, b = 7.07, CI: 2.35 - 11.79, t(16) = 3.09, p =0.007; Female, b = 6.48, CI: 1.24-11.72, t(16) = 2.55, p = 0.22), and IL-1 α (Male, b = 35.19, CI: 16.94 – 53.44, t(16) = 3.98, p = 0.001; Female, b = 28.89, CI: 6.49 – 51.29, t(16) = 2.66, p = 0.017). Moreover, both male and female offspring from MAA dams expressed higher levels of IL-6 (Male, b=5.11, CI: 1.67 – 8.54, t(16) = 3.07, p = 0.007; Female, b = 4.03, CI: 1.05 – 7.00, t(16) = 2.79, p =0.013) and TNFα (Male, b = 3.41, CI: 0.35 – 6.46, t(16) = 2.30, p = 0.035; Female, b = 2.52, CI: 0.66 – 4.38, t(16) = 2.79, p = 0.013), compared to sex-matched PBS controls. A decrease in IL-9 was also associated with both male and female offspring from MAA dams compared to controls (Male, b = -887.35, CI: -1626.55 - -148.16, t(16) = -2.48, p = 0.025; Female, b = -784.03, CI: -1527.74- -40.31, t(16) = -2.17, p = 0.045).

In addition to the broad increases in inflammatory cytokines in both sexes, there were additional sex-specific changes in several cytokines in response to MAA. In males (Figure 4) but not females, there was a significant increase in MIP-2, b= 21.28, Cl: 2.42 – 41.23, t(16)= 2.32, p= 0.034, and a significant decrease in IL-1β, b= -18.80, Cl: -36.50 - -1.11, t(16)= -2.19, p= 0.043, and IP-10, b= -215.76, Cl: -420.39 - -11.13, t(16)= -2.18, p= 0.045, compared to sex-matched PBS offspring. In addition, in MAA male offspring, an increase in G-CSF, b= 4.76, Cl: 0.04 –

9.49, t(16)= 2.08, p= 0.054, compared to PBS offspring was also observed, but it did not reach statistical significance $p<0.05$. Conversely in females (Figure 5), MAA-exposed offspring had an increase in KC levels compared to female offspring of PBS dams, b= 2.35, Cl: 0.90 – 3.79, t(16)= 3.35, p= 0.004. Similarly, increases in cytokines G-CSF, IL-2, MIP-2, IP-10, and MIP-1b were observed in female MAA offspring compared to PBS controls, but these did not reach statistical significance $p<0.09$ (Data not shown).

4.0 Discussion:

The fetal environment plays a vital role in offspring neurodevelopment and behavior, and the maternal response to allergies and asthma represents an increasingly common environmental factor that can impact fetal development. We hypothesized that allergic asthma exposure during pregnancy would result in sex-specific neuroinflammation in the brains of developing offspring in utero, based on previous studies on maternal asthma and ASD (Patel et al., 2020). As predicted, the analysis of the maternal serum following MAA challenge confirmed systemic inflammation consistent with the allergic-asthma immune phenotype. These elevations in maternal cytokines were met with concomitant elevations in neuroinflammatory signals in the fetal brain. There were common cytokines elevated in fetal brains in both males and females but also several cytokines were sex specific and may correspond to differences in behavior response seen in MAA models (Schwartzer et al., 2015; Schwartzer et al., 2017), and, also in humans (Patel et al., 2020). Interestingly, increases in fetal brain cytokines were paralleled by decreases in inflammatory cytokines in the placental tissue analysis. Together, our findings support the notion that maternal inflammation in response to MAA impacts the fetal neuroimmune environment during gestation.

In humans, an allergic asthma response is often associated with a systemic increase of IL-4 and IL-5, both of which have been linked to an increased likelihood of birthing a child later diagnosed with neurodevelopmental disorders when there are higher levels occurring during pregnancy (Goines et al., 2011; Jones et al., 2017). In case-control studies, Goines et al. also demonstrated that elevations in IL-5 and IL-4 together during gestation was associated with a 50% increased risk of ASD (Goines et al., 2011). Here, we showed that our model of MAA recapitulates the exposure to cytokines that are hallmarks of the allergic asthma response including increased levels of IL-4, IL-6, IL-5 IL-13, and IL-17. This global response may translate more closely to the human exposure than single cytokine exposures in isolation. These combined cytokine signals may be the central mechanistic factors that link MAA to the behavioral deficits, and shape brain chemistry and neurocircuitry, observed in our previous studies of this model (Schwartzer et al., 2015; Church al., 2021; Vogel Ciernia et al., 2018).

The placenta plays the role of a multipurpose organ for the fetus, acting as the lungs, gut, kidneys, and liver (Burton et al., 2015). In addition, the placenta acts as a communicator between the fetus and mother, relaying important environmental signals to prime the fetus to adapt to future postpartum insults (Barker et al., 2004). For example, extravillous trophoblast cells can interact with the maternal immune system by migrating to the uterine wall from the placenta (Burton et al., 2015). Acting in the converse direction, from mother to fetus, some cytokines such as IL-6, which are elevated in our MAA model, can cross the placental barrier (Dahlgren et al., 2006) and directly impact fetal development. A previous report by Hsiao et al. shows that IL-6 levels remain elevated in the placentas of maternal immune activated dams compared to saline controls 24 hours following immune induction with poly(I:C) (Hsiao et al., 2001). Although we report here an elevated IL-6 response in maternal serum, the placental tissue did not mirror the

findings by Hsiao et al. In fact, of the cytokines measured in our study, we did not observe significant elevations in any of the inflammatory markers measured. It is unclear yet why our findings do not mirror common changes shown in other poly(I:C) models of maternal immune activation, though these differences may represent a distinct mechanistic pathway unique to allergic asthma inflammation compared to other viral/bacterial models of maternal inflammation. Moreover, our measures of placental and brain cytokine makers were taken 4-hours after the final MAA induction, representing a single cross-sectional timepoint during the inflammatory cascade. Given that the cellular and molecular responses to allergic asthma change dynamically across several hours of exposures, there are likely other placental changes occurring earlier and/or later in the inflammatory time-course that were not captured in this single cross-sectional window. Future longitudinal measures are needed to capture a more complete timeline of placental changes.

Our model demonstrates that MAA induces a change in the inflammatory profile of offspring brains at E17.5. Among these changes, increases in GM-CSF, INFg, IL-1a, IL-6, and TNFα were found. These increases were seen in both male and female fetal brains. Postmortem tissue studies have found elevated TNFα, IL-6, IFNg, and GM-CSF in brains from ASD individuals compared to their typically developing counterparts (Li et al., 2009). Although our fetal brain analyses also shows elevations in these cytokines, we have yet to perform this analysis on whole brains from adults. However, we recently showed elevated IFNg in the hypothalamus of adult animals when exposed to MAA in early fetal development (Church et al., 2021). Of note, IL-6, which is normally expressed at low levels in the brain, plays a role in neurogenesis, cell growth, and myelination or demyelination. Moreover, IL-6 have been linked to altered neuronal cell adhesion, migration, and synaptic formation (Smith et al., 2007; Gruol et al., 2015).

TNFα has also been shown to have an impact on neuronal survival. Studies have suggested that the release of astroglial and microglial TNFα may be neuroprotective in a model of ischemic stroke (Batlle et al., 2015; Lambertsen et al., 2009). Microglia and astrocytes are also known producers of IL-1a (Salmeron et al., 2016), which was also found to be elevated in the brains of offspring in this model and, similar to TNFα, has been suggested to be neuroprotective in models of ischemic stroke (Salmeron et al., 2019). However, whether tissue damage leading to the release of TNFa or IL-1a in a neuroprotective role would need to be confirmed in the context of MAA. Receptors of GM-CSF, another cytokine elevated here in fetal brains after MAA, have been found on microglia, astrocytes, oligodendrocytes, and neurons with GM-CSF having different dose dependent effects on each cell type (Dame et al., 1999).

Ciernia et al. previously reported epigenetic alterations in microglia from adult offspring born to MAA dams. Given the early inflammatory changes observed in the fetal brains in the present study, initial cytokine signals could influence the epigenetic changes to microglia in MAA, as it is known that early life insults to microglia can impact their function (Bilbo and Schwarz 2009; Lenz and Nelson 2018). Of note, during neurodevelopment, microglia remove unneeded neuronal precursors (Cunningham et al., 2013), and when this function is disrupted, increases in neuronal connectivity activity may result (Liu et al., 2021). Expression data from microglia in our model suggests a deviation from normal functioning (Vogel Ciernia et al., 2018). Over-connectivity is a phenomenon that has been observed in some cases of ASD and has been linked to changes in social interactions, as well as increased restricted and repetitive behaviors (Conti et al., 2017; Maximo et al., 2014; Mills et al., 2016), changes that our model demonstrates (Schwartzer et al., 2015). The cascade of inflammatory factors, specifically IL-6, IL-1α, GM-CSF, and TNFα seen in the fetal brain after MAA, highlight an important set of

immune markers during fetal development that have neurodevelopmental impacts in shaping the brain architecture and function that may influence the behavioral, neuroinflammatory, and epigenetic alterations observed in adult offspring (Vogel Ciernia et al., 2018; Schwartzer et al., 2015; Church et al., 2021).

In addition to sex-independent changes in cytokine levels in MAA offspring, we also observed sex-specific changes in placental and fetal brain cytokines as well. Sex differences are commonly noted in neurodevelopmental disorders (Polyak et al., 2015; Li et al., 2016). For instance, ASD reports a 4:1 ratio in boys to girls (Kim et al., 2011; Werling et al., 2016; Patel et al., 2020). In the placenta, we observed a decrease in the expression of IL-4, IP-10, and RANTES in male placentas, and lower levels of MCP-1 and IL-17 in female placentas. Additionally, the brain cytokine analysis in our model showed a decrease in IL-1b and an increase in MIP-2 in the fetal brains of male offspring taken at E17.5, but not female. While previous reports have shown an increase in brain IL-1b following MIA induction in both male and female mice (Meyer et al., 2006), the driving mechanisms behind the observed sex difference in our model are yet unclear. The similarities in our findings to clinical reports highlight the significance of our model, and the contrasts to other animal models of maternal immune activation highlight both the novelty of the MAA model and the need for further investigation into mechanistic pathways.

Our fetal brain data reflect an analysis of whole brain homogenates, an important limitation that may be masking more region-specific shifts in brain neuroinflammation. For example, changes in IL-1b in response to maternal poly(I:C) exposure vary by region in the newly born offspring (Hsiao et al., 2014), and small elevations in one region, for example the frontal cortex, can become undetectable when pooled with other regions (e.g. hippocampus). As

a result, future studies will require analysis of region-specific changes in fetal brain tissue to get a better understand how MAA may be altering brain maturation and differentiation. It is also unclear yet how these changes in the maternal environment communicate changes through the maternal-fetal interface to influence fetal neurodevelopment.

These limitations notwithstanding, our data demonstrate that exposures to allergic asthma inflammation during pregnancy impacts cytokine levels in fetal placenta and brain in a sex-specific manner. Our findings underscore the importance of the maternal environment in shaping fetal brain development and further support behavioral and clinical findings linking MAA to offspring behavioral deficits and neurodevelopmental disorders. Our evidence of early life programming through maternal immune activation raises important questions surrounding the role of the placenta and immune signals of the brain in shaping offspring neurodevelopment and highlight the importance of understanding the maternal-fetal mechanisms that shape behavioral and mental health later in life.

References:

Abdallah, M. W., Larsen, N., Grove, J., Nørgaard-Pedersen, B., Thorsen, P., Mortensen, E. L., & Hougaard, D. M. (2013). Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, *14*(7), 528–538. https://doi.org/10.3109/15622975.2011.639803

Ahmad, S. F., Ansari, M. A., Nadeem, A., Bakheet, S. A., Al-Ayadhi, L. Y., Alasmari, A. F., Alanazi, M. M., Al-Mazroua, H. A., & Attia, S. M. (2020). Involvement of CD45 cells in the development of autism spectrum disorder through dysregulation of granulocyte-macrophage colony-stimulating factor, key inflammatory cytokines, and transcription factors. *International immunopharmacology*, *83*, 106466. https://doi.org/10.1016/j.intimp.2020.106466

Ali, Z., & Ulrik, C. S. (2013). Incidence and risk factors for exacerbations of asthma during pregnancy. *Journal of asthma and allergy*, *6*, 53–60. https://doi.org/10.2147/JAA.S43183

Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding, J. E., Owens, J. A., & Robinson, J. S. (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet (London, England)*, *341*(8850), 938–941. https://doi.org/10.1016/0140-6736(93)91224-a

Barker D. J. (2004). The developmental origins of adult disease. *Journal of the American College of Nutrition*, *23*(6 Suppl), 588S–595S. https://doi.org/10.1080/07315724.2004.10719428

Batlle, M., Ferri, L., Andrade, C., Ortega, F. J., Vidal-Taboada, J. M., Pugliese, M., Mahy, N., & Rodríguez, M. J. (2015). Astroglia-Microglia Cross Talk during Neurodegeneration in the Rat Hippocampus. *BioMed research international*, *2015*, 102419. https://doi.org/10.1155/2015/102419

Bilbo, S. D., & Schwarz, J. M. (2009). Early-life programming of later-life brain and behavior: a critical role for the immune system. *Frontiers in behavioral neuroscience*, *3*, 14. https://doi.org/10.3389/neuro.08.014.2009

Burton, G. J., & Fowden, A. L. (2015). The placenta: a multifaceted, transient organ. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *370*(1663), 20140066. https://doi.org/10.1098/rstb.2014.0066

Carter, A. S., Black, D. O., Tewani, S., Connolly, C. E., Kadlec, M. B., & Tager-Flusberg, H. (2007). Sex differences in toddlers with autism spectrum disorders. *Journal of autism and developmental disorders*, *37*(1), 86–97. https://doi.org/10.1007/s10803-006-0331-7

Church, J. S., Tamayo, J. M., Ashwood, P., & Schwartzer, J. J. (2021). Repeated allergic asthma in early versus late pregnancy differentially impacts offspring brain and behavior development. *Brain, behavior, and immunity*, *93*, 66–79. https://doi.org/10.1016/j.bbi.2020.12.014

Conti, E., Mitra, J., Calderoni, S., Pannek, K., Shen, K. K., Pagnozzi, A., Rose, S., Mazzotti, S., Scelfo, D., Tosetti, M., Muratori, F., Cioni, G., & Guzzetta, A. (2017). Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: A diffusion MRI study. *Human brain mapping*, *38*(5), 2333–2344. https://doi.org/10.1002/hbm.23520

Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Arch Pediatr Adolesc Med. 2005 Feb;159(2):151-7. doi: 10.1001/archpedi.159.2.151. PMID: 15699309.

Croen LA, Qian Y, Ashwood P, Zerbo O, Schendel D, Pinto-Martin J, Daniele Fallin M, Levy S, Schieve LA, Yeargin-Allsopp M, Sabourin KR, Ames JL. Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. Autism Res. 2019 Oct;12(10):1551-1561. doi: 10.1002/aur.2175. Epub 2019 Jul 17. PMID: 31317667; PMCID: PMC7784630.

Cunningham, C. L., Martínez-Cerdeño, V., & Noctor, S. C. (2013). Microglia regulate the number of neural precursor cells in the developing cerebral cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(10), 4216–4233. https://doi.org/10.1523/JNEUROSCI.3441-12.2013

Dahlgren K, Samuelsson AM, Jansson T, Halmong A. Interleukin-6 in the maternal cirulation reaches the rat fetus in mid-gestation. *Pediatr Res.* 2006;60:147–151.

Dame, J. B., Christensen, R. D., & Juul, S. E. (1999). The distribution of granulocyte-macrophage colony-stimulating factor and its receptor in the developing human fetus. *Pediatric research*, *46*(4), 358–366. https://doi.org/10.1203/00006450-199910000-00002

Fasmer OB, Halmøy A, Eagan TM, Oedegaard KJ, Haavik J. Adult attention deficit hyperactivity disorder is associated with asthma. BMC Psychiatry. 2011 Aug 7;11:128. doi: 10.1186/1471-244X-11-128. PMID: 21819624; PMCID: PMC3163523.

Fatemi, S. H., Reutiman, T. J., Folsom, T. D., Huang, H., Oishi, K., Mori, S., Smee, D. F., Pearce, D. A., Winter, C., Sohr, R., & Juckel, G. (2008). Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders. *Schizophrenia research*, *99*(1-3), 56–70. https://doi.org/10.1016/j.schres.2007.11.018

Garay, P. A., Hsiao, E. Y., Patterson, P. H., & McAllister, A. K. (2013). Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. *Brain, behavior, and immunity*, *31*, 54–68. https://doi.org/10.1016/j.bbi.2012.07.008

Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, Mahajan M, Manaa D, Pawitan Y, Reichert J, Ripke S, Sandin S, Sklar P, Svantesson O, Reichenberg A, Hultman CM, Devlin B, Roeder K, Buxbaum JD. Most genetic risk for autism resides with common variation. Nat Genet. 2014 Aug;46(8):881-5. doi: 10.1038/ng.3039. Epub 2014 Jul 20. PMID: 25038753; PMCID: PMC4137411.

Goines PE, Croen LA, Braunschweig D, Yoshida CK, Grether J, Hansen R, Kharrazi M, Ashwood P, Van de Water J. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Mol Autism. 2011 Aug 2;2:13. doi: 10.1186/2040-2392-2-13. PMID: 21810230; PMCID: PMC3170586.

Gong T, Lundholm C, Rejnö G, Bölte S, Larsson H, D'Onofrio BM, Lichtenstein P, Almqvist C. Parental asthma and risk of autism spectrum disorder in offspring: A population and family-based case-control study. Clin Exp Allergy. 2019 Jun;49(6):883-891. doi: 10.1111/cea.13353. Epub 2019 Feb 27. PMID: 30742718; PMCID: PMC6849600.

Gruol D. L. (2015). IL-6 regulation of synaptic function in the CNS. *Neuropharmacology*, *96*(Pt A), 42–54. https://doi.org/10.1016/j.neuropharm.2014.10.023

Guo, Y., Zhang, H., Yang, J., Liu, S., Bing, L., Gao, J., & Hao, A. (2013). Granulocyte colony-stimulating factor improves alternative activation of microglia under microenvironment of spinal cord injury. *Neuroscience*, *238*, 1–10. https://doi.org/10.1016/j.neuroscience.2013.01.047

Han, V.X., Patel, S., Jones, H.F. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. *Transl Psychiatry* **11,** 71 (2021). https://doi.org/10.1038/s41398-021-01198-w

Hisle-Gorman, E., Susi, A., Stokes, T., Gorman, G., Erdie-Lalena, C., & Nylund, C. M. (2018). Prenatal, perinatal, and neonatal risk factors of autism spectrum disorder. *Pediatric research*, *84*(2), 190–198. https://doi.org/10.1038/pr.2018.23

Hsiao, E. Y., & Patterson, P. H. (2011). Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. *Brain, behavior, and immunity*, *25*(4), 604–615. https://doi.org/10.1016/j.bbi.2010.12.017

Hughes, H. K., Mills Ko, E., Rose, D., & Ashwood, P. (2018). Immune Dysfunction and Autoimmunity as Pathological Mechanisms in Autism Spectrum Disorders. *Frontiers in cellular neuroscience*, *12*, 405. https://doi.org/10.3389/fncel.2018.00405

Instanes JT, Halmøy A, Engeland A, Haavik J, Furu K, Klungsøyr K. Attention-Deficit/Hyperactivity Disorder in Offspring of Mothers With Inflammatory and Immune System Diseases. Biol Psychiatry. 2017 Mar 1;81(5):452-459. doi: 10.1016/j.biopsych.2015.11.024. Epub 2015 Dec 9. PMID: 26809250.

Jiang, H. Y., Xu, L. L., Shao, L., Xia, R. M., Yu, Z. H., Ling, Z. X., Yang, F., Deng, M., & Ruan, B. (2016). Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. *Brain, behavior, and immunity*, *58*, 165–172. https://doi.org/10.1016/j.bbi.2016.06.005

Jones KL, Croen LA, Yoshida CK, Heuer L, Hansen R, Zerbo O, Ashwood P,Van de Water J (2017). Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. *Molecular Psychiatry*, 22, 273–279. doi:10.1038/mp.2016.77

Kim, Y. S., Leventhal, B. L., Koh, Y. J., Fombonne, E., Laska, E., Lim, E. C., Cheon, K. A., Kim, S. J., Kim, Y. K., Lee, H., Song, D. H., & Grinker, R. R. (2011). Prevalence of autism spectrum disorders in a total population sample. *The American journal of psychiatry*, *168*(9), 904–912. https://doi.org/10.1176/appi.ajp.2011.10101532

Krakowiak P, Goines PE, Tancredi DJ, Ashwood P, Hansen RL, Hertz-Picciotto I, & Van de Water J (2017). Neonatal cytokine profiles associated with autism spectrum disorder. *Biological Psychiatry*, 81, 442–445. doi:10.1016/j.biopsych.2015.08.00

Lambertsen, K. L., Clausen, B. H., Babcock, A. A., Gregersen, R., Fenger, C., Nielsen, H. H., Haugaard, L. S., Wirenfeldt, M., Nielsen, M., Dagnaes-Hansen, F., Bluethmann, H., Faergeman, N. J., Meldgaard, M., Deierborg, T., & Finsen, B. (2009). Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(5), 1319–1330. https://doi.org/10.1523/JNEUROSCI.5505-08.2009

Lenz, K. M., & Nelson, L. H. (2018). Microglia and Beyond: Innate Immune Cells As Regulators of Brain Development and Behavioral Function. *Frontiers in immunology*, *9*, 698. https://doi.org/10.3389/fimmu.2018.00698

Li, R., Ma, X., Wang, G., Yang, J., & Wang, C. (2016). Why sex differences in schizophrenia?. *Journal of translational neuroscience*, *1*(1), 37–42.

Li, X., Chauhan, A., Sheikh, A. M., Patil, S., Chauhan, V., Li, X. M., Ji, L., Brown, T., & Malik, M. (2009). Elevated immune response in the brain of autistic patients. *Journal of neuroimmunology*, *207*(1-2), 111–116. https://doi.org/10.1016/j.jneuroim.2008.12.002

Liu, Y. J., Spangenberg, E. E., Tang, B., Holmes, T. C., Green, K. N., & Xu, X. (2021). Microglia Elimination Increases Neural Circuit Connectivity and Activity in Adult Mouse Cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *41*(6), 1274–1287. https://doi.org/10.1523/JNEUROSCI.2140-20.2020

Liu, X., Dalsgaard, S., Munk-Olsen, T., Li, J., Wright, R. J., & Momen, N. C. (2019). Parental asthma occurrence, exacerbations and risk of attention-deficit/hyperactivity disorder. *Brain, behavior, and immunity*, *82*, 302–308. https://doi.org/10.1016/j.bbi.2019.08.198

Lyall, K., Ashwood, P., Van de Water, J., & Hertz-Picciotto, I. (2014). Maternal immune-mediated conditions, autism spectrum disorders, and developmental delay. *Journal of autism and developmental disorders*, *44*(7), 1546–1555. https://doi.org/10.1007/s10803-013-2017-2

Maenner MJ, Shaw KA, Bakian AV, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018. MMWR Surveill Summ 2021;70(No. SS-11):1–16. DOI: http://dx.doi.org/10.15585/mmwr.ss7011a1

Maximo, J. O., Cadena, E. J., & Kana, R. K. (2014). The implications of brain connectivity in the neuropsychology of autism. *Neuropsychology review*, *24*(1), 16–31. https://doi.org/10.1007/s11065-014-9250-0

Meakin, A. S., Saif, Z., Jones, A. R., Aviles, P., & Clifton, V. L. (2017). Review: Placental adaptations to the presence of maternal asthma during pregnancy. *Placenta*, *54*, 17–23. https://doi.org/10.1016/j.placenta.2017.01.123

Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, Yee BK, Feldon J. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. *J Neurosci.* 2006;26:4752–4762

Meyer U, Nyffeler M, Schwendener S, Knuesel I, Yee BK, Feldon J. Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge. *Neuropsychopharmacology.* 2008;33:441–456.

Mills, B. D., Pearce, H. L., Khan, O., Jarrett, B. R., Fair, D. A., & Lahvis, G. P. (2016). Prenatal domoic acid exposure disrupts mouse pro-social behavior and functional connectivity MRI. *Behavioural brain research*, *308*, 14–23. https://doi.org/10.1016/j.bbr.2016.03.039

Murphy, V. E., Gibson, P., Talbot, P. I., & Clifton, V. L. (2005). Severe asthma exacerbations during pregnancy. *Obstetrics and gynecology*, *106*(5 Pt 1), 1046–1054. https://doi.org/10.1097/01.AOG.0000185281.21716.02

Murphy, V. E., Clifton, V. L., & Gibson, P. G. (2006). Asthma exacerbations during pregnancy: incidence and association with adverse pregnancy outcomes. *Thorax*, *61*(2), 169–176. https://doi.org/10.1136/thx.2005.049718

Patel, S., Dale, R. C., Rose, D., Heath, B., Nordahl, C. W., Rogers, S., Guastella, A. J., & Ashwood, P. (2020). Maternal immune conditions are increased in males with autism spectrum disorders and are associated with behavioural and emotional but not cognitive co-morbidity. *Translational psychiatry*, *10*(1), 286. https://doi.org/10.1038/s41398-020-00976-2

Polyak, A., Rosenfeld, J. A., & Girirajan, S. (2015). An assessment of sex bias in neurodevelopmental disorders. *Genome medicine*, *7*(1), 94. https://doi.org/10.1186/s13073-015-0216-5

Salmeron, K., Aihara, T., Redondo-Castro, E., Pinteaux, E., & Bix, G. (2016). IL-1alpha induces angiogenesis in brain endothelial cells in vitro: implications for brain angiogenesis after acute injury. *Journal of neurochemistry*, *136*(3), 573–580. https://doi.org/10.1111/jnc.13422

Salmeron, K. E., Maniskas, M. E., Edwards, D. N., Wong, R., Rajkovic, I., Trout, A., Rahman, A. A., Hamilton, S., Fraser, J. F., Pinteaux, E., & Bix, G. J. (2019). Interleukin 1 alpha administration is neuroprotective and neuro-restorative following experimental ischemic stroke. *Journal of neuroinflammation*, *16*(1), 222. https://doi.org/10.1186/s12974-019-1599-9

Schneider A., Kruger C., Steigleder T., Weber D., Pitzer C., Laage R., et al. . (2005). The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. *J. Clin. Invest.* 115, 2083–2098. 10.1172/JCI23559

Schwartzer JJ, Careaga M, Chang C, Onore CE, Ashwood P. 2015. Allergic fetal priming leads to developmental, behavioral and neurobiological changes in mice. *Transl Psychiatry* 5:e543.

Schwartzer JJ, Careaga M, Coburn MA, Rose DR, Hughes HK, Ashwood P. Behavioral impact of maternal allergic-asthma in two genetically distinct mouse strains. Brain Behav Immun. 2017 Jul;63:99-107. doi: 10.1016/j.bbi.2016.09.007. Epub 2016 Sep 10. PMID: 27622677; PMCID: PMC5346064.

Smith, S. E., Li, J., Garbett, K., Mirnics, K., & Patterson, P. H. (2007). Maternal immune activation alters fetal brain development through interleukin-6. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *27*(40), 10695–10702. https://doi.org/10.1523/JNEUROSCI.2178-07.2007

Vogel Ciernia A, Careaga M, LaSalle JM, Ashwood P. Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. Glia. 2018 Mar;66(3):505-521. doi: 10.1002/glia.23261. Epub 2017 Nov 14. PMID: 29134693; PMCID: PMC5767155.

Wei, H., Zou, H., Sheikh, A. M., Malik, M., Dobkin, C., Brown, W. T., & Li, X. (2011). IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. *Journal of neuroinflammation*, *8*, 52. https://doi.org/10.1186/1742-2094-8-52

Werling, D. M., Parikshak, N. N., & Geschwind, D. H. (2016). Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders. *Nature communications*, *7*, 10717. https://doi.org/10.1038/ncomms10717

Zaretsky, M. V., Alexander, J. M., Byrd, W., & Bawdon, R. E. (2004). Transfer of inflammatory cytokines across the placenta. *Obstetrics and gynecology*, *103*(3), 546–550. https://doi.org/10.1097/01.AOG.0000114980.40445.83

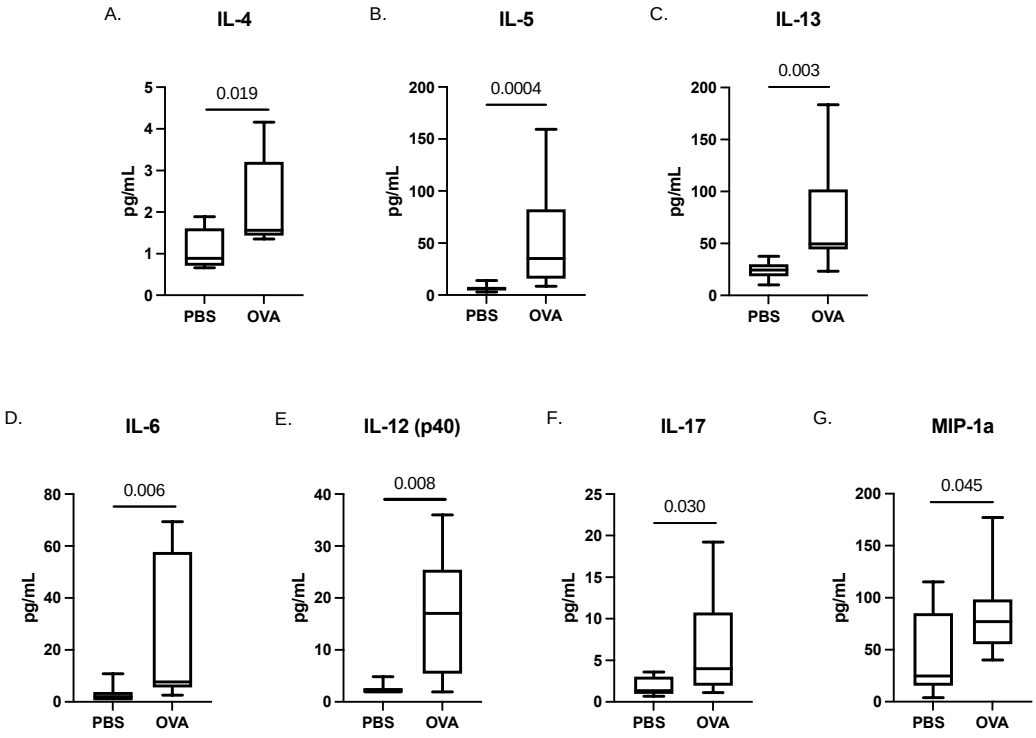

Figure. 1. Cytokine concentrations in maternal serum of dams exposed to PBS and OVA taken at GD 17.5. Serum was collected prior to fetal offspring and placenta collections. Cytokine levels were assessed using multiplex bead-based immunoassay. (A) IL-4, (B) IL-5, (C) IL-13, (D) IL-6, (E) IL-12 (p40), (F) IL-17, (G) MIP-1a are represented as pg/mL after being normalized to total protein content. * $P < 0.05$ compared to sex and timing matched control as determined by t-test followed by Mann-Whitney. PBS dams n=8, OVA dams n=10.

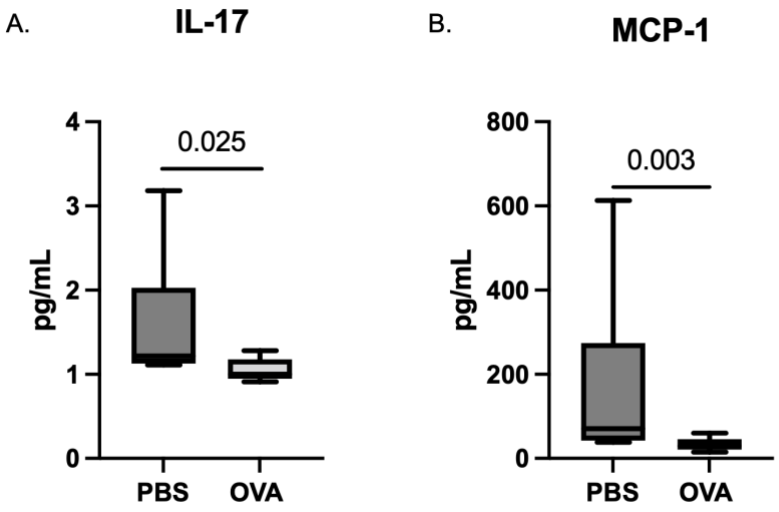

Figure 2. Cytokine concentrations in female placentas taken from dams exposed to PBS and OVA at GD 17.5 after final allergic asthma challenge. Cytokine levels were assessed using multiplex bead-based immunoassay. The concentrations of (A) IL-17 and (B) MCP-1 are represented as pg/mL after being normalized to total protein content. * $p < 0.05$ compared to sex and timing-matched control as determined by t-test followed by Mann-Whitney.

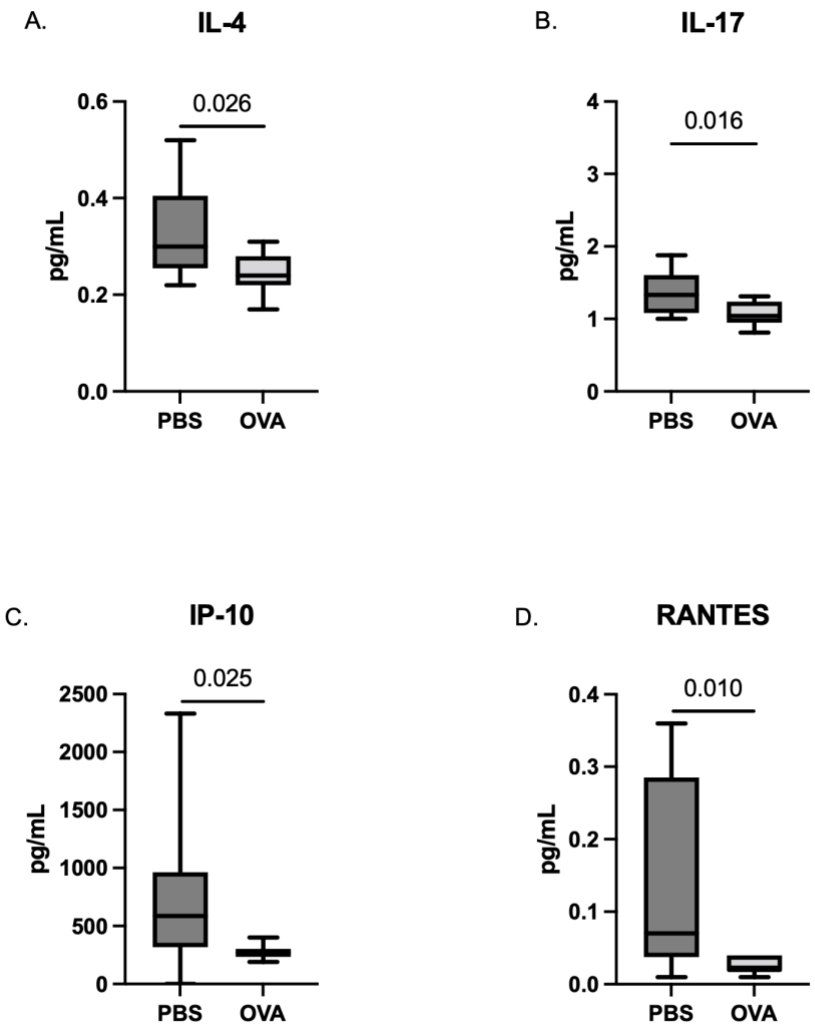

Figure 3. Cytokine concentrations in male placentas taken from dams exposed to PBS and OVA at GD 17.5 after final allergic asthma challenge. Cytokine levels were assessed using multiplex bead-based immunoassay. The concentrations of (A) IL-4, IL-17 (B), (C) IP-10, and (D) RANTES are represented as pg/mL after being normalized to total protein content. * $p < 0.05$ compared to sex and timing-matched control as determined by t-test followed by Mann-Whitney.

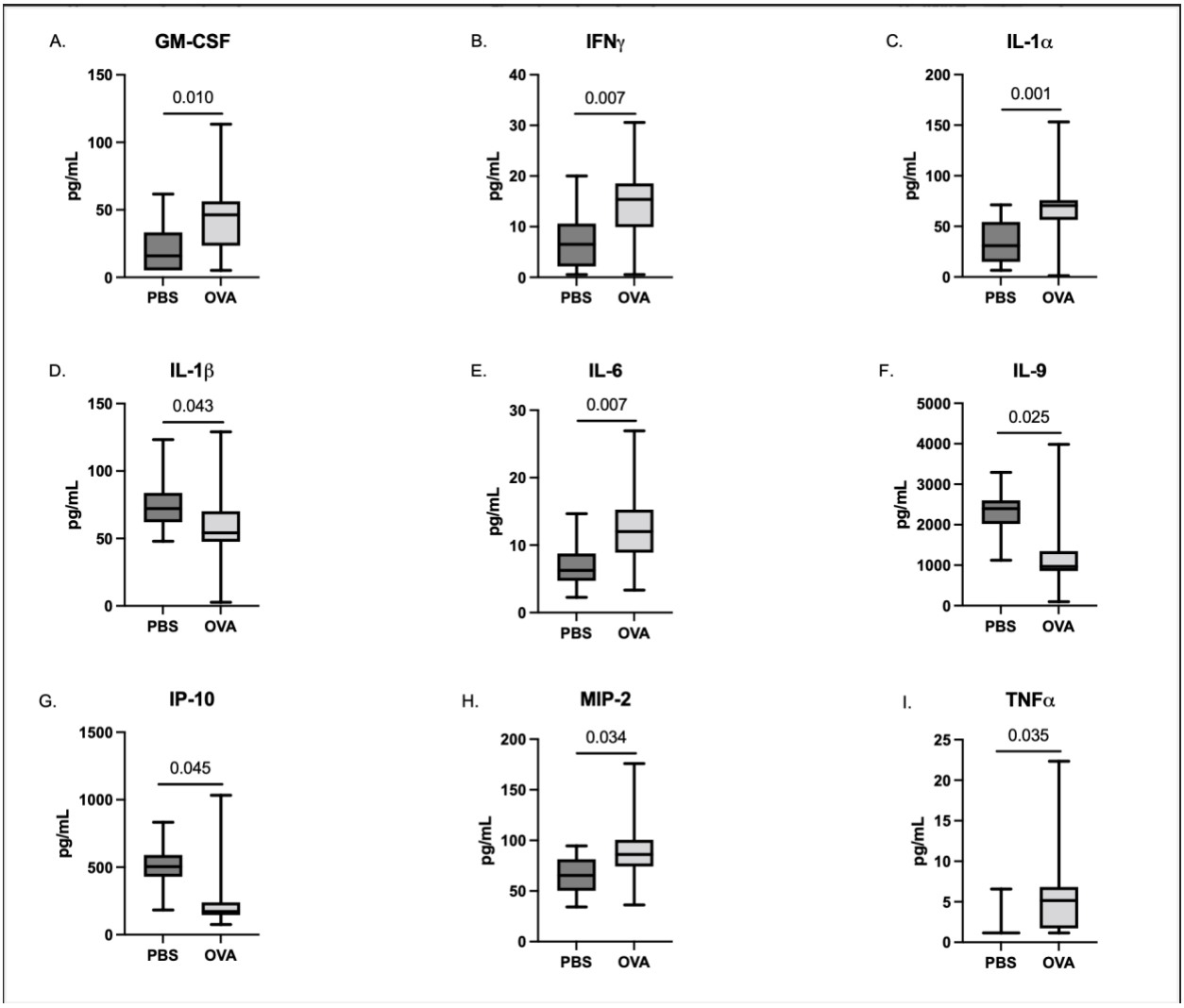

Figure 4. Cytokine concentrations in the male fetal brain (whole brain homogenates) taken at E17.5 following final maternal asthma challenge. Concentrations of cytokines from male and female offspring of OVA and PBS-exposed dams were assessed using multiplex bead-based immunoassay. The concentrations of (A) GM-CSF, (B) IFNγ, (C) IL-1α, (D) IL-1β, (E) IL-6, (F) IL-9, (G) IP-10, (H) MIP-2, and (I) TNFα are represented as pg/mL after being normalized to total protein content. * $p < 0.05$ compared to sex and timing-matched control as determined by multilevel mixed-effects modeling.

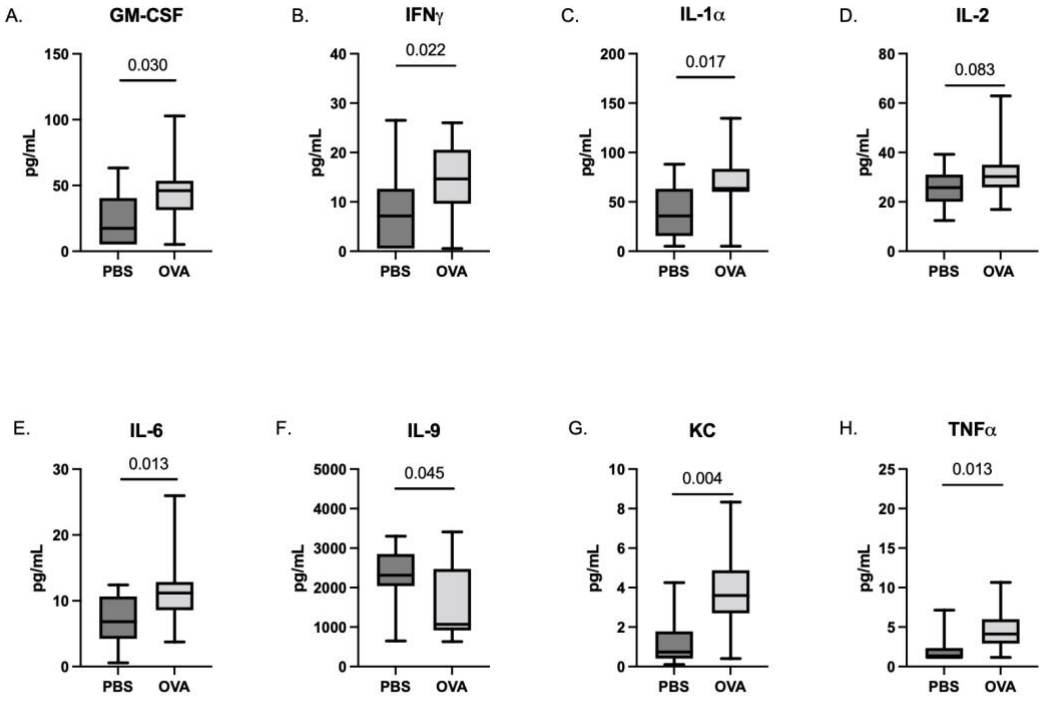

Figure 5. Cytokine concentrations in the female fetal brain (whole brain homogenates) taken at E17.5 following final maternal asthma challenge. Concentrations of cytokines from male and female offspring of OVA and PBS-exposed dams were assessed using multiplex bead-based immunoassay. The concentrations of (A) GM-CSF, (B) IFNγ, (C) IL-1α, (D) IL-2, (E) IL-6, (F) IL-9, (G) KC, (H) TNFα are represented as pg/mL after being normalized to total protein content. * $p < 0.05$ compared to sex and timing-matched control as determined by multilevel mixed-effects modeling.

Chapter 3: Characterizing the Neuroimmune Environment of Offspring in a Novel Model of Maternal Allergic Asthma and Particulate Matter Exposure

Abstract:

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by the presence of decreased social interactions and an increase in stereotyped and repetitive behaviors. Epidemiology studies suggests that cases of ASD are on the rise, with 1 in 44 children born in the United States later being diagnosed with ASD (Maenner et al., 2021). Similar to the rising incidence of ASD, rates of asthma are currently on the rise and studies have identified that the presence of asthma increases likelihood of birthing a child that is later diagnosed with ASD. Particulate matter (PM), via air pollution, is another environmental factor that is suspected to be associated with risk of neuropsychiatric disorders, including ASD, and exposure to PM is also known to worsen symptoms of asthma. Despite the links between asthma and PM to neuropsychiatric disorders, and the link between PM and worsening asthma symptoms, there is a lack of laboratory models investigating combined prenatal exposure to asthma and PM on offspring neurodevelopment. To begin addressing this gap in the research, we developed a novel mouse model that combines exposure to maternal allergic asthma (MAA) and ultrafine iron-soot (UIS), a common component of PM. Our findings add to our previous data on MAA, but also demonstrate novel findings on prenatal exposure of UIS alone, and MAA in combination with UIS (MAA-UIS), on offspring neurodevelopment. In the current study, female BALB/c mice were primed for allergic asthma with ovalbumin (OVA) prior to pregnancy. Following mating, and beginning on gestational day 2 (G2), dams were exposed to either aerosolized OVA or phosphate buffered saline (PBS) for 1 hour, depending on treatment group. Following the 1-hour exposure, pregnant females were then exposed to an aerosol of ultrafine iron-soot, or clean air

for controls, for 4 hours. Offspring brains from these treated dams were collected at postnatal days (P)15 and (P)35. Cortices and hippocampal regions were then isolated and assessed for changes in cytokines using a Luminex bead-based multiplex assay. Analyses identified changes in many cytokines across treatment groups at both timepoints in the cortex, including IL-1β, IL-2, IL-13, and IL-17, which remained elevated from P15 to P35 in all conditions. In the hippocampus at P15, elevations in cytokines were also identified across the treatment groups, namely IFNγ and IL-7. However, unlike the cortical measurements, the elevations in cytokines found in the hippocampus were only identified at P15 and did not persist into early adulthood. We also show that the combination of MAA and UIS exposure (MAA-UIS) during pregnancy resulted in an increase in microglia density in the hippocampus of offspring, as identified by IBA-1 staining. Together, these data indicate that MAA and UIS exposure alone, and the combination of these environmental stimuli in the MAA-UIS exposure group, result in changes in the neuroimmune environment of offspring that persist from adolescence into adulthood.

1.0 Introduction:

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by the presence of decreased social interactions and an increase in stereotyped and repetitive behaviors. Epidemiology studies suggests that cases of ASD are on the rise, with 1 in 44 children born in the United States later being diagnosed with ASD (Maenner et al., 2021), and ASD appears to disproportionately impact boys over girls at a 1:4 ratio (Kim et al., 2011; Werling et al., 2016). There does not appear to be a single cause for ASD, but research has shown that the etiology of some cases can be connected to heritable susceptibility from both genetic and environment sources. Depending on the study, genetic heritability can account for more than 50% of cases. However, having the susceptibility genes does not guarantee that a child will be diagnosed with ASD. In other words, many of the associated risk genes for ASD are common genetic variants in the population and carry a low risk (Gaugler et al., 2014). Given that genetic risk factors have only a moderate contribution to the observed cases of ASD (Devlin et al., 2011; Klei et al., 2012), it is important to identify environmental risk factors that can contribute to ASD incidence. Mounting clinical and epidemiological data underscore the notion that environmental factors play a large role in both the underlying development of ASD and their impact on the severity of individual behavioral characteristics (Hughes et al., 2018; Rossignol et al., 2014; Straughen et al., 2021; Raz et al., 2015; Volk et al., 2011; Volk et al., 2020; Patterson et al., 2009). Pregnancy, in particular, marks a critical period when environmental insults have long lasting effects on neurodevelopment.

The maternal immune system represents a key biological mechanism that links environmental factors to specific neurodevelopmental changes and later ASD diagnosis. For

example, clinical studies have linked hospitalizations for bacterial and viral infections during pregnancy to an increased rate of birthing a child that will later be diagnosed with ASD (Modabbernia et al., 2017; Tioleco et al., 2021; Atladóttir et al., 2010; Brown et al., 2014). Moreover, laboratories have developed animal models that often use bacterial or viral mimics, such as LPS or Poly(I:C) respectively, to induce maternal inflammation during pregnancy and demonstrate a causal change in offspring brain and behavioral health (Han et al., 2021). Despite the efficacy of these models in demonstrating a link between maternal immune signaling and offspring neurodevelopment, they are limited in representing many of the common environmental sources of immune activation. With perhaps the exception of SARS COV-19 in the recent years, hospitalizations for viral infections are less frequent, and a recent meta-analysis of viral infections during pregnancy as a risk for children later being diagnosed with ASD was not supported (Jiang et al., 2016). Importantly, infections via bacterial and viral pathogens are only two of many ways in which the immune system can become activated, and researchers have begun to investigate other common environmental influences that can have a substantial impact on the immune system and offspring brain development, most notably asthma.

Rates of asthma, like ASD, are currently on the rise and represent a highly prevalent chronic disease that more commonly impact ethnic/racial minority and socioeconomically disadvantaged groups in the US (Clougherty et al., 2007; Matsui et al., 2008). Importantly, chances of asthma exacerbations increase during pregnancy, and presence of asthma increases likelihood of birthing a child that is later diagnosed with ASD (Ali & Ulrik, 2013; Murphy, et al., 2005; Murphy et al., 2006; Croen et al., 2005; Croen et al., 2019; Gong et al., 2019; Abdallah et al., 2011; Hisle-Gorman et al., 2018; Lyall et al., 2014; Patel et al., 2020; Fasmer et al., 2011).

Asthma is an inflammatory disease of the lungs characterized by bronchial hyperresponsiveness, persistent inflammation, airflow obstruction and reduction of airflow (Gluck et al., 2006; Popa et al., 2021). The allergens associated with asthma can vary and include, but are not limited to rodent allergen, cockroach allergen, pollen, and house dust mite (Matsui et al., 2008; Jacquet 2013). Given the incidence of allergies and asthma in the United States (Blackwell et al., 2014; Fazel et al., 2018), and the high prevalence of allergic triggers in urban environments, including cockroach and mouse allergens, pollutants, and psychosocial stress (Leaderer et al., 2002; Matsui et al., 2008; Salo et al., 2008; Claeson et al., 2016), there is pressing need to better understand the causal effects of asthma during pregnancy and the impact of specific allergic triggers on offspring mental health.

Using our mouse model of maternal allergic asthma (MAA) to initiate an immune response in pregnant mice, we have previously reported systemic elevations in IL-4 and IL-5 in dams (Schwartzer et al., 2015; Tamayo et al., 2022) that parallel clinical reports associating increased IL-4 and IL-5 in mid-pregnancy maternal serum samples of mothers with children later diagnosed with ASD (Goines et al 2011). Not only do the dams in our MAA model produce the same allergic asthma cytokine profile observed in humans, but the offspring display characteristic ASD-like behaviors, such as decreased social interaction and increased repetitive-like behaviors (Schwartzer et al., 2015; Schwartzer et al., 2017; Church et al., 2021). Moreover, MAA produces transcriptional differences in microglia gene expression and neuroinflammation in both prenatal offspring and brain regions of adult mice (Ciernia et al., 2018; Church et al., 2021; Tamayo et al., 2022). These findings highlight the lasting changes that allergic asthma during pregnancy can have on offspring neurodevelopment. However, environmental insults

associated with ASD can vary, and exposure to these environmental factors do not necessarily occur in isolation for humans. In fact, individuals often encounter multiple inflammatory-inducing environmental stimulants simultaneously, and less is known about the potential synergistic effects of these exposures on maternal immune response and subsequent offspring development (Leaderer et al., 2002; Salo et al., 2008; Claeson et al., 2016).

Particulate matter (PM), via air pollution, is another environmental factor that is suspected to be associated with risk of neuropsychiatric disorders such as schizophrenia, attention deficit hyperactive disorder (ADHD), and ASD (Volk et al., 2011; Volk et al., 2013; Volk et al., 2020). Not only is PM linked to neurodevelopmental disruptions when exposures occur during pregnancy (Church et al 2018), it has also been tied to exacerbated asthma responses and could potentially cause new onset cases of asthma (Guarnieri & Balmes, 2014), and this is especially true for ultrafine PM (PM0.1; aerodynamic diameter < 0.1 μm) (Chan et al., 2011; Schraufnagel 2020; Chalupa et al., 2004). PM is a complex mixture of constituents that contains organic compounds, soot, metals and metal-oxides, nitrates, and other elements in varying quantities depending on its source (Steiner et al., 2016). As such, it is necessary to characterize individual components of PM in order to effectively regulate air quality for limiting environmental exposure to toxicants. Diesel is one common source of PM that has been identified as a risk factor for ASD (Kalkbrenner et al., 2015; Volk et al., 2011; Volk et al., 2013; Volk et al., 2020; von Ehrenstein et al., 2014; Roberts et al., 2013). Combustion-derived diesel exhaust includes soot particles containing elemental carbon as well as iron which is the most common transition metal found in PM (Zhou et al., 2003). Iron in PM can occur from fuel additives and as a product of normal engine wear (Mayer et al., 2010; Steiner et al., 2016), and

iron-soot (IS) exposure has previously been shown to cause oxidative lung injury, induce inflammation of the lungs, and can cross the blood brain barrier through nasal inhalation (Zhong et al., 2010; Zhou et al., 2003; Hopkins et al., 2018). These links to worsening of asthma symptoms and increased risk of neuropsychiatric disorders in offspring make PM exposure during pregnancy an important area that needs investigating.

There are many human studies and animal models investigating the links between gestational exposure to PM and neurodevelopmental outcomes in offspring (Volk et al., 2011; Volk et al., 2013; Volk et al., 2020; Bolton et al., 2017; Ahadullah et al., 2021), and also many investigations into the impacts of asthma as a risk factor for ASD, including our own (Schwartzer et al., 2015; Schwartzer et al., 2017; Ciernia et al., 2018; Church et al., 2021; Tamayo et al., 2022; Ali & Ulrik, 2013; Murphy, et al., 2005; Murphy et al., 2006; Croen et al., 2005; Croen et al., 2019; Gong et al., 2019; Abdallah et al., 2011; Hisle-Gorman et al., 2018; Lyall et al., 2013; Patel et al., 2020; Fasmer et al., 2011). However, these investigations consider asthma or PM only as independent risk factors for developing neuropsychiatric disorders. Despite the links between allergic asthma with ASD, and the link between PM and ASD, there are no studies investigating the neuroimmune outcome on offspring of these environmental factors in conjunction. This is an apparent oversight given that PM exposure can worsen symptoms of asthma (Guarnieri & Balmes, 2014), and the source of environmental factors contributing to ASD are likely multifaceted. In this study, we hypothesized that when mice are exposed to MAA and ultrafine iron-soot (UIS) particles during pregnancy (MAA-UIS), the neuroimmune outcomes will show heightened inflammation in offspring compared to that of MAA or UIS exposure alone. We also suspected that, because microglia can respond to changes

in the neuroimmune environment through proliferation and are suspected to be associated with ASD behaviors (Hughes et al., 2020; Davoli-Ferreira et al., 2021), we would see signs of functional differences in the frontal cortex and hippocampus. Two brain regions implicated as being impacted developmentally in neuropsychiatric disorders such as ASD (Ecker 2017; Hughes et al., 2020; Richards et al., 2020; Banker et al., 2021). Using our established mouse paradigm of maternal aerosol exposure to study offspring outcomes, we demonstrate that MAA and UIS alone, or MAA-UIS combined, alter the neuroimmune profile in the brains of offspring that is sustained from adolescence into early adulthood.

2.0 Methods:

2.1 Animals

BALB/c male and female mice were obtained from breeding pairs originally purchased from Envigo Laboratories (Livermore, CA, USA) and maintained at University of California, Davis at the Center for Health and Environment, Davis, CA. Mice were housed with same-sex littermates and kept at ambient room temperature (23°C) on a 12 h light/dark cycle (lights on at 0800 h) within ventilated cages with water and food provided ad libitum. All procedures were performed with approval by University of California Davis Institutional Animal Care and Use Committee and according to guidelines established by National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2 Maternal allergic asthma and ultra-fine soot particle exposure

Female BALB/c (n= 8/group) mice were sensitized with 10ug of ovalbumin (OVA, Sigma, St. Louis, MO, USA) and 1 mg (Al) OH3 dissolved in 200ul of phosphate buffered saline (PBS) injected intraperitoneally at 7 and 8 weeks of age. Control dams were injected with PBS vehicle alone. Dams were then mated with age matched males and checked daily for the presence of a seminal plug which was noted as gestational day (GD) 0.5. Beginning on gestational day 2 (G2), dams were exposed to either aerosolized OVA or phosphate buffered saline (PBS) for 1 hour, depending on treatment group. Following the 1-hour exposure, mice were placed in a 20 cm x 43 cm x 18cm polycarbonate whole-body chamber for exposure to an aerosol of ultrafine iron-soot (target concentration of 200 ug/m^3), including 40 ug/m^3 of iron-oxide nanoparticles, or sham control (AIR). The total iron-soot generated was cooled and diluted with filtered air to achieve

the desired concentration prior to reaching the exposure chambers. Mice were exposed for 4 hours/day on G2, G4, G6, G9, G11, G13, G16, and G18 to PBS or OVA [MAA condition] and Air or ultrafine iron-soot [UIS condition], resulting in a total of four treatment groups: PBS/AIR (PBS-AIR), MAA/AIR (MAA), PBS/UIS (UIS), MAA/UIS (MAA-UIS). Following the last day of aerosol exposure (G18) pregnant mice were left undisturbed through parturition and offspring were either sacrificed at postnatal day (P)15 or weaned at P21, housed with same-sex littermates, and sacrificed at P35 for brain tissue analysis.

2.3 Generation and characterization of particles

Ultrafine iron-soot particles were generated as previously described by Hopkins et al. (Hopkins et al., 2018). Briefly, particle generation was obtained using a laminar diffusion flame system by mixing ethylene gas, the primary hydrocarbon fuel, and acetylene gas to compensate for the effect of iron-oxide to suppress soot formation. By reaching vapor phase of iron pentacarbonyl by warming to 20°C with combusted argon carrier gas (all Sigma-Aldrich Chemical Co., St. Louis, MO) in the presence of an ethylene/acetylene vapor mix, the source of iron was generated. The result of these combusted reactants generated a hetero-disperse aerosol of ultrafine iron oxide particles (Fe_2O_3) and associated soot. Further details of the system and particle generation can be found (Jasinski et al., 2006, Pinkerton et al., 2008; Hopkins et al., 2018). In order to simulate unhealthy air quality conditions, an average particle concentration of 200 $\mu g/m^3$ was selected, as this reflects a concentration that is reminiscent of heavy pollution days seen with many poor air quality days in many parts of the world, including China (Pui et al., 2014, Connor, 2015).

2.4 Cardiac perfusion and brain tissue collections

At postnatal days 15 and 35, offspring were collected from their home cages, anesthetized using isoflurane (2-4% inhalation) and underwent transcardial perfusion. Briefly, a lateral incision was made in the abdominal wall below the rib cage. With curved scissors, an incision was made in the diaphragm and cuts were made along the ribs to the collarbone to allow the sternum to be lifted. Once exposed, the heart was inserted with a 15-gauge perfusion needle into the ascending aorta for entry of perfuse, and an incision was made into the right atrium to create an outlet for drainage. Using a perfusion pump, 20 mL of PBS was slowly pumped through the circulatory system to reach adequate clearing. Whole brains were removed and dissected into hemispheric halves and one half was further dissected into cortical and hippocampal regions, flash frozen with liquid nitrogen and stored at -80°C for later use. The remaining half was placed in 4% PFA for fixation, 24 hours following this, the halves were then placed in 30% sucrose for 24 hours for cryoprotection. Cryoprotected tissues were then embedded in optimal cutting temperature (O.C.T.) media and frozen at -80°C.

2.5 Tissue sectioning and staining

Frozen tissue embedded in O.C.T. was sectioned with a Leica Instruments cryostat at 20 µm. Free-floating tissue sections were stored in PBS containing 0.01% sodium azide. Sections were incubated in 1:1000 rabbit-anti IBA-1 (Wako, Neuss, Germany) with 10% normal goat serum (NGS) and 0.2% triton X-100 at 4°C for 24 hours, followed by 1 hour incubation with goat-anti rabbit biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) in 5% NGS for 1 hour at room temperature. Tissue sections were then incubated with avidin-biotinylated HRP complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) at room temperature.

Visualization of labeling was conducted using 3,3’ -diaminobenzidine (DAB) solution in the presence of peroxidase (HRP) enzyme. All sections were thoroughly rinsed 3 times with 1xPBS between staining steps. Sections were mounted onto glass Superfrost Plus microscope slides and cover slipped with VectaMount Permanent Mounting Medium. Once dry, 20x images were taken on a brightfield microscope and stitched together using Photoshop version 22.3 (Adobe Inc., San Jose, CA, 2023). A macros script in ImageJ version 1.53 (U.S. National Institutes of Health, Bethesda Maryland, 2022) was used to quantify microglia in order to limit user bias.

2.6 Stereology

IBA-1 positive microglia were identified using stereological methods. IBA-1 cell counts were made on brightfield microscope (Nikon Eclipse Ci, Nikon, Tokyo) at 20x magnification and images were taken using NIS Elements v.4.0 (Nikon Instruments Inc. 1300 Walt Whitman Road Melville, NY 11747-3064, U.S.A.). Image analysis was performed using a macros script in ImageJ version 1.53 (U.S. National Institutes of Health, Bethesda Maryland, 2022) to quantify microglia. A total of 3-9 sections per brain region were collected. Microglia cell counts were taken from infralimbic, and anterior cingulate cortical areas of the frontal cortex, and the dentate gyrus, CA1, CA2, and CA3 of the hippocampus. Microglia were identified by IBA-1 positive cell body staining.

2.7 Multiplex Bead-Based Cytokine Analysis

Analysis of serum cytokines was performed using a multiplex mouse 25-plex bead immunoassay (Milliplex Mouse Cytokine/Chemokine Magnetic Bead Panel #MCYTMAG70PMX25BK). The following cytokines were quantified: G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5,

IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, KC, MCP-1, MIP-1α, MIP-1β, MIP-2, RANTES, and TNF-α. Standards and reagents were all prepared according to the manufacturers' recommendations. Each brain sample was diluted to a standardized concentration and run in duplicate. Twenty-five microliters of sample, standards, or blanks were loaded into a 96-well plate with appropriate amounts of assay buffer and matrix solution. The plate was then incubated overnight with antibody-coupled magnetic beads. The following day, after a series of washes, the plate was incubated with biotinylated detection antibody on a shaker for 30 min. Washes were performed using a Bio-Plex handheld magnet (Bio-Rad Laboratories, Hercules, CA, USA). After the final wash, the plate was analyzed using a Bio-Rad Bio-Plex 200 plate reader (Bio-Rad Laboratories, Hercules, CA, USA). The following were the minimal amounts of detectable cytokine concentration: G-CSF: 1.7 pg/mL; GM-CSF: 10.9 pg/mL; IFNγ: 1.1 pg/mL; IL-1α: 10.3 pg/mL; IL-1β: 5.4 pg/mL; IL-2: 1.0 pg/mL; IL-4: 0.4 pg/mL; IL-5: 1.0 pg/mL; IL-6: 1.1 pg/mL; IL-7: 1.4 pg/mL; IL-9: 17.3 pg/mL; IL-10: 2.0 pg/mL; IL-12 (p40): 3.9 pg/mL; IL-12 (p70): 4.8 pg/mL; IL-13: 7.8 pg/mL; IL-15: 7.4 pg/mL; IL-17: 0.5 pg/mL; IP-10: 0.8 pg/mL; KC: 2.3 pg/mL; MCP-1: 6.7 pg/mL; MIP-1α: 7.7 pg/mL; MIP-1β: 11.9 pg/mL; MIP-2: 30.6 pg/mL; RANTES: 2.7 pg/mL; TNF-α: 2.3 pg/mL. Sample concentrations that fell below minimal detection value were given a proxy value of half the limit of detection for statistical comparisons.

2.8 Statistical analysis

Brain cytokine data were analyzed using linear-mixed effects modeling to control for the unexplained residual variance that could be originating from litter-to-litter variations due to the hierarchical data structure in which statistical independence of observation is violated (Lazic and

Essioux, 2013; Lazic et al., 2018, Brauer and Curtin, 2018; Schielzeth and Nakagawa, 2013; Wainwright et al., 2007). Models were built with the lme package of R version 4.2.2 using forward a froward-stepwise approach. First a random-effects only model was constructed with litter set as the random effect. Then fixed effects for treatment (PBS or MAA; AIR or UI) and sex (male or female) were added followed by a full model containing both treatment, sex, and their interaction. Model fit was assessed using the likelihood ratios test and the best model was selected based on the Akaike Information Criterion (AIC). For models with significant interactions, fixed effects were further analyzed using pairwise comparisons of estimated marginal means.

3.0 Results:

3.1 P15 offspring cortex cytokines

Offspring brains were collected and micro-dissected into cortical and hippocampal sections at P15. Homogenates of each section were then analyzed for cytokine concentration. Multilevel mixed-effects modeling was used to control for within-litter variability and inclusions of fixed effects was determined using forward stepwise regression. For all cytokines measured, the inclusion of sex-difference did not significantly improve model fit (Supplementary Tables). In the cortex, several cytokines were elevated in both male and female offspring of MAA, UIS, and MAA-UIS dams, many of which are generally considered inflammatory and potentially neurotoxic with prolonged exposure. Among these, three cytokines (IL-1β, IL-2, IL-17) were found to be significantly elevated in all treatment groups. For example, exposure to MAA, UIS, and the combination resulted in an average of two to three fold increase of IL-1β in the cortex, MAA ($p = 0.004$), UIS ($p < 0.001$), and MAA-UIS ($p < 0.001$), compared to age-matched PBS-AIR controls (see supplementary tables for additional statistics information). Similarly, it was found that there were significant increases in IL-2 in the MAA ($p = 0.002$), UIS ($p = 0.001$), and MAA-UIS ($P < 0.001$) groups, along with increased IL-17 in MAA ($p = 0.001$), UIS ($p < 0.001$), and MAA-UIS ($p < 0.001$).

For several inflammatory markers, only the presence of UIS, with or without the addition of MAA, significantly altered cytokine concentrations in the cortex. Specifically, the combined exposure of MAA-UIS increased concentration of IL-13 ($p < 0.001$), IP-10 ($p = 0.049$), and MIP-1α ($p = 0.041$), and UIS alone resulting in an approximately 70 pg/mL ($p = 0.001$), 13 pg/mL ($p = 0.017$), and 65 pg/mL ($p = 0.004$) increase in IL-13 and IP-10, and MIP-1a

respectively. Conversely, MAA-UIS ($p = 0.041$) and UIS ($p = 0.030$) resulted in a decrease in IL-9 concentration by 425–456 pg/mL.

For IL-1α, a significant increase in cortical concentration was observed in MAA exposure alone ($p = 0.025$) and in combination with UIS ($p = 0.006$) compared to PBS-Air offspring, while no statistically significant differences were observed in the UIS-alone condition. Moreover, MAA ($p = 0.001$) and UIS ($p = 0.005$) both increased IL-10 concentration, but this increase was absent in the MAA-UIS group ($p = 0.122$). Of the cytokines investigated, IL-7 was the only immune marker elevated by MAA treatment ($p = 0.030$), but not UIS alone ($p = 0.822$), or MAA-UIS ($p = 0.699$). No sex-by-treatment interactions were identified in P15 offspring.

3.2 P35 offspring cortex cytokines

Littermates from the P15 cytokine investigation were left undisturbed until P35, at which time brains were collected and micro-dissected into cortical and hippocampal sections. Mixed-effects models that included sex were not significantly improved over treatment-alone models for all cytokines measured (Supplementary Tables). However, models that included treatment conditions revealed changes in several cytokines in the cortices of both sexes of MAA, UIS, and MAA-UIS offspring at P35 in a similar manner to what was identified in P15 offspring. Many of the cytokine changes observed at P15 remained altered in the P35 offspring cortex. For instance, it was revealed that there was an effect of MAA, UIS, and MAA-UIS combined on IL-2 and IL-17 in P35 offspring compared to PBS-Air control mice (IL-2: MAA $p = 0.006$, UIS $p = 0.043$, MAA-UIS $p = 0.001$; IL-17: MAA $p < 0.001$, UIS $p = 0.043$, MAA-UIS $p = 0.001$) (see supplementary tables for additional statistical information). Similar increases were also revealed

for IL-13 (MAA $p = 0.002$, UIS $p = 0.005$, MAA-UIS $p = 0.002$) and KC (MAA $p = 0.004$, UIS $p = 0.037$, MAA-UIS $p < 0.001$), and a trend for IL-1β (MAA $p = 0.046$, UIS $p = 0.008$, MAA-UIS $p = 0.063$).

For several cytokines, the individual contribution of UIS or MAA impacted specific inflammatory profiles. For example, UIS treatment alone ($p = 0.026$) and in combination MAA-UIS ($p = 0.002$) significantly increased concentrations of IL-1α, with a trend observed in offspring of the MAA alone condition ($p = 0.064$). Moreover, MAA treatment with UIS resulted in an increase in IL-9 ($p = 0.018$) and MIP-1α ($p < 0.001$) and these increases were also present in the MAA alone condition ($p = 0.015$ and $p = 0.033$, respectively) but not in the UIS only mice ($p > 0.1$). In addition to these MAA-induced elevations in IL-9, concomitant decreases in IL-4 concentrations were observed in MAA ($p = 0.028$) and MAA-UIS ($p = 0.015$). IL-4 was the only cytokine investigated in P35 offspring that was revealed to be decreased due to treatment with MAA but not UIS ($p = 0.088$). Of all the cytokines investigated, IP-10 concentration was the only identified cytokine to be increased by one treatment only (MAA $p = 0.010$), with neither UIS ($p = 0.435$), or MAA-UIS ($p = 0.694$), reaching significance.

3.3 P15 offspring hippocampus cytokines

Along with cortical sections, hippocampal homogenates from the same offspring brains were analyzed at P15 for cytokine concentrations. For all cytokines measured, the inclusion of sex in multi-level models did not significantly improve model fit (Supplementary Tables). Similar to what was uncovered in the respective cortical sections, several inflammatory cytokines were elevated in male and female offspring of MAA, UIS, MAA-UIS dams.

Interestingly, none of the cytokines investigated reached statistical significance in all three treatment groups. However, we suspect that this may be a result of a lack of statistical power given the strong trends observed across analytes. For instance, IL-7 concentrations were significantly increased in the hippocampus of MAA ($p = 0.009$) and MAA-UIS ($p < 0.001$) offspring, while the UIS group was did not reach the statistical threshold for significance ($p = 0.051$). In a similar manner, IFNγ was revealed to be elevated by UIS ($p = 0.008$) and MAA-UIS ($p = 0.004$), with similar elevations observed the MAA group ($p = 0.056$). MAA-UIS exposure was also revealed to have a treatment effect on IL-12(p40) ($p = 0.028$), as did UIS alone ($p = 0.028$), but these increases were not present in the hippocampus of offspring born from the MAA alone condition ($p = 0.151$). Concentrations of IL-17 in the offspring hippocampus were elevated at P15 in response to maternal UIS exposure alone ($p = 0.002$) and in combination MAA ($p = 0.051$). However, these increase in IL-17 were not detected in MAA-only offspring hippocampus ($p = 0.135$).

The chemokine CXCL1, referred to as KC, was only observed to be increased in the hippocampus of offspring who were exposed to the combination of MAA-UIS ($p = 0.044$), but not MAA alone ($p = 0.480$) or UIS alone ($p = 0.254$), suggesting a potential synergistic effect of the two environmental exposures. In contrast, several cytokines were elevated by MAA and UIS alone compared to the PBS-AIR control offspring, but these elevations did not reach statistical significance in the combined MAA-UIS condition. These increases included IL-1β (MAA $p = 0.047$, UIS $p = 0.038$), IL-2 (MAA $p = 0.026$, UIS $p = 0.002$), IL-13 (MAA $p = 0.034$, UIS $p = 0.011$), and RANTES (MAA $p = 0.041$, UIS p 0.034). Additionally, two cytokines were found to

be impacted by MAA alone compared to control, and not the UIS or MAA-UIS treatment groups. Specifically, IL-15 ($p = 0.009$) and MIP-1b ($p = 0.028$) were found to be elevated by MAA, but not UIS or MAA-UIS.

3.4 P35 offspring hippocampus cytokines

Hippocampal homogenates from P35 offspring were also assessed for cytokine differences, however, multilevel mixed-effects modeling did not reveal any significant effects of treatment or sex in on the concentration of all cytokines measured ($p > 0.05$, see Supplementary Materials).

3.5 Microglia density in the hippocampus and frontal cortex of MAA, UIS, and MAA-UIS of P15 Offspring

Microglia within a 554.7 µm by 1232.1 µm box spanning the anterior cingulate cortex, prelimbic area, and infralimbic area were counted using ImageJ by an investigator blinded to treatment condition. There were no significant changes in the microglia density between treatment groups in the frontal cortex of p15 mice. Analysis of microglia density in the dentate gyrus, CA1, CA2, and CA3 regions of the hippocampus in p15 mice showed significant differences between the PBS-air group and the MAA-UIS group ($p = 0.0166$).

4.0 Discussion:

Among the many well established environmental factors that can impact fetal neurodevelopment, asthma and air pollution represent two major sources of immune stimulation that are on the rise, making them a significant concern for pregnant individuals. Based on previous studies of maternal immune activation, and PM exposure during pregnancy, we hypothesized that the combination of these two environmental stimuli would result in an exacerbated neuroimmune response in offspring. Although the appearance of a synergistic effect of MAA and UIS exposure combined was limited, we did identify increases in cytokine concentrations across all treatment groups in the cortex and hippocampus. Importantly, some of these elevations appear to be sustained across treatment groups from adolescence into early adulthood in the cortex, demonstrating lasting impacts of these gestational exposures on the neuroimmune environment later in life. Although we identified increases in cytokines in the hippocampus within all treatment groups at P15, these did not remain elevated into early adulthood. Overall, these data show that MAA and UIS environmental stimuli can result in an altered neuroimmune environment that persist from juvenile to adult timepoints.

The allergic response in the lungs of individuals with asthma is characterized by an influx of immune cells, such as neutrophils, mast cells, macrophages, and T-helper (T_H)2 cells. Our mouse model of MAA previously showed elevated IL-4, IL-5, IL-13, IL-17, and IFNγ in the lung and peripheral blood of mice exposed to aerosolized OVA during pregnancy (Schwartzer et al., 2017; Church et al., 2021; Tamayo et al., 2022). These increases in maternal serum cytokines result in neuro-immune signaling changes in the fetal brain during in utero development (Tamayo et al., 2022). Our present data extend these findings by revealing evidence of increases

in cortical and hippocampal cytokines in juvenile mice of MAA dams. In the cortex, for example, MAA alone increased IL-1β, IL-2, IL-17, IL-1α, IL-10, and IL-17, and in the hippocampus, we identified IFNγ, IL-1β, IL-2, IL-7, IL-13, IL-15, MIP-1β, and RANTES as being elevated in P15 offspring of MAA-exposed dams. These observed increases in MAA-AIR compared to PBS-AIR controls demonstrates the independent neurodevelopmental impact of allergic inflammation during pregnancy on offspring neuroinflammation. In addition to these findings in the MAA alone treatment group, we also identified UIS treatment (in the absence of MAA) resulting in an increase of cytokine concentration in juvenile offspring, specifically, in IL-1β, IL-2, IL-13, IL-17, IP-10, MIP-1α, and IL-10. Moreover, increases in cytokines as a result of UIS exposure alone were also identified in the hippocampus of juvenile offspring. Specifically, elevated IFNγ, IL-1β, IL-2, IL-7, IL-12(p40), IL-13, IL-17, and RANTES. To the authors' knowledge, investigations into these neurobiological outcomes in offspring under UIS exposure during gestation have not been previously reported, highlighting the novelty of our model and findings.

In addition to the independent effects of MAA or UIS treatment on cytokine concentrations in the cortex and hippocampus of juvenile offspring, these elevations were most often coupled with elevations in the MAA-UIS combined treatment group. Most notably, we observed elevations in the cortex of IL-1β, IL-2, IL-13, and IL-17 in dual-exposed MAA-UIS offspring. These cytokines were increased in the MAA and UIS single treatment groups as well as the MAA-UIS treatment group at P15, and they remained elevated into the P35 timepoint. Although IL-13 in the P15 MAA group alone, and IL-1β in the P35 MAA-UIS group, did not reach the criteria for statistical significance ($p < 0.05$), at $p = 0.061$ and $p = 0.063$ respectively

our data suggest the presence of a trend that is perhaps the result of limited statistical power in our model. Despite this, our results suggest a sustained elevation in these four cytokines from P15 to P35 as a result of both MAA and UIS that have the potential for long-lasting impacts on neurodevelopment in the cortex of these offspring.

Consistent with the pleiotropic nature of cytokines in the CNS, IL-1β, IL-2, IL-13, and IL-17 have all been identified as having neurotrophic properties. Indeed, high concentrations (500 ng/mL), of IL-1β can have neurotoxic effects on neurons when exposed for 3-5 days (Park et al., 2018), and IL-17 is detected at high levels in the CNS in multiple sclerosis and associated with the neuroinflammatory pathology of the disease (Lock et al., 2002; Kebir et al., 2007). Compared to these neurotoxic concentrations, our data represent moderate increases in cytokines with less than 2.5-fold increases in treatment groups compared to PBS-AIR controls, and may not represent overt inflammation per se. Instead, these smaller changes in brain cytokine levels during the juvenile period may be biologically significant given their alternative functions in promoting neuronal survival and neurodevelopment. For example, IL-1β acts as a chemokine that guides neurite outgrowth in cortical neurons (Ma et al., 2014), and IL-17 in initiating the release of brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF) and nerve growth factors (NGF) associated with neuronal cell survival and repair (Milovanovic et al., 2020). Taken from this view, these cytokines which are generally considered overtly inflammatory, may be having a more subtle impact on the neuroarchitecture of offspring brains than models finding dramatic increases in concentration of IL-1β and IL-17.

Further demonstrating the neuropoietic nature of these cytokines within the CNS and adding to the idea that the moderate increases observed in this model may be altering the neuropatterning of the offspring brains, IL-2 has been found to have neurotropic properties and is necessary for proper cytoarchitecture in development (Beck et al., 2004; Beck et al., 2007). In addition, IL-13 is often considered anti-inflammatory in the CNS, with some studies pointing to a neuroprotective impact in CNS diseases and injuries (Miao et al., 2020; Guglielmetti et al., 2016; Le Blon et al., 2016; Pan et al., 2013). Both IL-2 and IL-13 are among several cytokines that are known to decrease in concentration at P14 under homeostatic conditions, and this developmental timepoint in mice is characterized by a high degree of synaptogenesis and pruning (Garay et al., 2013; Morato Torres et al., 2020). In contrast, our model which investigated cytokine concentrations at P15, still within this window of high synaptogenesis, showed increased IL-2 and IL-13, representing a shift in homeostatic load. Taken together, it may be that these sustained moderate increases in cytokines of the cortex are changing the trajectory of cortical development and promoting altered connectivity in the cortex linked to behavioral changes such as decreased social interaction and repetitive behaviors previously identified in our model (Schwartzer et al., 2015; Church et al., 2020). This phenomenon of altered connectivity has also been implicated in the core behaviors associated with ASD, specifically the social deficits and restrictive and repetitive behaviors (Conti et al., 2017; Maximo et al., 2014; Mills et al., 2016).

Similar to our findings in the cortex, we also identified elevations in several cytokines, most notably IFNγ and IL-7, at P15 in the hippocampus of MAA alone, in UIS alone, and MAA-UIS offspring compared to controls. IFNγ receptors are present on both neurons and glia (Ottum

et al., 2015), and in the hippocampus, IFNγ appears to play a role in synaptogenesis (Lee et al., 2006). Some investigators have found that overexpression resulted in increased neurogenesis in the dentate gyrus, and because of its neuromodulatory effects, it has been suggested that this may impact cognition and social behavior as a result (Flood et al., 2019; Filiano et al., 2016; Baron et al., 2008). Additionally, IL-7 promotes survival and neurite outgrowth in hippocampal neurons (Macia et al., 2010; Michaelson et al., 1996). Considering the effects of IL-7 and IFNγ, and that the hippocampus is a major neurogenic niche in the developing brain, future studies may benefit from investigating the potential for hippocampal overgrowth in offspring brain in response to UIS or allergic asthma inflammation during pregnancy. Indeed, this phenomenon of hippocampal overgrowth has been identified in cases of ASD (Groen et al., 2010; Murphy et al., 2012, Rojas et al., 2006), and has been implicated in the deficits associated with emotion perception and sensory processing in ASD individuals (Groen et al., 2010, Banker et al., 2021). Curiously, our observed increases in hippocampal cytokine concentrations at P15 were not observed in any treatment group of P35 offspring compared to PBS-AIR controls. Although we can only speculate about these findings, it may be that these changes resolve during adolescence when additional brain maturation may be present to compensate for developmental overgrowth much in the same way that volumetric increases in the hippocampus of ASD individuals do not persist into adulthood (Groen et al., 2010; Rojas et al., 2006). Although IL-7 and IFNγ are only two examples of cytokines that we found elevated among the treatment groups, they illustrate the broader findings that treatment with MAA, UIS, or MAA-UIS can alter the hippocampal neuroimmune environment with potential consequences to behavioral outcomes.

Prenatal insults have been shown to have lifelong impacts on microglia function and are suspected to play a prominent role in neurodevelopmental disorders (Ciernia et al., 2018; Eggen et al., 2013; Knuesel et al., 2014; Slusarczyk et al., 2015; Giovanoli et al., 2016). In ASD, some postmortem studies have identified differences in microglia density and morphology in brains of individuals (Koyama et al., 2015; Morgan et al., 2010; Morgan et al., 2014). In our previous study of MAA, we found DNA methylation differences in adult microglia, and several of these changes occurred in regulatory genes that are shared among some ASD individuals (Ciernia et al., 2017). Given these findings, we sought to examine the density of microglia in the P15 brains of our MAA and UIS exposure model. We observed a significant increase in microglia density within the hippocampus of offspring exposed to the combination of MAA and UIS, but these increases were not present in the frontal cortex. One plausible explanation for why these increases were only observed in the hippocampus may be due to the higher density of microglia known to be present in the hippocampus. This higher density of microglia is thought to make the hippocampus more vulnerable to inflammation (Réus et al., 2015; Choi et al., 2011), and disruptions in the dentate gyrus have been linked to neurodevelopmental disorders (Cai et al., 2018; Yagishita et al., 2017). Our findings of increased microglia density in the hippocampus of MAA-UIS offspring mirror data from another maternal immune activation (MIA) model that utilizes the viral mimic poly(I:C). Specifically, Juckel et al. reported an increase in microglia density in the hippocampus but not the cortex of offspring born from immune-activated dams (Juckel et al., 2011). Similarly, another study of MIA using LPS stimulation that showed an increase in microglia density in the hippocampus (Diz-Chaves et al., 2013). While it is difficult to make conclusions about microglial function based on density data alone, our observed difference in the hippocampus in combination with similar report from other MIA models

(Juckel et al., 2011; Diz-Chaves et al., 2013) suggest that asthma allergy mediated immune activation during pregnancy can result in a deviation from homeostatic activity in the offspring hippocampus.

Although we did not collect maternal data in this preliminary study, data from previous MAA studies demonstrates increased systemic inflammation characteristic of an allergic asthma response, specifically with increased IL-4, IL-5, and IL-13 (Schwartzer et al., 2017; Church et al., 2020; Tamayo et al., 2022), suggesting the potential for a similar response in dams of MAA in the current model. Speculation about the systemic impacts of UIS on maternal immune system, however, is difficult. Many studies of PM exposure suggest IL-6, IL-8, and TNFα as the main cytokines upregulated in response to PM exposure (Mitschik et al., 2008; Silbajoris et al., 2011; Musah et al., 2012; Gong et al., 2022; Longhin et al., 2018). This difference in cytokine response highlights the potential reason we see differences in the impact between MAA and UIS in our model. However, models of PM can vary widely in the size of PM and composition (Mitschik et al., 2008), making speculation about the maternal response in the UIS groups, and the potential role this plays in offspring neurodevelopment, difficult. This variation in PM studies underscores the need for future investigations to identify the maternal cytokine milieu in this model.

While our findings do not necessarily demonstrate an additive effect of MAA and UIS with regard to the cytokines we investigated, we did see a synergistic impact of MAA-UIS on microglia density in the hippocampus. This finding demonstrates the potential for additive effects of maternal asthma exposure when coupled with PM exposure. Independently, studies

have shown in both humans and animal models that PM exposure during pregnancy can increase susceptibility to offspring developing asthma (Mortimer et al., 2008; Wang et al., 2013; Hua et al., 2023). This increased susceptibility of asthma in offspring is also seen in children of asthmatic mothers (Martel et al., 2009; Mattes et al., 2013; Murphy 2022), suggesting the potential for systemic immune disfunction when these two stimuli during pregnancy are combined. The findings in this unique model of MAA and UIS exposure highlight the importance of investigating the impact of these closely linked and prevalent environmental factors.

Our data add to our previous studies on the impact of MAA on fetal brain development, showing here that this model impacts region-specific cytokine concentrations in both the juvenile and adolescent periods. In addition, to the investigators' knowledge this was the first study to assess the impact of ultrafine iron-soot exposure during gestation on offspring neurobiology. Moreover, our identification of regional changes in cytokine concentrations implicate a potential driving force behind our previously identified behavioral changes in the MAA model. Taken together, these data highlight the importance of understanding the impact that common environmental stimuli can have on fetal development, and the potential for these stimuli to have long lasting changes in offspring.

References:

Maenner,M.J.; Shaw, K.A.; Bakian, A.V.; Bilder, D.A.; Durkin,M.S.; Esler, A.; Furnier, S.M.; Hallas, L.; Hall-Lande, J.; Hudson, A.; et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018. Morb. Mortal. Wkly. Rep. Surveill. Summ. 2021, 70, 1–16.

Kim, Y. S., Leventhal, B. L., Koh, Y. J., Fombonne, E., Laska, E., Lim, E. C., Cheon, K. A., Kim, S. J., Kim, Y. K., Lee, H., Song, D. H., & Grinker, R. R. (2011). Prevalence of autism spectrum disorders in a total population sample. *The American journal of psychiatry*, *168*(9), 904–912. https://doi.org/10.1176/appi.ajp.2011.10101532

Werling, D. M., Parikshak, N. N., & Geschwind, D. H. (2016). Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders. *Nature communications*, *7*, 10717. https://doi.org/10.1038/ncomms10717

Gaugler, T., Klei, L., Sanders, S. J., Bodea, C. A., Goldberg, A. P., Lee, A. B., Mahajan, M., Manaa, D., Pawitan, Y., Reichert, J., Ripke, S., Sandin, S., Sklar, P., Svantesson, O., Reichenberg, A., Hultman, C. M., Devlin, B., Roeder, K., & Buxbaum, J. D. (2014). Most genetic risk for autism resides with common variation. *Nature genetics*, *46*(8), 881–885. https://doi.org/10.1038/ng.3039

Devlin, B., Melhem, N., & Roeder, K. (2011). Do common variants play a role in risk for autism? Evidence and theoretical musings. *Brain research*, *1380*, 78–84. https://doi.org/10.1016/j.brainres.2010.11.026

Klei, L., Sanders, S. J., Murtha, M. T., Hus, V., Lowe, J. K., Willsey, A. J., Moreno-De-Luca, D., Yu, T. W., Fombonne, E., Geschwind, D., Grice, D. E., Ledbetter, D. H., Lord, C., Mane, S. M., Martin, C. L., Martin, D. M., Morrow, E. M., Walsh, C. A., Melhem, N. M., Chaste, P., … Devlin, B. (2012). Common genetic variants, acting additively, are a major source of risk for autism. *Molecular autism*, *3*(1), 9. https://doi.org/10.1186/2040-2392-3-9

Hughes, H. K., Mills Ko, E., Rose, D., & Ashwood, P. (2018). Immune Dysfunction and Autoimmunity as Pathological Mechanisms in Autism Spectrum Disorders. *Frontiers in cellular neuroscience*, *12*, 405. https://doi.org/10.3389/fncel.2018.00405

Rossignol, D. A., Genuis, S. J., & Frye, R. E. (2014). Environmental toxicants and autism spectrum disorders: a systematic review. *Translational psychiatry*, *4*(2), e360. https://doi.org/10.1038/tp.2014.4

Straughen, J. K., Sitarik, A. R., Johnson, C. C., Wegienka, G., Ownby, D. R., Johnson-Hooper, T. M., Allo, G., Levin, A. M., & Cassidy-Bushrow, A. E. (2021). Prenatal IgE as a Risk Factor for the Development of Childhood Neurodevelopmental Disorders. *Frontiers in pediatrics*, *9*, 601092. https://doi.org/10.3389/fped.2021.601092

Raz, R., Roberts, A. L., Lyall, K., Hart, J. E., Just, A. C., Laden, F., & Weisskopf, M. G. (2015). Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case-control analysis within the Nurses' Health Study II Cohort. *Environmental health perspectives*, *123*(3), 264–270. https://doi.org/10.1289/ehp.1408133

Volk, H. E., Hertz-Picciotto, I., Delwiche, L., Lurmann, F., & McConnell, R. (2011). Residential proximity to freeways and autism in the CHARGE study. *Environmental health perspectives*, *119*(6), 873–877. https://doi.org/10.1289/ehp.1002835

Volk, H. E., Park, B., Hollingue, C., Jones, K. L., Ashwood, P., Windham, G. C., Lurman, F., Alexeeff, S. E., Kharrazi, M., Pearl, M., Van de Water, J., & Croen, L. A. (2020). Maternal immune response and air pollution exposure during pregnancy: insights from the Early Markers for Autism (EMA) study. *Journal of neurodevelopmental disorders*, *12*(1), 42. https://doi.org/10.1186/s11689-020-09343-0

Patterson P. H. (2009). Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behavioural brain research*, *204*(2), 313–321. https://doi.org/10.1016/j.bbr.2008.12.016

Modabbernia, A., Velthorst, E., & Reichenberg, A. (2017). Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. *Molecular autism*, *8*, 13. https://doi.org/10.1186/s13229-017-0121-4

Tioleco, N., Silberman, A. E., Stratigos, K., Banerjee-Basu, S., Spann, M. N., Whitaker, A. H., & Turner, J. B. (2021). Prenatal maternal infection and risk for autism in offspring: A meta-analysis. *Autism research : official journal of the International Society for Autism Research*, *14*(6), 1296–1316. https://doi.org/10.1002/aur.2499

Atladóttir, H. O., Thorsen, P., Østergaard, L., Schendel, D. E., Lemcke, S., Abdallah, M., & Parner, E. T. (2010). Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *Journal of autism and developmental disorders*, *40*(12), 1423–1430. https://doi.org/10.1007/s10803-010-1006-y

Brown, A. S., Sourander, A., Hinkka-Yli-Salomäki, S., McKeague, I. W., Sundvall, J., & Surcel, H. M. (2014). Elevated maternal C-reactive protein and autism in a national birth cohort. *Molecular psychiatry*, *19*(2), 259–264. https://doi.org/10.1038/mp.2012.197

Han, V. X., Patel, S., Jones, H. F., Nielsen, T. C., Mohammad, S. S., Hofer, M. J., Gold, W., Brilot, F., Lain, S. J., Nassar, N., & Dale, R. C. (2021). Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. *Translational psychiatry*, *11*(1), 71. https://doi.org/10.1038/s41398-021-01198-w

Jiang, H. Y., Xu, L. L., Shao, L., Xia, R. M., Yu, Z. H., Ling, Z. X., Yang, F., Deng, M., & Ruan, B. (2016). Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. *Brain, behavior, and immunity*, *58*, 165–172. https://doi.org/10.1016/j.bbi.2016.06.005

Clougherty, J. E., Levy, J. I., Kubzansky, L. D., Ryan, P. B., Suglia, S. F., Canner, M. J., & Wright, R. J. (2007). Synergistic effects of traffic-related air pollution and exposure to violence on urban asthma etiology. *Environmental health perspectives*, *115*(8), 1140–1146. https://doi.org/10.1289/ehp.9863

Matsui, E. C., Hansel, N. N., McCormack, M. C., Rusher, R., Breysse, P. N., & Diette, G. B. (2008). Asthma in the inner city and the indoor environment. *Immunology and allergy clinics of North America*, *28*(3), 665–x. https://doi.org/10.1016/j.iac.2008.03.004

Ali, Z., & Ulrik, C. S. (2013). Incidence and risk factors for exacerbations of asthma during pregnancy. *Journal of asthma and allergy*, *6*, 53–60. https://doi.org/10.2147/JAA.S43183

Murphy, V. E., Gibson, P., Talbot, P. I., & Clifton, V. L. (2005). Severe asthma exacerbations during pregnancy. *Obstetrics and gynecology*, *106*(5 Pt 1), 1046–1054. https://doi.org/10.1097/01.AOG.0000185281.21716.02

Murphy, V. E., Clifton, V. L., & Gibson, P. G. (2006). Asthma exacerbations during pregnancy: incidence and association with adverse pregnancy outcomes. *Thorax*, *61*(2), 169–176. https://doi.org/10.1136/thx.2005.049718

Croen, L. A., Grether, J. K., Yoshida, C. K., Odouli, R., & Van de Water, J. (2005). Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Archives of pediatrics & adolescent medicine*, *159*(2), 151–157. https://doi.org/10.1001/archpedi.159.2.151

Croen, L. A., Qian, Y., Ashwood, P., Zerbo, O., Schendel, D., Pinto-Martin, J., Daniele Fallin, M., Levy, S., Schieve, L. A., Yeargin-Allsopp, M., Sabourin, K. R., & Ames, J. L. (2019). Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. *Autism research : official journal of the International Society for Autism Research*, *12*(10), 1551–1561. https://doi.org/10.1002/aur.2175

Gong, T., Lundholm, C., Rejnö, G., Bölte, S., Larsson, H., D'Onofrio, B. M., Lichtenstein, P., & Almqvist, C. (2019). Parental asthma and risk of autism spectrum disorder in offspring: A population and family-based case-control study. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, *49*(6), 883–891. https://doi.org/10.1111/cea.13353

Abdallah, M. W., Larsen, N., Grove, J., Nørgaard-Pedersen, B., Thorsen, P., Mortensen, E. L., & Hougaard, D. M. (2013). Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, *14*(7), 528–538. https://doi.org/10.3109/15622975.2011.639803

Hisle-Gorman, E., Susi, A., Stokes, T., Gorman, G., Erdie-Lalena, C., & Nylund, C. M. (2018). Prenatal, perinatal, and neonatal risk factors of autism spectrum disorder. *Pediatric research*, *84*(2), 190–198. https://doi.org/10.1038/pr.2018.23

Lyall, K., Ashwood, P., Van de Water, J., & Hertz-Picciotto, I. (2014). Maternal immune-mediated conditions, autism spectrum disorders, and developmental delay. *Journal of autism and developmental disorders*, *44*(7), 1546–1555. https://doi.org/10.1007/s10803-013-2017-2

Patel, S., Dale, R. C., Rose, D., Heath, B., Nordahl, C. W., Rogers, S., Guastella, A. J., & Ashwood, P. (2020). Maternal immune conditions are increased in males with autism spectrum disorders and are associated with behavioural and emotional but not cognitive co-morbidity. *Translational psychiatry*, *10*(1), 286. https://doi.org/10.1038/s41398-020-00976-2

Fasmer, O. B., Halmøy, A., Eagan, T. M., Oedegaard, K. J., & Haavik, J. (2011). Adult attention deficit hyperactivity disorder is associated with asthma. *BMC psychiatry*, *11*, 128. https://doi.org/10.1186/1471-244X-11-128

Gluck, J. C., & Gluck, P. A. (2006). The effect of pregnancy on the course of asthma. *Immunology and allergy clinics of North America*, *26*(1), 63–80. https://doi.org/10.1016/j.iac.2005.10.008

Popa, M., Peltecu, G., Gica, N., Ciobanu, A. M., Botezatu, R., Gica, C., Steriade, A., & Panaitescu, A. M. (2021). Asthma in Pregnancy. Review of Current Literature and Recommendations. *Maedica*, *16*(1), 80–87. https://doi.org/10.26574/maedica.2020.16.1.80

Jacquet A. (2013). Innate immune responses in house dust mite allergy. *ISRN allergy*, *2013*, 735031. https://doi.org/10.1155/2013/735031

Blackwell, D. L., Lucas, J. W., & Clarke, T. C. (2014). Summary health statistics for U.S. adults: national health interview survey, 2012. *Vital and health statistics. Series 10, Data from the National Health Survey*, (260), 1–161.

Fazel, N., Kundi, M., Jensen-Jarolim, E., Pali-Schöll, I., Kazemzadeh, A., Abdizadeh, M. F., Esmaily, H., Akbarzadeh, R., & Ahmadi, R. (2018). Prospective cohort study of pregnancy complications and birth outcomes in women with asthma. *Archives of gynecology and obstetrics*, *298*(2), 279–287. https://doi.org/10.1007/s00404-018-4800-y

Leaderer, B. P., Belanger, K., Triche, E., Holford, T., Gold, D. R., Kim, Y., Jankun, T., Ren, P., McSharry Je, J. E., Platts-Mills, T. A., Chapman, M. D., & Bracken, M. B. (2002). Dust mite, cockroach, cat, and dog allergen concentrations in homes of asthmatic children in the northeastern United States: impact of socioeconomic factors and population density. *Environmental health perspectives*, *110*(4), 419–425. https://doi.org/10.1289/ehp.02110419

Salo, P. M., Arbes, S. J., Jr, Crockett, P. W., Thorne, P. S., Cohn, R. D., & Zeldin, D. C. (2008). Exposure to multiple indoor allergens in US homes and its relationship to asthma. *The Journal of allergy and clinical immunology*, *121*(3), 678–684.e2. https://doi.org/10.1016/j.jaci.2007.12.1164

Claeson, A. S., Palmquist, E., Lind, N., & Nordin, S. (2016). Symptom-trigger factors other than allergens in asthma and allergy. *International journal of environmental health research*, *26*(4), 448–457. https://doi.org/10.1080/09603123.2015.1135314

Schwartzer, J. J., Careaga, M., Chang, C., Onore, C. E., & Ashwood, P. (2015). Allergic fetal priming leads to developmental, behavioral and neurobiological changes in mice. *Translational psychiatry*, *5*(4), e543. https://doi.org/10.1038/tp.2015.40

Tamayo, J. M., Rose, D., Church, J. S., Schwartzer, J. J., & Ashwood, P. (2022). Maternal Allergic Asthma Induces Prenatal Neuroinflammation. *Brain sciences*, *12*(8), 1041. https://doi.org/10.3390/brainsci12081041

Goines, P. E., Croen, L. A., Braunschweig, D., Yoshida, C. K., Grether, J., Hansen, R., Kharrazi, M., Ashwood, P., & Van de Water, J. (2011). Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. *Molecular autism*, *2*, 13. https://doi.org/10.1186/2040-2392-2-13

Schwartzer, J. J., Careaga, M., Coburn, M. A., Rose, D. R., Hughes, H. K., & Ashwood, P. (2017). Behavioral impact of maternal allergic-asthma in two genetically distinct mouse strains. *Brain, behavior, and immunity*, *63*, 99–107. https://doi.org/10.1016/j.bbi.2016.09.007

Church, J. S., Tamayo, J. M., Ashwood, P., & Schwartzer, J. J. (2021). Repeated allergic asthma in early versus late pregnancy differentially impacts offspring brain and behavior development. *Brain, behavior, and immunity*, *93*, 66–79. https://doi.org/10.1016/j.bbi.2020.12.014

Vogel Ciernia, A., Careaga, M., LaSalle, J. M., & Ashwood, P. (2018). Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. *Glia*, *66*(3), 505–521. https://doi.org/10.1002/glia.23261

Volk, H. E., Lurmann, F., Penfold, B., Hertz-Picciotto, I., & McConnell, R. (2013). Traffic-related air pollution, particulate matter, and autism. *JAMA psychiatry*, *70*(1), 71–77. https://doi.org/10.1001/jamapsychiatry.2013.266

Church, J. S., Tijerina, P. B., Emerson, F. J., Coburn, M. A., Blum, J. L., Zelikoff, J. T., & Schwartzer, J. J. (2018). Perinatal exposure to concentrated ambient particulates results in autism-like behavioral deficits in adult mice. *Neurotoxicology*, *65*, 231–240. https://doi.org/10.1016/j.neuro.2017.10.007

Guarnieri, M., & Balmes, J. R. (2014). Outdoor air pollution and asthma. *Lancet (London, England)*, *383*(9928), 1581–1592. https://doi.org/10.1016/S0140-6736(14)60617-6

Chan, J. K., Fanucchi, M. V., Anderson, D. S., Abid, A. D., Wallis, C. D., Dickinson, D. A., Kumfer, B. M., Kennedy, I. M., Wexler, A. S., & Van Winkle, L. S. (2011). Susceptibility to inhaled flame-generated ultrafine soot in neonatal and adult rat lungs. Toxicological sciences : an official journal of the Society of Toxicology, 124(2), 472–486. https://doi.org/10.1093/toxsci/kfr233

Schraufnagel D. E. (2020). The health effects of ultrafine particles. Experimental & molecular medicine, 52(3), 311–317. https://doi.org/10.1038/s12276-020-0403-3

Chalupa, D. C., Morrow, P. E., Oberdörster, G., Utell, M. J., & Frampton, M. W. (2004). Ultrafine particle deposition in subjects with asthma. Environmental health perspectives, 112(8), 879–882. https://doi.org/10.1289/ehp.6851

Steiner, S., Bisig, C., Petri-Fink, A., & Rothen-Rutishauser, B. (2016). Diesel exhaust: current knowledge of adverse effects and underlying cellular mechanisms. *Archives of toxicology*, *90*(7), 1541–1553. https://doi.org/10.1007/s00204-016-1736-5

Kalkbrenner, A. E., Windham, G. C., Serre, M. L., Akita, Y., Wang, X., Hoffman, K., Thayer, B. P., & Daniels, J. L. (2015). Particulate matter exposure, prenatal and postnatal windows of susceptibility, and autism spectrum disorders. *Epidemiology (Cambridge, Mass.)*, *26*(1), 30–42. https://doi.org/10.1097/EDE.0000000000000173

von Ehrenstein, O. S., Aralis, H., Cockburn, M., & Ritz, B. (2014). In utero exposure to toxic air pollutants and risk of childhood autism. *Epidemiology (Cambridge, Mass.)*, *25*(6), 851–858. https://doi.org/10.1097/EDE.0000000000000150

Roberts, A. L., Lyall, K., Hart, J. E., Laden, F., Just, A. C., Bobb, J. F., Koenen, K. C., Ascherio, A., & Weisskopf, M. G. (2013). Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. *Environmental health perspectives*, *121*(8), 978–984. https://doi.org/10.1289/ehp.1206187

Zhou, Y. M., Zhong, C. Y., Kennedy, I. M., Leppert, V. J., & Pinkerton, K. E. (2003). Oxidative stress and NFkappaB activation in the lungs of rats: a synergistic interaction between soot and iron particles. *Toxicology and applied pharmacology*, *190*(2), 157–169. https://doi.org/10.1016/s0041-008x(03)00157-1

Mayer, A., Ulrich, A., Czerwinski, J., and Mooney, J., "Metal-Oxide Particles in Combustion Engine Exhaust," SAE Technical Paper 2010-01-0792, 2010, https://doi.org/10.4271/2010-01-0792.

Zhong, C. Y., Zhou, Y. M., Smith, K. R., Kennedy, I. M., Chen, C. Y., Aust, A. E., & Pinkerton, K. E. (2010). Oxidative injury in the lungs of neonatal rats following short-term exposure to ultrafine iron and soot particles. *Journal of toxicology and environmental health. Part A*, *73*(12), 837–847. https://doi.org/10.1080/15287391003689366

Hopkins, L. E., Laing, E. A., Peake, J. L., Uyeminami, D., Mack, S. M., Li, X., Smiley-Jewell, S., & Pinkerton, K. E. (2018). Repeated Iron-Soot Exposure and Nose-to-brain Transport of Inhaled Ultrafine Particles. *Toxicologic pathology*, *46*(1), 75–84. https://doi.org/10.1177/0192623317729222

Bolton, J. L., Marinero, S., Hassanzadeh, T., Natesan, D., Le, D., Belliveau, C., Mason, S. N., Auten, R. L., & Bilbo, S. D. (2017). Gestational Exposure to Air Pollution Alters Cortical Volume, Microglial Morphology, and Microglia-Neuron Interactions in a Sex-Specific Manner. Frontiers in synaptic neuroscience, 9, 10. https://doi.org/10.3389/fnsyn.2017.00010

Ahadullah, Yau, S. Y., Lu, H. X., Lee, T. M. C., Guo, H., & Chan, C. C. H. (2021). PM2.5 as a potential risk factor for autism spectrum disorder: Its possible link to neuroinflammation, oxidative stress and changes in gene expression. Neuroscience and biobehavioral reviews, 128, 534–548. https://doi.org/10.1016/j.neubiorev.2021.06.043

Hughes, H. K., & Ashwood, P. (2020). Overlapping evidence of innate immune dysfunction in psychotic and affective disorders. Brain, behavior, & immunity - health, 2, 100038. https://doi.org/10.1016/j.bbih.2020.100038

Davoli-Ferreira, M., Thomson, C. A., & McCoy, K. D. (2021). Microbiota and Microglia Interactions in ASD. Frontiers in immunology, 12, 676255. https://doi.org/10.3389/fimmu.2021.676255

Ecker C. (2017). The neuroanatomy of autism spectrum disorder: An overview of structural neuroimaging findings and their translatability to the clinical setting. Autism : the international journal of research and practice, 21(1), 18–28. https://doi.org/10.1177/1362361315627136

Richards, R., Greimel, E., Kliemann, D., Koerte, I. K., Schulte-Körne, G., Reuter, M., & Wachinger, C. (2020). Increased hippocampal shape asymmetry and volumetric ventricular asymmetry in autism spectrum disorder. NeuroImage. Clinical, 26, 102207. https://doi.org/10.1016/j.nicl.2020.102207

Banker, S. M., Gu, X., Schiller, D., & Foss-Feig, J. H. (2021). Hippocampal contributions to social and cognitive deficits in autism spectrum disorder. Trends in neurosciences, 44(10), 793–807. https://doi.org/10.1016/j.tins.2021.08.005

Jasinski, J., Pinkerton, K. E., Kennedy, I. M., & Leppert, V. J. (2006). Spatially resolved energy electron loss spectroscopy studies of iron oxide nanoparticles. Microscopy and microanalysis : the official journal of Microscopy Society of America, Microbeam Analysis Society, Microscopical Society of Canada, 12(5), 424–431. https://doi.org/10.1017/S1431927606060491

Pinkerton, K. E., Zhou, Y., Zhong, C., Smith, K. R., Teague, S. V., Kennedy, I. M., & Ménache, M. G. (2008). Mechanisms of particulate matter toxicity in neonatal and young adult rat lungs. Research report (Health Effects Institute), (135), 3–52

Pui DYH, Chen SC, and Zuo Z (2014). PM2.5 in China: Measurements, sources, visibility and health effects, and mitigation. Particuology, 13, 1–26.

Connor N (2015). Choking smog more than 50 times health guidelines blankets China. The Telegraph, 9 11 2015.

Lazic, S. E., & Essioux, L. (2013). Improving basic and translational science by accounting for litter-to-litter variation in animal models. *BMC neuroscience*, *14*, 37. https://doi.org/10.1186/1471-2202-14-37

Lazic, S. E., Clarke-Williams, C. J., & Munafò, M. R. (2018). What exactly is 'N' in cell culture and animal experiments?. *PLoS biology*, *16*(4), e2005282. https://doi.org/10.1371/journal.pbio.2005282

Brauer, M., & Curtin, J. J. (2018). Linear mixed-effects models and the analysis of nonindependent data: A unified framework to analyze categorical and continuous independent variables that vary within-subjects and/or within-items. *Psychological methods*, *23*(3), 389–411. https://doi.org/10.1037/met0000159

Schielzeth, H. and Nakagawa, S. (2013), Nested by design: model fitting and interpretation in a mixed model era. Methods Ecol Evol, 4: 14-24. https://doi.org/10.1111/j.2041-210x.2012.00251.x

Wainwright, P. E., Leatherdale, S. T., & Dubin, J. A. (2007). Advantages of mixed effects models over traditional ANOVA models in developmental studies: a worked example in a mouse model of fetal alcohol syndrome. *Developmental psychobiology*, *49*(7), 664–674. https://doi.org/10.1002/dev.20245

Park, S. Y., Kang, M. J., & Han, J. S. (2018). Interleukin-1 beta promotes neuronal differentiation through the Wnt5a/RhoA/JNK pathway in cortical neural precursor cells. *Molecular brain*, *11*(1), 39. https://doi.org/10.1186/s13041-018-0383-6

Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S. J., Oksenberg, J. R., Raine, C. S., Heller, R., & Steinman, L. (2002). Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nature medicine*, *8*(5), 500–508. https://doi.org/10.1038/nm0502-500

Kebir, H., Kreymborg, K., Ifergan, I., Dodelet-Devillers, A., Cayrol, R., Bernard, M., Giuliani, F., Arbour, N., Becher, B., & Prat, A. (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nature medicine, 13(10), 1173–1175. https://doi.org/10.1038/nm1651

Ma, L., Li, X. W., Zhang, S. J., Yang, F., Zhu, G. M., Yuan, X. B., & Jiang, W. (2014). Interleukin-1 beta guides the migration of cortical neurons. *Journal of neuroinflammation*, *11*, 114. https://doi.org/10.1186/1742-2094-11-114

Milovanovic, J., Arsenijevic, A., Stojanovic, B., Kanjevac, T., Arsenijevic, D., Radosavljevic, G., Milovanovic, M., & Arsenijevic, N. (2020). Interleukin-17 in Chronic Inflammatory Neurological Diseases. *Frontiers in immunology*, *11*, 947. https://doi.org/10.3389/fimmu.2020.00947

Beck, R. D., Jr, Wasserfall, C., Ha, G. K., Cushman, J. D., Huang, Z., Atkinson, M. A., & Petitto, J. M. (2005). Changes in hippocampal IL-15, related cytokines, and neurogenesis in IL-2 deficient mice. *Brain research*, *1041*(2), 223–230. https://doi.org/10.1016/j.brainres.2005.02.010

Beck, R. D., Jr, King, M. A., Ha, G. K., Cushman, J. D., Huang, Z., & Petitto, J. M. (2005). IL-2 deficiency results in altered septal and hippocampal cytoarchitecture: relation to development and neurotrophins. *Journal of neuroimmunology*, *160*(1-2), 146–153. https://doi.org/10.1016/j.jneuroim.2004.11.006

Miao, W., Zhao, Y., Huang, Y., Chen, D., Luo, C., Su, W., & Gao, Y. (2020). IL-13 Ameliorates Neuroinflammation and Promotes Functional Recovery after Traumatic Brain Injury. *Journal of immunology (Baltimore, Md. : 1950)*, *204*(6), 1486–1498. https://doi.org/10.4049/jimmunol.1900909

Guglielmetti, C., Le Blon, D., Santermans, E., Salas-Perdomo, A., Daans, J., De Vocht, N., Shah, D., Hoornaert, C., Praet, J., Peerlings, J., Kara, F., Bigot, C., Mai, Z., Goossens, H., Hens, N., Hendrix, S., Verhoye, M., Planas, A. M., Berneman, Z., van der Linden, A., … Ponsaerts, P. (2016). Interleukin-13 immune gene therapy prevents CNS inflammation and demyelination via alternative activation of microglia and macrophages. *Glia*, *64*(12), 2181–2200. https://doi.org/10.1002/glia.23053

Le Blon, D., Guglielmetti, C., Hoornaert, C., Quarta, A., Daans, J., Dooley, D., Lemmens, E., Praet, J., De Vocht, N., Reekmans, K., Santermans, E., Hens, N., Goossens, H., Verhoye, M., Van der Linden, A., Berneman, Z., Hendrix, S., & Ponsaerts, P. (2016). Intracerebral transplantation of interleukin 13-producing mesenchymal stem cells limits microgliosis, oligodendrocyte loss and demyelination in the cuprizone mouse model. *Journal of neuroinflammation*, *13*(1), 288. https://doi.org/10.1186/s12974-016-0756-7

Pan, H. C., Yang, C. N., Hung, Y. W., Lee, W. J., Tien, H. R., Shen, C. C., Sheehan, J., Chou, C. T., & Sheu, M. L. (2013). Reciprocal modulation of C/EBP-α and C/EBP-β by IL-13 in activated microglia prevents neuronal death. *European journal of immunology*, *43*(11), 2854–2865. https://doi.org/10.1002/eji.201343301

Garay, P. A., Hsiao, E. Y., Patterson, P. H., & McAllister, A. K. (2013). Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. *Brain, Behavior, and Immunity*, 31, 54– 68.

Morato Torres, C. A., Wassouf, Z., Zafar, F., Sastre, D., Outeiro, T. F., & Schüle, B. (2020). The Role of Alpha-Synuclein and Other Parkinson's Genes in Neurodevelopmental and Neurodegenerative Disorders. International journal of molecular sciences, 21(16), 5724. https://doi.org/10.3390/ijms21165724

Conti, E.; Mitra, J.; Calderoni, S.; Pannek, K.; Shen, K.K.; Pagnozzi, A.; Rose, S.; Mazzotti, S.; Scelfo, D.; Tosetti, M.; et al. Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: A diffusion MRI study. Hum. Brain Mapp. 2017, 38, 2333–2344

Maximo, J.; Cadena, E.J.; Kana, R.K. The Implications of Brain Connectivity in the Neuropsychology of Autism. Neuropsychol. Rev. 2014, 24, 16–31

Mills, B.D.; Pearce, H.L.; Khan, O.; Jarrett, B.R.; Fair, D.A.; Lahvis, G.P. Prenatal domoic acid exposure disrupts mouse pro-social behavior and functional connectivity MRI. Behav. Brain Res. 2016, 308, 14–23.

Ottum, P. A., Arellano, G., Reyes, L. I., Iruretagoyena, M., & Naves, R. (2015). Opposing Roles of Interferon-Gamma on Cells of the Central Nervous System in Autoimmune Neuroinflammation. Frontiers in immunology, 6, 539. https://doi.org/10.3389/fimmu.2015.00539

Lee, J., Kim, S. J., Son, T. G., Chan, S. L., & Mattson, M. P. (2006). Interferon-gamma is up-regulated in the hippocampus in response to intermittent fasting and protects hippocampal neurons against excitotoxicity. Journal of neuroscience research, 83(8), 1552–1557. https://doi.org/10.1002/jnr.20831

Flood, L., Korol, S. V., Ekselius, L., Birnir, B., & Jin, Z. (2019). Interferon-γ potentiates GABAA receptor-mediated inhibitory currents in rat hippocampal CA1 pyramidal neurons. Journal of neuroimmunology, 337, 577050. https://doi.org/10.1016/j.jneuroim.2019.577050

Filiano, A. J., Xu, Y., Tustison, N. J., Marsh, R. L., Baker, W., Smirnov, I., Overall, C. C., Gadani, S. P., Turner, S. D., Weng, Z., Peerzade, S. N., Chen, H., Lee, K. S., Scott, M. M., Beenhakker, M. P., Litvak, V., & Kipnis, J. (2016). Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour. Nature, 535(7612), 425–429.

Baron, R., Nemirovsky, A., Harpaz, I., Cohen, H., Owens, T., & Monsonego, A. (2008). IFN-gamma enhances neurogenesis in wild-type mice and in a mouse model of Alzheimer's disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 22(8), 2843–2852. https://doi.org/10.1096/fj.08-105866

Macia, L., Viltart, O., Delacre, M., Sachot, C., Héliot, L., Di Santo, J. P., & Wolowczuk, I. (2010). Interleukin-7, a new cytokine targeting the mouse hypothalamic arcuate nucleus: role in body weight and food intake regulation. PloS one, 5(4), e9953. https://doi.org/10.1371/journal.pone.0009953

Michaelson, M. D., Mehler, M. F., Xu, H., Gross, R. E., & Kessler, J. A. (1996). Interleukin-7 is trophic for embryonic neurons and is expressed in developing brain. Developmental biology, 179(1), 251–263. https://doi.org/10.1006/dbio.1996.0255

Groen, W., Teluij, M., Buitelaar, J., & Tendolkar, I. (2010). Amygdala and hippocampus enlargement during adolescence in autism. Journal of the American Academy of Child and Adolescent Psychiatry, 49(6), 552–560. https://doi.org/10.1016/j.jaac.2009.12.023

Murphy, C. M., Deeley, Q., Daly, E. M., Ecker, C., O'Brien, F. M., Hallahan, B., Loth, E., Toal, F., Reed, S., Hales, S., Robertson, D. M., Craig, M. C., Mullins, D., Barker, G. J., Lavender, T., Johnston, P., Murphy, K. C., & Murphy, D. G. (2012). Anatomy and aging of the amygdala and hippocampus in autism spectrum disorder: an in vivo magnetic resonance imaging study of Asperger syndrome. *Autism research : official journal of the International Society for Autism Research*, *5*(1), 3–12. https://doi.org/10.1002/aur.227

Rojas, D. C., Peterson, E., Winterrowd, E., Reite, M. L., Rogers, S. J., & Tregellas, J. R. (2006). Regional gray matter volumetric changes in autism associated with social and repetitive behavior symptoms. BMC psychiatry, 6, 56. https://doi.org/10.1186/1471-244X-6-56

Eggen, B. J. L., Raj, D., Hanisch, U. K., & Boddeke, H. W. G. M. (2013). Microglial phenotype and adaptation. *Journal of Neuroimmune Pharmacology*, *8*, 807-823.

Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. 2014. Maternal immune activation and abnormal brain development across cns disorders. Nat Rev Neurol 10:643–660.

Ślusarczyk, J., Trojan, E., Głombik, K., Budziszewska, B., Kubera, M., Lasoń, W., Popiołek-Barczyk, K., Mika, J., Wędzony, K., & Basta-Kaim, A. (2015). Prenatal stress is a vulnerability factor for altered morphology and biological activity of microglia cells. Frontiers in cellular neuroscience, 9, 82.

Giovanoli, S., Weber-Stadlbauer, U., Schedlowski, M., Meyer, U., & Engler, H. (2016). Prenatal immune activation causes hippocampal synaptic deficits in the absence of overt microglia anomalies. *Brain, behavior, and immunity*, *55*, 25-38.

Koyama, R., & Ikegaya, Y. (2015). Microglia in the pathogenesis of autism spectrum disorders. *Neuroscience research*, *100*, 1-5.

Morgan, J. T., Chana, G., Pardo, C. A., Achim, C., Semendeferi, K., Buckwalter, J., ... & Everall, I. P. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological psychiatry*, *68*(4), 368-376.

Morgan, J. T., Barger, N., Amaral, D. G., & Schumann, C. M. (2014). Stereological study of amygdala glial populations in adolescents and adults with autism spectrum disorder. *PloS one*, *9*(10), e110356.

Réus, G. Z., Fries, G. R., Stertz, L., Badawy, M., Passos, I. C., Barichello, T., ... & Quevedo, J. (2015). The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience*, *300*, 141-154.

Choi, J. H., & Won, M. H. (2011). Microglia in the normally aged hippocampus. *Laboratory animal research*, *27*(3), 181-187.

Cai, Y., Tang, X., Chen, X., Li, X., Wang, Y., Bao, X., ... & Fan, X. (2018). Liver X receptor β regulates the development of the dentate gyrus and autistic-like behavior in the mouse. *Proceedings of the National Academy of Sciences*, *115*(12), E2725-E2733.

Yagishita, K., Suzuki, R., Mizuno, S., Katoh-Semba, R., Sadakata, T., Sano, Y., ... & Shinoda, Y. (2017). CAPS2 deficiency affects environmental enrichment-induced adult neurogenesis and differentiation/survival of newborn neurons in the hippocampal dentate gyrus. *Neuroscience Letters*, *661*, 121-125.

Juckel G, Manitz MP, Brüne M, Friebe A, Heneka MT, Wolf RJ. Microglial activation in a neuroinflammational animal model of schizophrenia--a pilot study. Schizophr Res. 2011 Sep;131(1-3):96-100. doi: 10.1016/j.schres.2011.06.018. Epub 2011 Jul 12. PMID: 21752601.

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018.

Diz-Chaves, Y., Astiz, M., Bellini, M. J., & Garcia-Segura, L. M. (2013). Prenatal stress increases the expression of proinflammatory cytokines and exacerbates the inflammatory response to LPS in the hippocampal formation of adult male mice. *Brain, behavior, and immunity*, *28*, 196–206. https://doi.org/10.1016/j.bbi.2012.11.013

Mitschik, S., Schierl, R., Nowak, D., & Jörres, R. A. (2008). Effects of particulate matter on cytokine production in vitro: a comparative analysis of published studies. Inhalation toxicology, 20(4), 399–414. https://doi.org/10.1080/08958370801903784

Silbajoris, R., Osornio-Vargas, A. R., Simmons, S. O., Reed, W., Bromberg, P. A., Dailey, L. A., & Samet, J. M. (2011). Ambient particulate matter induces interleukin-8 expression through an alternative NF-κB (nuclear factor-kappa B) mechanism in human airway epithelial cells. Environmental health perspectives, 119(10), 1379–1383. https://doi.org/10.1289/ehp.1103594

Musah, S., DeJarnett, N., & Hoyle, G. W. (2012). Tumor necrosis factor-α mediates interactions between macrophages and epithelial cells underlying proinflammatory gene expression induced by particulate matter. Toxicology, 299(2-3), 125–132. https://doi.org/10.1016/j.tox.2012.05.014

Gong, C., Chu, M., Yang, J., Gong, X., Han, B., Chen, L., Bai, Z., Wang, J., & Zhang, Y. (2022). Ambient fine particulate matter exposures and human early placental inflammation. Environmental pollution (Barking, Essex : 1987), 315, 120446. https://doi.org/10.1016/j.envpol.2022.120446

Longhin, E., Holme, J. A., Gualtieri, M., Camatini, M., & Øvrevik, J. (2018). Milan winter fine particulate matter (wPM2.5) induces IL-6 and IL-8 synthesis in human bronchial BEAS-2B cells, but specifically impairs IL-8 release. Toxicology in vitro : an international journal published in association with BIBRA, 52, 365–373. https://doi.org/10.1016/j.tiv.2018.07.016

Mortimer, K., Neugebauer, R., Lurmann, F., Alcorn, S., Balmes, J., & Tager, I. (2008). Air pollution and pulmonary function in asthmatic children: effects of prenatal and lifetime exposures. Epidemiology (Cambridge, Mass.), 19(4), 550–562. https://doi.org/10.1097/EDE.0b013e31816a9dcb

Wang, P., You, D., Saravia, J., Shen, H., & Cormier, S. A. (2013). Maternal exposure to combustion generated PM inhibits pulmonary Th1 maturation and concomitantly enhances postnatal asthma development in offspring. Particle and fibre toxicology, 10, 29. https://doi.org/10.1186/1743-8977-10-29

Hua, L., Ju, L., Xu, H., Li, C., Sun, S., Zhang, Q., Cao, J., & Ding, R. (2023). Outdoor air pollution exposure and the risk of asthma and wheezing in the offspring. Environmental science and pollution research international, 30(6), 14165–14189. https://doi.org/10.1007/s11356-022-23094-6

Mattes, J., Murphy, V. E., Powell, H., & Gibson, P. G. (2014). Prenatal origins of bronchiolitis: protective effect of optimised asthma management during pregnancy. Thorax, 69(4), 383–384. https://doi.org/10.1136/thoraxjnl-2013-203388

Martel, M. J., Rey, E., Beauchesne, M. F., Malo, J. L., Perreault, S., Forget, A., & Blais, L. (2009). Control and severity of asthma during pregnancy are associated with asthma incidence in offspring: two-stage case-control study. The European respiratory journal, 34(3), 579–587. https://doi.org/10.1183/09031936.00074608

Murphy V. E. (2022). Asthma in pregnancy - Management, maternal co-morbidities, and long-term health. Best practice & research. Clinical obstetrics & gynaecology, 85(Pt A), 45–56. https://doi.org/10.1016/j.bpobgyn.2022.06.005

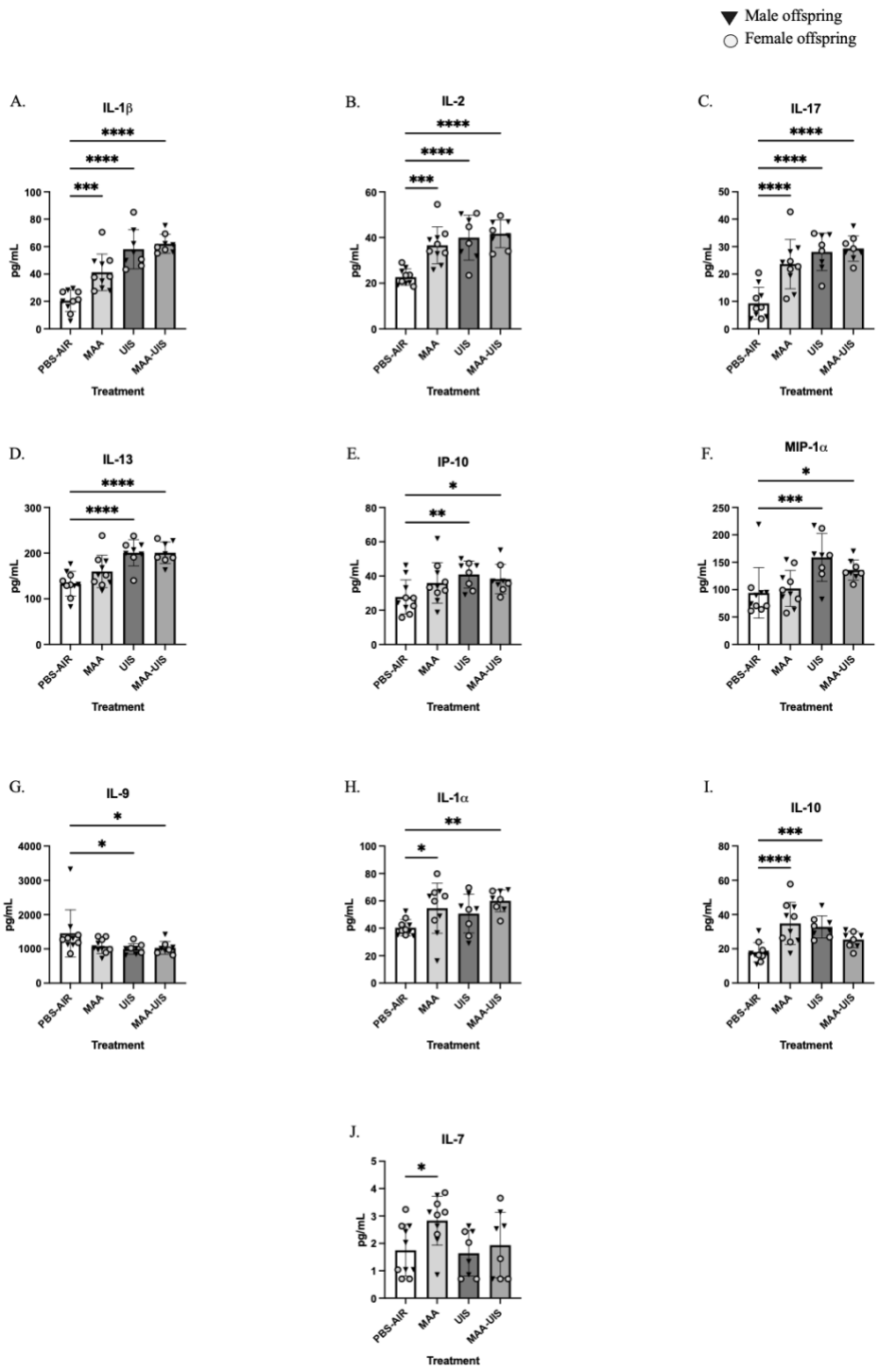

Figure 1. Cortical cytokine concentrations in P15 offspring brains

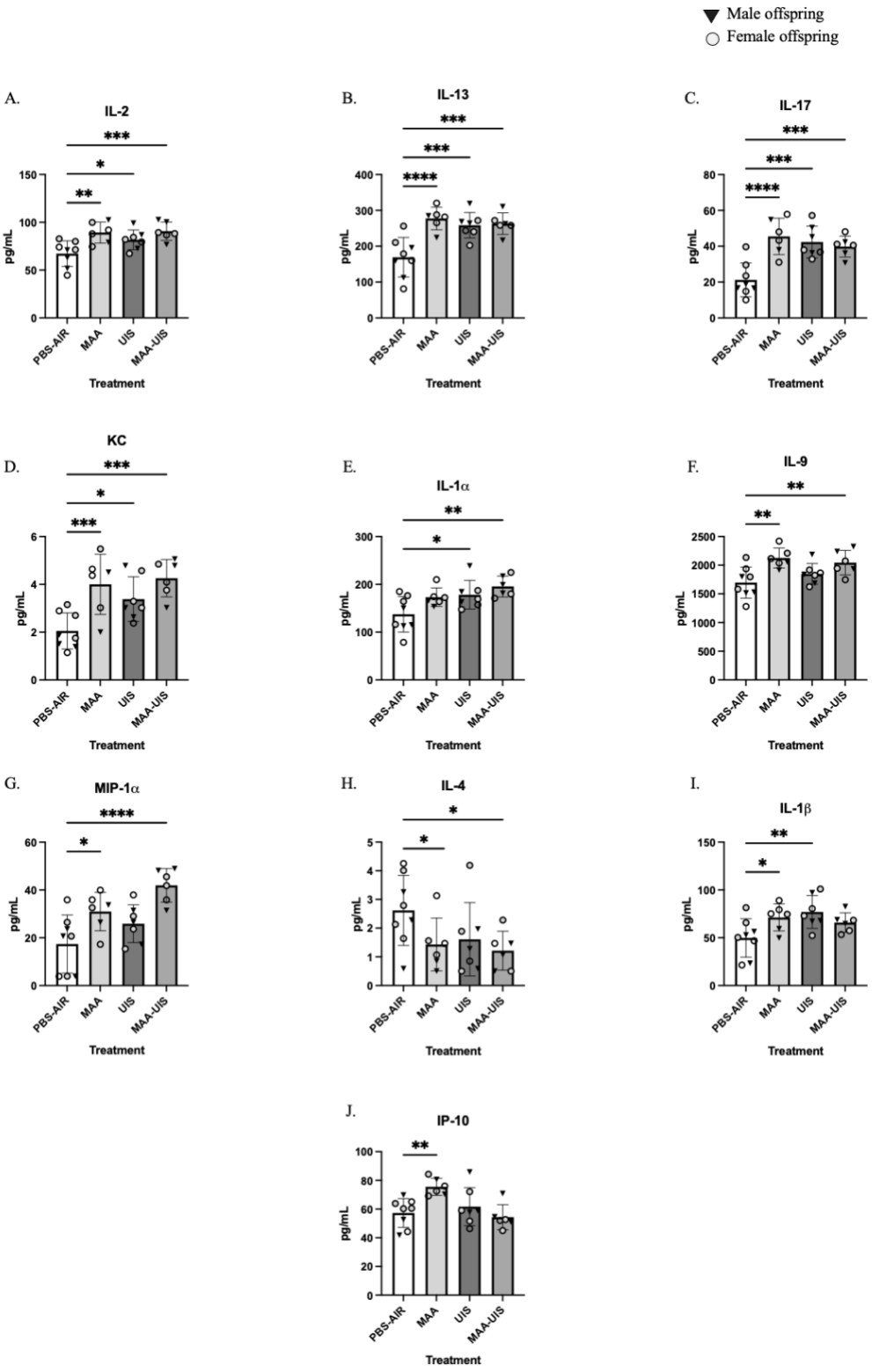

Figure 2. Cortical cytokine concentrations in P35 offspring brains

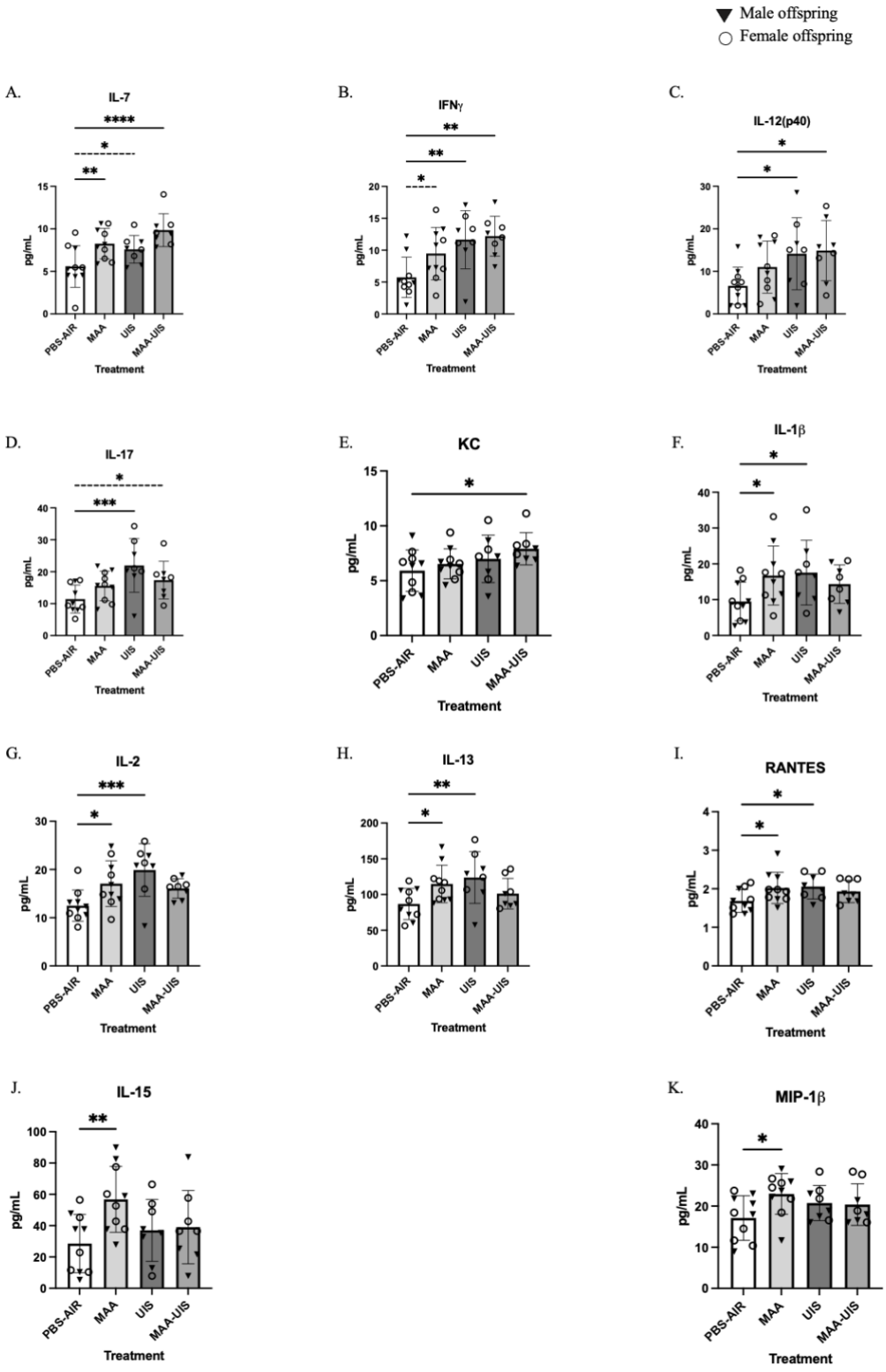

Figure 3. Hippocampal cytokine concentrations in P15 offspring brains

Figure 1. Cortical cytokine concentrations in P15 offspring brains exposed to PBS-AIR, MAA, UIS, or MAA-UIS. Cytokines were assessed using multiplex bead-based immunoassay. (A) IL-1β, (B) IL-2, (C) IL-17, (D) IL-13, (E) IP-10, (F) MIP-1α, (G) IL-19, (H) IL-1α, (I) IL-10, and (J) IL-7 are represented as pg/mL after being normalized to total protein content. Statistical significance determined by multilevel mixed-effects modeling.

Figure 2. Cortical cytokine concentrations in P35 offspring brains exposed to PBS-AIR, MAA, UIS, or MAA-UIS. Cytokines were assessed using multiplex bead-based immunoassay. (A) IL-2, (B) IL-13, (C) IL-17, (D) KC, (E) IL-1α, (F) IL-9, (G) MIP-1α, (H) IL-1, (I) IL-1β, and (J) IP-10 are represented as pg/mL after being normalized to total protein content. Statistical significance determined by multilevel mixed-effects modeling.

Figure 3. Hippocampal cytokine concentrations in P15 offspring brains exposed to PBS-AIR, MAA, UIS, or MAA-UIS. Cytokines were assessed using multiplex bead-based immunoassay. (A) IL-7, (B) IFNγ, (C) IL-12(p40), (D) IL-17, (E) KC, (F) IL-1β, (G) IL-2, (H) IL-13, (I) RANTES, (J) IL-15, (K) MIP-1β are represented as pg/mL after being normalized to total protein content. Statistical significance determined by multilevel mixed-effects modeling.

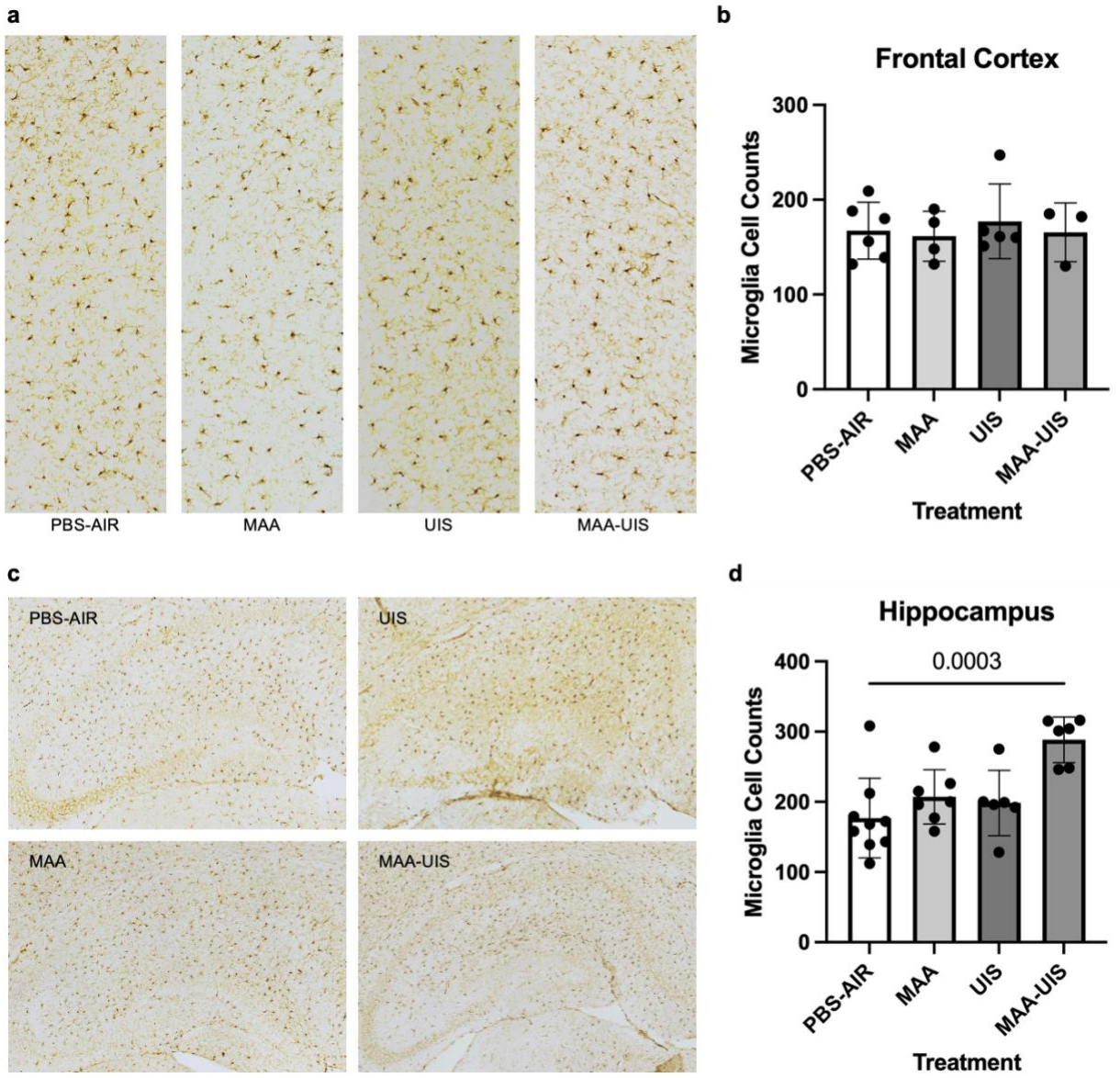

Figure 4. (a and c) Staining for IBA-1 with diaminobenzidine (DAB) was used to label microglia in coronal sections of p15 mice in the (a) frontal cortex and (b) hippocampus. (b and d) Quantification of microglial density in the (c) frontal cortex and (d) hippocampus. Statistical significance determined via one-way ANOVA. In the frontal cortex, n=6 (PBS-AIR), n=4 (MAA), n=5 (UIS), n=3 (MAA-UIS). In the hippocampus, n=9 (PBS-AIR), n=7 (MAA), n=6 (UIS), n=6 (MAA-UIS).

Chapter 3: Particulate Matter Exposure and Allergen Sensitization During Preconception: Is There a Synergistic Impact on Offspring Neurodevelopment?

Abstract:

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by social impairments and communication deficits, along with repetitive and restricted behaviors. Epidemiology studies suggests that cases of ASD are on the rise, with 1 in 44 children born in the United States later being diagnosed with ASD (Maenner et al., 2021). Much like the rising incidence of ASD, rates of asthma diagnosis are on the rise, and studies have identified that the presence of asthma during pregnancy increases likelihood of birthing a child that is later diagnosed with ASD. Air pollution has been well studied for its ability to exacerbate existing asthma, but it has also been linked to worsening of asthma symptoms when exposure occurs at asthma onset or allergen sensitization. Previously, we showed that maternal allergic asthma (MAA) using ovalbumin can have lasting impacts on offspring behavior and neurobiology. However, it has yet to be demonstrated whether these changes can be mirrored using a human allergen, such as house dust mite allergen (HDM). Additionally, because asthma severity is linked to increased symptom severity of ASD in humans, we suspect that PM exposure during allergen sensitization will increase asthma symptom severity in dams, which will result in increased inflammation during pregnancy and altered cytokine responses in offspring. In the current study, female BALB/c mice were exposed to PM during allergen sensitization to HDM prior to pregnancy. Following mating, pregnant mice were challenged with HDM at gestational days (GD)12 and GD15. Offspring brains from these treated dams were collected at postnatal days (P)15. Cortical regions were then isolated and assessed for changes in cytokines using a

Luminex bead-based multiplex assay. Analyses identified a decrease in cortical IFNγ in the PM alone treatment group and PM with HDM sensitization group (HDM/PM). We also identified elevated MIP-2 in the cortex of HDM/PM offspring. Using this novel MAA paradigm, we demonstrate that PM exposure during asthma sensitization with HDM, in preconception, and subsequent HDM allergic challenge, may synergistically impact neurodevelopment of offspring.

1.0 Introduction:

Autism spectrum disorders (ASD) are a group of heterogenous neurodevelopmental disorders that begin to manifest in early childhood and consist of social impairments and communication deficits, along with repetitive and restricted behaviors. The severity of these behaviors can vary widely among individuals, can have lifelong impacts on mental and physical wellbeing of ASD individuals (Simonoff et al., 2008; Zafeiriou et al., 2007), and is considered to have a male dominance (Kim et al., 2011; Werling et al., 2016). ASD represents a serious concern for society, with current estimates of 1 in 44 children born in the US later diagnosed with ASD (Maenner et al., 2021). Additionally, ASD can have a serious impact on the lives of the individuals, their families, and the healthcare system (Kogan et al., 2008). A single etiology for ASD remains elusive, however, a mix of common genetic variants and environmental exposures is most often postulated as the most likely cause (Hughes et al., 2023). While many studies have identified genetic variants and groups of variants that can be associated with ASD (Pugsley et al., 2022; Gaugler et al., 2014), these represent only a fraction of cases (Devlin et al., 2011; Klei et al., 2012). In addition to the genetic contribution to cases of ASD, many clinical studies have identified environmental factors associated with ASD risk, and/or the impact severity of behavioral impairments (Hughes et al., 2018; Rossignol et al., 2014; Straughen et al., 2021; Raz et al., 2015; Volk et al., 2011; Volk et al., 2020; Patterson et al., 2009). A common underlying theme among many of these environmental risk factors is the activation of the maternal immune system. Indeed, both clinical and animal models have found that maternal inflammation during pregnancy can increase incidence of neurodevelopmental disorders (NDD) in offspring, including ASD (Hughes et al., 2018; Rossignol et al., 2014; Straughen et al., 2021; Raz et al., 2015; Volk et al., 2011; Volk et al., 2020; Patterson et al., 2009). Maternal exposure to

toxicants and air pollution, as well as immune conditions such as autoimmunity and asthma have all been linked to NDD (Han et al., 2021). Although animal models of infection are widely used to investigate maternal inflammation and links to NDD in offspring, recent meta-analyses and clinical reports do not necessarily support infection as a causal factor for ASD incidence (Atladóttir et al., 2010; Brown et al., 2014; Jiang et al, 2016; Brynge et al., 2022).

Interestingly, similar to rates of ASD, asthma rates are also on the rise (Clougherty et al., 2007; Matsui et al., 2008). Compared to the T helper type 1 (T_H1) inflammatory response initiated by infection, allergic asthma primarily represents a T helper type 2 (T_H2) dominant immune mediated response. Recent epidemiological studies have identified a link between maternal asthma during pregnancy and later ASD diagnosis in offspring (Ali & Ulrik, 2013; Murphy, et al., 2005; Murphy et al., 2006; Croen et al., 2005; Croen et al., 2019; Gong et al., 2019; Abdallah et al., 2011; Hisle-Gorman et al., 2018; Lyall et al., 2014; Patel et al., 2020; Fasmer et al., 2011). Importantly, asthma was found to be linked to increased risk for ASD even when treated (Croen et al., 2019). Asthma is a heterogenous airway disease most commonly characterized by bronchial hyperresponsiveness that leads to constriction, obstruction, and airway remodeling (Gans and Gavrilova, 2020; Popa et al., 2021; Mims 2015). Much like ASD, there does not appear to be a single cause of asthma, but genetic and environmental factors have both been associated (Harb et al., 2015; Mims 2015). Allergic asthma develops through sensitization, or initial immune recognition of a foreign body (allergen), and that leads to a hypersensitivity response to subsequent exposures, or challenges, to the same allergen. Allergens of allergic asthma can vary, but one of the most widely studied, and one of the most common airborne allergens in industrialized countries, is house dust mite (*Dermatophagoides sp*., HDM)

(Jacquet A. 2013; Erwin & Platts-Mills 2005). It has been estimated that over 50% of asthmatics are sensitized to HDM (de Vries et al., 2005). Currently, there are no studies investigating HDM associated allergic asthma during pregnancy on offspring neurodevelopment and given the link between maternal asthma and ASD incidence, this is an understudied area that needs further investigation.

Using a mouse model of maternal allergic asthma (MAA), initiated by ovalbumin (OVA) sensitization and challenge, we demonstrated that MAA during pregnancy results in disrupted neurobiology in offspring, and altered behaviors (Schwartzer et al., 2015; Schwartzer et al., 2017; Ciernia et al., 2018; Church et al., 2021; Tamayo et al., 2022). The offspring of MAA dams display two core behaviors associated with ASD, specifically a decrease in social interaction and increased repetitive-like behaviors (Schwartzer et al., 2015; Church et al., 2021). Additionally, MAA offspring show elevations in pro-inflammatory cytokines in fetal brains, and disrupted cytokine signaling was also reported in adult offspring (Tamayo et al., 2022; Church et al., 2021). Transcriptional changes in microglia regulatory genes, some of which are among the top SFARI risk genes for ASD, were also shown in adult offspring (Ciernia et al., 2018). Taken together, these data suggest that MAA results in neurobiological changes that begin in utero and can persist into adulthood. However, this has not been demonstrated in a model using a more common human related airborne allergen such as HDM.

Air pollution has been well studied for its ability to exacerbate asthma symptoms (Wu et al., 2018). Air pollution includes many components, such as traffic related air pollution (TRAP), gaseous compounds, and particulate matter (PM). Separately, each of these have been shown to

impact lung function, and have been linked to airway-hyperresponsiveness, induction of oxidative stress, and airway remodeling (Guarnieri & Balmes 2014). Not only have studies investigated its ability to exacerbate established asthma, but it has also been linked to worsening of asthma symptoms when exposure occurs at asthma onset or allergen sensitization (Mortimer et al., 2008; Bowatte et al., 2015; Fuertes & Heinrich 2015). This phenomenon has now been demonstrated in animal models. In a mouse model, it was shown that allergic asthma initiated through either OVA or HDM sensitization in combination with PM exposure was able to worsen asthma symptoms when subsequent challenge occurred with allergen only (Castaneda et al. 2017; Castañeda et al., 2018). Previously, we showed that MAA using OVA results in neurobiological impacts on offspring that can last into adulthood (Schwartzer et al., 2015; Schwartzer et al., 2017; Ciernia et al., 2018; Church et al., 2021; Tamayo et al., 2022). However, it has yet to be shown whether these changes can be mirrored using a human allergen, such as HDM. Additionally, because asthma severity is linked to increased symptom severity of ASD in humans, we suspect that PM exposure during allergen sensitization will increase asthma symptom severity in dams, which will result in increased inflammation during pregnancy and altered cytokine responses in offspring. Using this novel MAA paradigm, we demonstrate that HDM alone can alter neurobiology of offspring, and that PM exposure during asthma sensitization with HDM can exacerbate this response.

2.0 Methods:

2.1 Animals

Male and female BALB/C mouse breeding pairs were purchased from Envigo Laboratories (Livermore, CA, USA) and maintained at University of California, Davis at the Center for Health and the Environment (Davis, CA, USA). Mice were housed with same-sex littermates in ventilated cages prior to breeding and kept on a 12-hour light/dark cycle at 23°C ambient room temperature and food and water provided *ad libitum*. Mice were bred at 8 weeks of age. All procedures were performed with approval by University of California Davis Institutional Animal Care and Use Committee and according to guidelines established by National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2 Ambient PM collection and extraction

Ambient PM collection was performed in summer of 2011 at a sampling site located in Downtown Sacramento, CA. The location is a two-story building on the northeast corner of T St. and 13th St. The location is surrounded by a mixture of residential, commercial, and industrial sources, and is roughly a quarter mile from a major freeway interchange. Inductively coupled ion mass spectrophotometry (IC-MS) was used to identify chemical composition, water soluble inorganic and organic ions identified by ion chromatography and atomic absorption spectrophotometry (AAS), thermal desorption gas chromatography-mass spectroscopy (GC-MS) used for molecular organic compound identification, and thermal optical reflectance for elemental and organic carbon identification.

2.3 Particulate matter and house dust mite exposure

Female mice were randomly separated into 4 groups and allergen sensitization was performed with a) 33.3 μg PM, b) 25 μg house dust mite, c) 33.3 μg PM and 25 μg house dust mite, or d) 25 μL PBS control, intranasally three times, every other day, during the span of 1 week, 1 week prior to mating. At 10 weeks of age, mice were mated and checked for seminal plugs, and plug presence was noted as GD0. During pregnancy, dams were challenged with intranasal instillations of 25 μL of HDM or 25 μL of PBS control on gestational days 12 and 15. The sensitization/challenge groups were thus as follows: (1) PBS/PBS (2) PM/PBS, (3) HDM/HDM, or (4) HDM + PM/HDM

2.4 Maternal serum, placenta, and fetal brain tissue collection

Pregnant mice on GD15 (3-7 dams per group) were euthanized and cardiac puncture was performed immediately to collect 500 ml of whole blood collected, which was allowed to clot for 30 minutes at room temperature. Blood was then centrifuged for 10 minutes at 10,000 x g at 4°C. Serum was harvested and stored at -80°C. In addition, each placenta and fetal brain was extracted, flash frozen in liquid nitrogen, and individually stored at -80°C until further processing. Additional pregnant mice per conditions were left undisturbed and allowed to give birth normally.

2.5 Cardiac perfusion and brain tissue collections of P15 offspring

At postnatal days 15 and 35, offspring were collected from their home cages, anesthetized using isoflurane (2-4% inhalation) and underwent transcardial perfusion. Briefly, a lateral incision was made in the abdominal wall below the rib cage. With curved scissors, an incision was made in

the diaphragm and cuts were made along the ribs to the collarbone to allow the sternum to be lifted. Once exposed, the heart was inserted with a 15-gauge perfusion needle into the ascending aorta for entry of perfuse, and an incision was made into the right atrium to create an outlet for drainage. Using a perfusion pump, 20 mL of PBS was slowly pumped through the circulatory system to reach adequate clearing. Whole brains were removed and dissected into hemispheric halves and one half was further dissected into cortical, hippocampal, and cerebellar regions, flash frozen with liquid nitrogen and stored at -80°C for later use. The remaining half was placed in 4% PFA for fixation, 24 hours following this, the halves were then placed in 30% sucrose for 24 hours for cryoprotection. Cryoprotected tissues were then embedded in optimal cutting temperature (O.C.T.) media and frozen at -80°C.

2.6 Multiplex bead-based cytokine analysis

Maternal serum cytokines and cortical sections from P15 offspring were measured using a multiplex mouse 25-plex bead-based immunoassay. The concentrations of the following cytokine were assessed: G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, KC, MCP-1, MIP-1α, MIP-1β, MIP-2, RANTES, and TNF-α. Standards and reagents were prepared following Milliplex Mouse cytokine/chemokine magnetic bead protocol. All serum samples were run in duplicate. 25 µl of serum or standard was added to each well containing assay buffer or matrix solution after washing and incubated overnight with antibody-coupled magnetic beads. The following day, the plate was washed according to the protocol using a handheld magnetic plate and incubated with a biotinylated detection antibody for 1 hour on a shaker. The plate was then incubated with streptavidin-phycoerythrin for 30 minutes on a shaker. The plate was washed following protocol

instructions and analyzed on a Bio-Rad Bio-Plex 200 plate reader (Bio-Rad Laboratories, Hercules, CA, USA). The following are the minimal amounts of detectable cytokine concentrations: G-CSF: 1.7 pg/mL; GM-CSF: 10.9 pg/mL; IFNγ: 1.1 pg/mL; IL-1α: 10.3 pg/mL; IL-1β: 5.4 pg/mL; IL-2: 1.0 pg/mL; IL-4: 0.4 pg/mL; IL-5: 1.0 pg/mL; IL-6: 1.1 pg/mL; IL-7: 1.4 pg/mL; IL-9: 17.3 pg/mL; IL-10: 2.0 pg/mL; IL-12 (p40): 3.9 pg/mL; IL-12 (p70): 4.8 pg/mL; IL-13: 7.8 pg/mL; IL-15: 7.4 pg/mL; IL-17: 0.5 pg/mL; IP-10: 0.8 pg/mL; KC: 2.3 pg/mL; MCP-1: 6.7 pg/mL; MIP-1α: 7.7 pg/mL; MIP-1β: 11.9 pg/mL; MIP-2: 30.6 pg/mL; RANTES: 2.7 pg/mL; TNF-α: 2.3 pg/mL. Sample concentrations that fell below minimal detection value were given a proxy value of half the limit of detection for statistical comparisons.

2.7 Statistical analyses

Data are expressed as means ± standard error of the mean (SEM). All comparisons were assessed by one-way ANOVA followed by Fisher's LSD test using GraphPad PrismVersion 9.5.1 (GraphPad Software, San Diego, CA, USA). A value of $P < 0.05$ was considered statistically significant.

3.0 Results:

3.1 Maternal serum cytokine concentrations

Following the final allergen induction at GD15, the maternal sera was collected from dams of all treatment groups and analyzed for cytokine concertation. Maternal serum from dams exposed to house dust mite (HDM) and particulate matter (PM) showed increased levels of T_H2 allergic asthma associated cytokine IL-5 ($p = 0.0001$) compared to PBS control group. IL-5 was also elevated in HDM group compared to PBS control ($p = 0.0159$), confirming allergic inflammation in response to HDM treatment. Other cytokines with pro-inflammatory effects, MIP-1α and IP-10, were also significantly increased in the HDM/PM dam serum compared to the control group ($p = 0.0411$; $p = 0.0002$; respectively). IP-10 was also elevated in the HDM group compared to PBS control ($p = 0.0054$). G-CSF, which has both pro and anti-inflammatory effects, was significantly elevated in the HDM/PM ($p = 0.0041$) group compared to the control group, and HDM group compared to control ($p = 0.0165$). (Fig. 1)

3.2 P15 Offspring Cortical cytokines

Offspring brains were collected, and cortical sections were removed at P15. Homogenates of each cortical section were then analyzed for cytokine concentration. One-way ANOVA was used to compare means of each treatment group. Two cytokines were found to be significantly altered compared to PBS control. IFNγ was decreased in both the HDM/PM treatment group ($p = 0.0120$) and PM ($p = 0.0055$) treatment groups compared to PBS control. Conversely, MIP-2 was found to be elevated in the HDM/PM treatment group when compared to PBS control ($p = 0.0077$), but also compared to PM treatment alone ($p = 0.0042$), and HDM treatment alone ($p = 0.0155$), suggesting a synergistic effect of the dual sensitization treatment. (Fig. 2)

4.0 Discussion:

There is now substantial evidence that MAA during pregnancy can have lasting impacts on the neurodevelopment of offspring, with evidence from epidemiological studies identifying the potential for asthma severity worsening neurodevelopmental outcomes. Based on previous studies of MAA, and studies demonstrating that PM exposure during allergen sensitization can increase symptom severity, we hypothesized that combined PM exposure with HDM allergen sensitization, and MAA exposure during pregnancy, would result in worse neurobiological outcomes in offspring than MAA alone. We report here that this exposure paradigm may result in cytokine dysregulation in the cortex of P15 offspring, and these changes in cytokine concentration could be evidence for the presence of aberrant behaviors in offspring.

Allergic asthma is commonly characterized by lung inflammation, airway hyperresponsiveness, and bronchoconstriction, along with local and systemic elevations in inflammatory cytokines (Gans and Gavrilova, 2020; Popa et al., 2021; Mims 2015). In this novel model of MAA using HDM sensitization and challenge, we identified systemic elevation in some cytokines associated with an allergic response. Specifically, we identified elevations in IL-5, which is a key cytokine involved in the inflammatory response in allergic asthma, and is crucial for the recruitment, development, activation, and survival of eosinophils (O'Byrne et al., 2001; Principe et al., 2021). IL-5 appears to have a significant link to HDM mediated allergic asthma specifically, and elevated presence of IL-5 may be used as an early identifying marker of HDM allergic asthma in human children (Weber-Chrysochoou et al., 2014; Weber-Chrysochoou et al., 2007). Elevations in IL-4 and IL-13, the other two cytokine mediators associated with human HDM allergic asthma, appears to occur over time with subsequent exposures (Weber-

Chrysochoou et al., 2014; Weber-Chrysochoou et al., 2007). It may be that, because our paradigm used 2 HDM allergen challenges, compared to more chronic models of HDM allergic asthma that use 3 or more challenges, this was not enough to see elevations in IL-4 and IL-13 as has been previously described in other HDM asthma mouse models (Sun et al., 2020; Malaviya et al., 2021; Rothenberg et al., 2011). Additionally, HDM allergen can vary between lot numbers and different lots may produce differences in sensitization and immune response upon challenge (Cyphert-Daly et al., 2019). We also identified increases in MIP-1a, which has been seen elevated in the bronchoalveolar lavage fluid of asthma patients (Alam et al., 1996). In peripheral blood mononuclear cells (PBMC) isolated from intermittent and severe asthma patients, MIP-1a was found to be increased (Rojas-Dotor et al., 2013). IP-10 has also been identified as playing a role in asthma and is increased in persistent asthma patients and contributes to airway inflammation in mouse models of asthma (Rojas-Dotor et al., 2013; Medoff et al., 2002). Similar to our model, others have described IP-10 increases in response to HDM allergen exposure in mice (Mohamed et al., 2015; Bunting et al., 2013). G-CSF is more commonly associated with eosinophilic asthma but there is evidence of its elevation as an early response to HDM (Bunting et al., 2013; Kim et al., 2020). Taken together, our model shows moderate increases in asthma associated cytokines in response to HDM asthma challenge during pregnancy.

These increases in maternal serum cytokines resulted in neuro-immune signaling changes in juvenile offspring brains. Specifically, we identified a decrease in IFNγ in the PM alone and HDM/PM groups, and an increase in MIP-2 in the HDM/PM group at P15. Generally, there is pleiotropy associated with cytokines of the CNS, and indeed, IFNγ has been identified in neurodegeneration and tissue destruction (Strickland et al., 2018; Wolf et al. 2002), but it has

also been identified with neuroprotection (Garg et al., 2009; Kipnis et al., 2002). Filiano et al. found that IFNγ deficient mice had social deficits, but not anxiety or motor deficits (Filiano et al., 2016). These IFNγ deficient mice also displayed aberrant hyperconnectivity in fronto-cortical/insular regions, and it was shown that attenuating IFNγ in neurons of the prefrontal cortex decreased social preference in the 3-chamber social approach task (Filiano et al., 2016). Interestingly, social deficits are one of the core features of ASD, and hyperconnectivity has also been identified in some regions of the cortex in ASD individuals (Conti et al., 2017; Maximo et al., 2014; Mills et al., 2016). Given the previously described connections between decreased IFNγ concentrations in the cortex and resulting behavioral alterations, mice from our HDM MAA model will need to be assessed for ASD relevant behaviors in future studies.

Although we demonstrate that both PM exposure alone and HDM/PM exposure resulted in decreased IFNγ concentration, the potential for the neurodevelopmental dysfunction in the HDM/PM group may be worse, as evidenced by the synergistic impact that HDM/PM exposure had on the concentration of MIP-2 in the cortex of P15 offspring. MIP-2 is released by astrocytes and microglia in the brain, has associations with demyelination, and could have cytotoxic effects on neurons (Sharafeldin et al., 2000; Kalehua et al., 2004; Zhao et al., 2021). It is most often associated with a neuroinflammatory response, which has been demonstrated in models of LPS-induced neuroinflammation (Zhao et al., 2021; Astrup et al., 2019) and could promote blood-brain barrier breakdown allowing peripheral immune cell recruitment into the brain (Kalehua et al., 2004). Taken together, the decrease in IFNγ and the increase in MIP-2 in the cortex of HDM/PM offspring suggest disruptions in offspring neurodevelopment as a result of

preconception PM exposure and asthma development, highlighting a need for further investigation.

Together findings represent only a snapshot of the impact HDM/PM sensitization and MAA during pregnancy has on offspring neurodevelopment, additional studies are planned in the remaining tissues that were collected from this study. To expand on the current findings of cortical cytokine changes; we will also analyze hippocampal and cerebellar brain sections for cytokine analysis at P15. Our previous findings in MAA demonstrated elevated fetal brain cytokines taken at gestational day (GD)17.5, as well as changes in placental cytokines at the same timepoint (Tamayo et al., 2022). In this study, we collected fetal brains and placenta at GD15, and will extend our previous findings using this novel model of MAA. We also reported previously that MAA results in neurobiological changes that persist into adulthood (Church et al., 2021). Cortical, hippocampal, and cerebellar brain sections were also collected at P35, which will inform on whether our findings persist into adulthood in this HDM/PM MAA model. Because our findings on IFNγ suggest the potential for hyperconnectivity, histological studies into synaptic connectivity in P15 and P35 offspring brains will also be performed. Additionally, analysis on maternal bronchoalveolar lavage fluid and maternal serum will be done to identify local inflammation and antibody titers associated with the allergic inflammatory response during pregnancy. The limitations of this preliminary study notwithstanding, we demonstrate here that PM exposure during HDM allergen sensitization, and subsequent MAA challenge in pregnancy, may result in worse neurodevelopmental impacts on offspring than MAA alone.

References:

Maenner, M.J.; Shaw, K.A.; Bakian, A.V.; Bilder, D.A.; Durkin,M.S.; Esler, A.; Furnier, S.M.; Hallas, L.; Hall-Lande, J.; Hudson, A.; et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018. Morb. Mortal. Wkly. Rep. Surveill. Summ. 2021, 70, 1–16.

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry*, *47*(8), 921–929. https://doi.org/10.1097/CHI.0b013e318179964f

Zafeiriou, D. I., Ververi, A., & Vargiami, E. (2007). Childhood autism and associated comorbidities. *Brain & development*, *29*(5), 257–272. https://doi.org/10.1016/j.braindev.2006.09.003

Kim, Y. S., Leventhal, B. L., Koh, Y. J., Fombonne, E., Laska, E., Lim, E. C., Cheon, K. A., Kim, S. J., Kim, Y. K., Lee, H., Song, D. H., & Grinker, R. R. (2011). Prevalence of autism spectrum disorders in a total population sample. *The American journal of psychiatry*, *168*(9), 904–912. https://doi.org/10.1176/appi.ajp.2011.10101532

Werling, D. M., Parikshak, N. N., & Geschwind, D. H. (2016). Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders. *Nature communications*, *7*, 10717. https://doi.org/10.1038/ncomms10717

Kogan, M. D., Strickland, B. B., Blumberg, S. J., Singh, G. K., Perrin, J. M., & van Dyck, P. C. (2008). A national profile of the health care experiences and family impact of autism spectrum disorder among children in the United States, 2005-2006. *Pediatrics*, *122*(6), e1149–e1158. https://doi.org/10.1542/peds.2008-1057

Hughes, H. K., R J Moreno, & Ashwood, P. (2023). Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain, behavior, and immunity*, *108*, 245–254. https://doi.org/10.1016/j.bbi.2022.12.001

Pugsley, K., Scherer, S. W., Bellgrove, M. A., & Hawi, Z. (2022). Environmental exposures associated with elevated risk for autism spectrum disorder may augment the burden of deleterious de novo mutations among probands. *Molecular psychiatry*, *27*(1), 710–730. https://doi.org/10.1038/s41380-021-01142-w

Gaugler, T., Klei, L., Sanders, S. J., Bodea, C. A., Goldberg, A. P., Lee, A. B., Mahajan, M., Manaa, D., Pawitan, Y., Reichert, J., Ripke, S., Sandin, S., Sklar, P., Svantesson, O., Reichenberg, A., Hultman, C. M., Devlin, B., Roeder, K., & Buxbaum, J. D. (2014). Most genetic risk for autism resides with common variation. *Nature genetics*, *46*(8), 881–885. https://doi.org/10.1038/ng.3039

Devlin, B., Melhem, N., & Roeder, K. (2011). Do common variants play a role in risk for autism? Evidence and theoretical musings. Brain research, 1380, 78–84. https://doi.org/10.1016/j.brainres.2010.11.026

Klei, L., Sanders, S. J., Murtha, M. T., Hus, V., Lowe, J. K., Willsey, A. J., Moreno-De-Luca, D., Yu, T. W., Fombonne, E., Geschwind, D., Grice, D. E., Ledbetter, D. H., Lord, C., Mane, S. M., Martin, C. L., Martin, D. M., Morrow, E. M., Walsh, C. A., Melhem, N. M., Chaste, P., … Devlin, B. (2012). Common genetic variants, acting additively, are a major source of risk for autism. Molecular autism, 3(1), 9. https://doi.org/10.1186/2040-2392-3-9

Hughes, H. K., Mills Ko, E., Rose, D., & Ashwood, P. (2018). Immune Dysfunction and Autoimmunity as Pathological Mechanisms in Autism Spectrum Disorders. *Frontiers in cellular neuroscience*, *12*, 405. https://doi.org/10.3389/fncel.2018.00405

Rossignol, D. A., Genuis, S. J., & Frye, R. E. (2014). Environmental toxicants and autism spectrum disorders: a systematic review. *Translational psychiatry*, *4*(2), e360. https://doi.org/10.1038/tp.2014.4

Straughen, J. K., Sitarik, A. R., Johnson, C. C., Wegienka, G., Ownby, D. R., Johnson-Hooper, T. M., Allo, G., Levin, A. M., & Cassidy-Bushrow, A. E. (2021). Prenatal IgE as a Risk Factor for the Development of Childhood Neurodevelopmental Disorders. *Frontiers in pediatrics*, *9*, 601092. https://doi.org/10.3389/fped.2021.601092

Raz, R., Roberts, A. L., Lyall, K., Hart, J. E., Just, A. C., Laden, F., & Weisskopf, M. G. (2015). Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case-control analysis within the Nurses' Health Study II Cohort. *Environmental health perspectives*, *123*(3), 264–270. https://doi.org/10.1289/ehp.1408133

Volk, H. E., Hertz-Picciotto, I., Delwiche, L., Lurmann, F., & McConnell, R. (2011). Residential proximity to freeways and autism in the CHARGE study. *Environmental health perspectives*, *119*(6), 873–877. https://doi.org/10.1289/ehp.1002835

Volk, H. E., Park, B., Hollingue, C., Jones, K. L., Ashwood, P., Windham, G. C., Lurman, F., Alexeeff, S. E., Kharrazi, M., Pearl, M., Van de Water, J., & Croen, L. A. (2020). Maternal immune response and air pollution exposure during pregnancy: insights from the Early Markers for Autism (EMA) study. *Journal of neurodevelopmental disorders*, *12*(1), 42. https://doi.org/10.1186/s11689-020-09343-0

Patterson P. H. (2009). Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behavioural brain research*, *204*(2), 313–321. https://doi.org/10.1016/j.bbr.2008.12.016

Han, V. X., Patel, S., Jones, H. F., Nielsen, T. C., Mohammad, S. S., Hofer, M. J., Gold, W., Brilot, F., Lain, S. J., Nassar, N., & Dale, R. C. (2021). Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. *Translational psychiatry*, *11*(1), 71. https://doi.org/10.1038/s41398-021-01198-w

Atladóttir, H. O., Thorsen, P., Østergaard, L., Schendel, D. E., Lemcke, S., Abdallah, M., & Parner, E. T. (2010). Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *Journal of autism and developmental disorders*, *40*(12), 1423–1430. https://doi.org/10.1007/s10803-010-1006-y

Brown, A. S., Sourander, A., Hinkka-Yli-Salomäki, S., McKeague, I. W., Sundvall, J., & Surcel, H. M. (2014). Elevated maternal C-reactive protein and autism in a national birth cohort. *Molecular psychiatry*, *19*(2), 259–264. https://doi.org/10.1038/mp.2012.197

Jiang, H. Y., Xu, L. L., Shao, L., Xia, R. M., Yu, Z. H., Ling, Z. X., Yang, F., Deng, M., & Ruan, B. (2016). Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. *Brain, behavior, and immunity*, *58*, 165–172. https://doi.org/10.1016/j.bbi.2016.06.005

Brynge, M., Sjöqvist, H., Gardner, R. M., Lee, B. K., Dalman, C., & Karlsson, H. (2022). Maternal infection during pregnancy and likelihood of autism and intellectual disability in children in Sweden: a negative control and sibling comparison cohort study. *The lancet. Psychiatry*, *9*(10), 782–791. https://doi.org/10.1016/S2215-0366(22)00264-4

Clougherty, J. E., Levy, J. I., Kubzansky, L. D., Ryan, P. B., Suglia, S. F., Canner, M. J., & Wright, R. J. (2007). Synergistic effects of traffic-related air pollution and exposure to violence on urban asthma etiology. *Environmental health perspectives*, *115*(8), 1140–1146. https://doi.org/10.1289/ehp.9863

Matsui, E. C., Hansel, N. N., McCormack, M. C., Rusher, R., Breysse, P. N., & Diette, G. B. (2008). Asthma in the inner city and the indoor environment. *Immunology and allergy clinics of North America*, *28*(3), 665–x. https://doi.org/10.1016/j.iac.2008.03.004

Ali, Z., & Ulrik, C. S. (2013). Incidence and risk factors for exacerbations of asthma during pregnancy. *Journal of asthma and allergy*, *6*, 53–60. https://doi.org/10.2147/JAA.S43183

Murphy, V. E., Gibson, P., Talbot, P. I., & Clifton, V. L. (2005). Severe asthma exacerbations during pregnancy. *Obstetrics and gynecology*, *106*(5 Pt 1), 1046–1054. https://doi.org/10.1097/01.AOG.0000185281.21716.02

Murphy, V. E., Clifton, V. L., & Gibson, P. G. (2006). Asthma exacerbations during pregnancy: incidence and association with adverse pregnancy outcomes. *Thorax*, *61*(2), 169–176. https://doi.org/10.1136/thx.2005.049718

Croen, L. A., Grether, J. K., Yoshida, C. K., Odouli, R., & Van de Water, J. (2005). Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Archives of pediatrics & adolescent medicine*, *159*(2), 151–157. https://doi.org/10.1001/archpedi.159.2.151

Croen, L. A., Qian, Y., Ashwood, P., Zerbo, O., Schendel, D., Pinto-Martin, J., Daniele Fallin, M., Levy, S., Schieve, L. A., Yeargin-Allsopp, M., Sabourin, K. R., & Ames, J. L. (2019). Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. *Autism research : official journal of the International Society for Autism Research*, *12*(10), 1551–1561. https://doi.org/10.1002/aur.2175

Gong, T., Lundholm, C., Rejnö, G., Bölte, S., Larsson, H., D'Onofrio, B. M., Lichtenstein, P., & Almqvist, C. (2019). Parental asthma and risk of autism spectrum disorder in offspring: A population and family-based case-control study. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, *49*(6), 883–891. https://doi.org/10.1111/cea.13353

Abdallah, M. W., Larsen, N., Grove, J., Nørgaard-Pedersen, B., Thorsen, P., Mortensen, E. L., & Hougaard, D. M. (2013). Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, *14*(7), 528–538. https://doi.org/10.3109/15622975.2011.639803

Hisle-Gorman, E., Susi, A., Stokes, T., Gorman, G., Erdie-Lalena, C., & Nylund, C. M. (2018). Prenatal, perinatal, and neonatal risk factors of autism spectrum disorder. *Pediatric research*, *84*(2), 190–198. https://doi.org/10.1038/pr.2018.23

Lyall, K., Ashwood, P., Van de Water, J., & Hertz-Picciotto, I. (2014). Maternal immune-mediated conditions, autism spectrum disorders, and developmental delay. *Journal of autism and developmental disorders*, *44*(7), 1546–1555. https://doi.org/10.1007/s10803-013-2017-2

Patel, S., Dale, R. C., Rose, D., Heath, B., Nordahl, C. W., Rogers, S., Guastella, A. J., & Ashwood, P. (2020). Maternal immune conditions are increased in males with autism spectrum disorders and are associated with behavioural and emotional but not cognitive co-morbidity. *Translational psychiatry*, *10*(1), 286. https://doi.org/10.1038/s41398-020-00976-2

Fasmer, O. B., Halmøy, A., Eagan, T. M., Oedegaard, K. J., & Haavik, J. (2011). Adult attention deficit hyperactivity disorder is associated with asthma. *BMC psychiatry*, *11*, 128. https://doi.org/10.1186/1471-244X-11-128

Croen, L. A., Qian, Y., Ashwood, P., Zerbo, O., Schendel, D., Pinto-Martin, J., Daniele Fallin, M., Levy, S., Schieve, L. A., Yeargin-Allsopp, M., Sabourin, K. R., & Ames, J. L. (2019). Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. *Autism research : official journal of the International Society for Autism Research*, *12*(10), 1551–1561. https://doi.org/10.1002/aur.2175

Gans, M. D., & Gavrilova, T. (2020). Understanding the immunology of asthma: Pathophysiology, biomarkers, and treatments for asthma endotypes. *Paediatric respiratory reviews*, *36*, 118–127. https://doi.org/10.1016/j.prrv.2019.08.002

Mims J. W. (2015). Asthma: definitions and pathophysiology. *International forum of allergy & rhinology*, *5 Suppl 1*, S2–S6. https://doi.org/10.1002/alr.21609

Jacquet A. (2013). Innate immune responses in house dust mite allergy. ISRN allergy, 2013, 735031. https://doi.org/10.1155/2013/735031

Erwin, E. A., & Platts-Mills, T. A. (2005). Allergens. Immunology and allergy clinics of North America, 25(1), 1–14. https://doi.org/10.1016/j.iac.2004.09.008

de Vries, M. P., van den Bemt, L., van der Mooren, F. M., Muris, J. W., & van Schayck, C. P. (2005). The prevalence of house dust mite (HDM) allergy and the use of HDM-impermeable bed covers in a primary care population of patients with persistent asthma in the Netherlands. *Primary care respiratory journal : journal of the General Practice Airways Group*, *14*(4), 210–214. https://doi.org/10.1016/j.pcrj.2005.04.005

Schwartzer, J. J., Careaga, M., Chang, C., Onore, C. E., & Ashwood, P. (2015). Allergic fetal priming leads to developmental, behavioral and neurobiological changes in mice. Translational psychiatry, 5(4), e543. https://doi.org/10.1038/tp.2015.40

Schwartzer, J. J., Careaga, M., Coburn, M. A., Rose, D. R., Hughes, H. K., & Ashwood, P. (2017). Behavioral impact of maternal allergic-asthma in two genetically distinct mouse strains. Brain, behavior, and immunity, 63, 99–107. https://doi.org/10.1016/j.bbi.2016.09.007

Vogel Ciernia, A., Careaga, M., LaSalle, J. M., & Ashwood, P. (2018). Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. *Glia*, *66*(3), 505–521. https://doi.org/10.1002/glia.23261

Church, J. S., Tamayo, J. M., Ashwood, P., & Schwartzer, J. J. (2021). Repeated allergic asthma in early versus late pregnancy differentially impacts offspring brain and behavior development. Brain, behavior, and immunity, 93, 66–79. https://doi.org/10.1016/j.bbi.2020.12.014

Tamayo, J. M., Rose, D., Church, J. S., Schwartzer, J. J., & Ashwood, P. (2022). Maternal Allergic Asthma Induces Prenatal Neuroinflammation. *Brain sciences*, *12*(8), 1041. https://doi.org/10.3390/brainsci12081041

Wu, J. Z., Ge, D. D., Zhou, L. F., Hou, L. Y., Zhou, Y., & Li, Q. Y. (2018). Effects of particulate matter on allergic respiratory diseases. *Chronic diseases and translational medicine*, *4*(2), 95–102. https://doi.org/10.1016/j.cdtm.2018.04.001

Guarnieri, M., & Balmes, J. R. (2014). Outdoor air pollution and asthma. Lancet (London, England), 383(9928), 1581–1592. https://doi.org/10.1016/S0140-6736(14)60617-6

Mortimer, K., Neugebauer, R., Lurmann, F., Alcorn, S., Balmes, J., & Tager, I. (2008). Early-lifetime exposure to air pollution and allergic sensitization in children with asthma. *The Journal of asthma : official journal of the Association for the Care of Asthma*, *45*(10), 874–881. https://doi.org/10.1080/02770900802195722

Bowatte, G., Lodge, C., Lowe, A. J., Erbas, B., Perret, J., Abramson, M. J., Matheson, M., & Dharmage, S. C. (2015). The influence of childhood traffic-related air pollution exposure on asthma, allergy and sensitization: a systematic review and a meta-analysis of birth cohort studies. *Allergy*, *70*(3), 245–256. https://doi.org/10.1111/all.12561

Fuertes, E., & Heinrich, J. (2015). The influence of childhood traffic-related air pollution exposure on asthma, allergy and sensitization. *Allergy*, *70*(10), 1350–1351. https://doi.org/10.1111/all.12611

Castañeda, A. R., Bein, K. J., Smiley-Jewell, S., & Pinkerton, K. E. (2017). Fine particulate matter ($PM_{2.5}$) enhances allergic sensitization in BALB/c mice. *Journal of toxicology and environmental health. Part A*, *80*(4), 197–207. https://doi.org/10.1080/15287394.2016.1222920

Castañeda, A. R., Vogel, C. F. A., Bein, K. J., Hughes, H. K., Smiley-Jewell, S., & Pinkerton, K. E. (2018). Ambient particulate matter enhances the pulmonary allergic immune response to house dust mite in a BALB/c mouse model by augmenting Th2- and Th17-immune responses. *Physiological reports*, *6*(18), e13827. https://doi.org/10.14814/phy2.13827

O'Byrne, P. M., Inman, M. D., & Parameswaran, K. (2001). The trials and tribulations of IL-5, eosinophils, and allergic asthma. *Journal of allergy and clinical immunology*, *108*(4), 503-508.

Principe, S., Porsbjerg, C., Bolm Ditlev, S., Kjærsgaard Klein, D., Golebski, K., Dyhre-Petersen, N., ... & Maitland-van der Zee, A. H. (2021). Treating severe asthma: Targeting the IL-5 pathway. *Clinical & Experimental Allergy*, *51*(8), 992-1005.

Weber-Chrysochoou, C., Crisafulli, D., Kemp, A. S., Britton, W. J., Marks, G. B., & CAPS Investigators (2014). Allergen-specific IL-5 responses in early childhood predict asthma at age eight. *PloS one*, *9*(5), e97995. https://doi.org/10.1371/journal.pone.0097995

Weber-Chrysochoou, C., Crisafulli, D., Almqvist, C., Li, Q., Kemp, A. S., Britton, W. J., Marks, G. B., & Childhood Asthma Prevention Study Investigators (2007). IL-5 T-cell responses to house dust mite are associated with the development of allergen-specific IgE responses and asthma in the first 5 years of life. *The Journal of allergy and clinical immunology*, *120*(2), 286–292. https://doi.org/10.1016/j.jaci.2007.06.034

Sun, X., Hou, T., Cheung, E., Iu, T. N., Tam, V. W., Chu, I. M., Tsang, M. S., Chan, P. K., Lam, C. W., & Wong, C. K. (2020). Anti-inflammatory mechanisms of the novel cytokine interleukin-38 in allergic asthma. *Cellular & molecular immunology*, *17*(6), 631–646. https://doi.org/10.1038/s41423-019-0300-7

Malaviya, R., Zhou, Z., Raymond, H., Wertheimer, J., Jones, B., Bunting, R., Wilkinson, P., Madireddy, L., Hall, L., Ryan, M., & Rao, T. S. (2021). Repeated exposure of house dust mite induces progressive airway inflammation in mice: Differential roles of CCL17 and IL-13. *Pharmacology research & perspectives*, *9*(3), e00770. https://doi.org/10.1002/prp2.770

Rothenberg, M. E., Wen, T., Shik, D., Cole, E. T., Mingler, M. M., & Munitz, A. (2011). IL-13 receptor α1 differentially regulates aeroallergen-induced lung responses. *Journal of immunology (Baltimore, Md. : 1950)*, *187*(9), 4873–4880. https://doi.org/10.4049/jimmunol.1004159

Cyphert-Daly, J. M., Yang, Z., Ingram, J. L., Tighe, R. M., & Que, L. G. (2019). Physiologic response to chronic house dust mite exposure in mice is dependent on lot characteristics. *The Journal of allergy and clinical immunology*, *144*(5), 1428–1432.e8. https://doi.org/10.1016/j.jaci.2019.07.019

Alam, R., York, J., Boyars, M., Stafford, S., Grant, J. A., Lee, J., ... & Ida, N. (1996). Increased MCP-1, RANTES, and MIP-1alpha in bronchoalveolar lavage fluid of allergic asthmatic patients. *American journal of respiratory and critical care medicine*, *153*(4), 1398-1404.

Rojas-Dotor, S., Segura-Méndez, N. H., Miyagui-Namikawa, K., & Mondragón-González, R. (2013). Expression of resistin, CXCR3, IP-10, CCR5 and MIP-1α in obese patients with different severity of asthma. *Biological research*, *46*(1), 13-20.

Medoff, B. D., Sauty, A., Tager, A. M., Maclean, J. A., Smith, R. N., Mathew, A., ... & Luster, A. D. (2002). IFN-γ-inducible protein 10 (CXCL10) contributes to airway hyperreactivity and airway inflammation in a mouse model of asthma. *The Journal of Immunology*, *168*(10), 5278-5286.

Mohamed A. Ghonim, Kusma Pyakurel, Salome V. Ibba, Jeffrey Wang, Paulo Rodriguez, Amir A. Al-Khami, Matthew R. Lammi, Hogyoung Kim, Arnold H. Zea, Christian Davis, Samuel Okpechi, Dorota Wyczechowska, Kamel Al-Ghareeb, Moselhy S. Mansy, Augusto Ochoa, Amarjit S. Naura, A. Hamid Boulares; PARP is activated in human asthma and its inhibition by olaparib blocks house dust mite-induced disease in mice. *Clin Sci (Lond)* 1 December 2015; 129 (11): 951–962. doi: https://doi.org/10.1042/CS2015012

Bunting, R. A., Wertheimer, J., Zhou, Z., Cochlin, K., Deutsch, H., Raymond, H., Yurkow, E., Ogden, C. A., & Kilgore, K. (2013). In-depth assessment of acute and chronic house dust mite-induced asthma mouse models. *Journal of Inflammation (London, England)*, *10*(Suppl 1), P2. https://doi.org/10.1186/1476-9255-10-S1-P2

Kim, Y. M., Kim, H., Lee, S., Kim, S., Lee, J. U., Choi, Y., Park, H. W., You, G., Kang, H., Lee, S., Park, J. S., Park, Y., Park, H. S., Park, C. S., & Lee, S. W. (2020). Airway G-CSF identifies neutrophilic inflammation and contributes to asthma progression. *The European respiratory journal*, *55*(2), 1900827. https://doi.org/10.1183/13993003.00827-2019

O'Garra, A., Steinman, L., & Gijbels, K. (1997). CD4+ T-cell subsets in autoimmunity. Current opinion in immunology, 9(6), 872–883. https://doi.org/10.1016/s0952-7915(97)80192-6

Strickland, M. R., Koller, E. J., Deng, D. Z., Ceballos-Diaz, C., Golde, T. E., & Chakrabarty, P. (2018). Ifngr1 and Stat1 mediated canonical Ifn-γ signaling drives nigrostriatal degeneration. *Neurobiology of disease*, *110*, 133–141. https://doi.org/10.1016/j.nbd.2017.11.007

Wolf, S. A., Fisher, J., Bechmann, I., Steiner, B., Kwidzinski, E., & Nitsch, R. (2002). Neuroprotection by T-cells depends on their subtype and activation state. *Journal of neuroimmunology*, *133*(1-2), 72–80. https://doi.org/10.1016/s0165-5728(02)00367-3

Garg, S. K., Kipnis, J., & Banerjee, R. (2009). IFN-gamma and IL-4 differentially shape metabolic responses and neuroprotective phenotype of astrocytes. *Journal of neurochemistry*, *108*(5), 1155–1166. https://doi.org/10.1111/j.1471-4159.2009.05872.x

Kipnis, J., Mizrahi, T., Yoles, E., Ben-Nun, A., & Schwartz, M. (2002). Myelin specific Th1 cells are necessary for post-traumatic protective autoimmunity. *Journal of neuroimmunology*, *130*(1-2), 78–85. https://doi.org/10.1016/s0165-5728(02)00219-9

Filiano, A. J., Xu, Y., Tustison, N. J., Marsh, R. L., Baker, W., Smirnov, I., Overall, C. C., Gadani, S. P., Turner, S. D., Weng, Z., Peerzade, S. N., Chen, H., Lee, K. S., Scott, M. M., Beenhakker, M. P., Litvak, V., & Kipnis, J. (2016). Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour. *Nature*, *535*(7612), 425–429. https://doi.org/10.1038/nature18626

Conti, E.; Mitra, J.; Calderoni, S.; Pannek, K.; Shen, K.K.; Pagnozzi, A.; Rose, S.; Mazzotti, S.; Scelfo, D.; Tosetti, M.; et al. Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: A diffusion MRI study. Hum. Brain Mapp. 2017, 38, 2333–2344

Maximo, J.; Cadena, E.J.; Kana, R.K. The Implications of Brain Connectivity in the Neuropsychology of Autism. Neuropsychol. Rev. 2014, 24, 16–31

Mills, B.D.; Pearce, H.L.; Khan, O.; Jarrett, B.R.; Fair, D.A.; Lahvis, G.P. Prenatal domoic acid exposure disrupts mouse pro-social behavior and functional connectivity MRI. Behav. Brain Res. 2016, 308, 14–23.

Sharafeldin, A., Eltayeb, R., Pashenkov, M., & Bakhiet, M. (2000). Chemokines are produced in the brain early during the course of experimental African trypanosomiasis. *Journal of neuroimmunology*, *103*(2), 165–170. https://doi.org/10.1016/s0165-5728(99)00238-6

Kalehua, A. N., Nagel, J. E., Whelchel, L. M., Gides, J. J., Pyle, R. S., Smith, R. J., Kusiak, J. W., & Taub, D. D. (2004). Monocyte chemoattractant protein-1 and macrophage inflammatory protein-2 are involved in both excitotoxin-induced neurodegeneration and regeneration. *Experimental cell research*, *297*(1), 197–211. https://doi.org/10.1016/j.yexcr.2004.02.031

Zhao, X., Liu, S., Yang, X., Liu, Y., Liu, G., Fan, K., & Ma, J. (2021). Cathepsin C aggravates neuroinflammation via promoting production of CCL2 and CXCL2 in glial cells and neurons in a cryogenic brain lesion. *Neurochemistry international*, *148*, 105107. https://doi.org/10.1016/j.neuint.2021.105107

Astrup, L. B., Skovgaard, K., Rasmussen, R. S., Iburg, T. M., Agerholm, J. S., Aalbæk, B., Jensen, H. E., Nielsen, O. L., Johansen, F. F., Heegaard, P. M. H., & Leifsson, P. S. (2019). Staphylococcus aureus infected embolic stroke upregulates Orm1 and Cxcl2 in a rat model of septic stroke pathology. *Neurological research*, *41*(5), 399–412. https://doi.org/10.1080/01616412.2019.1573455

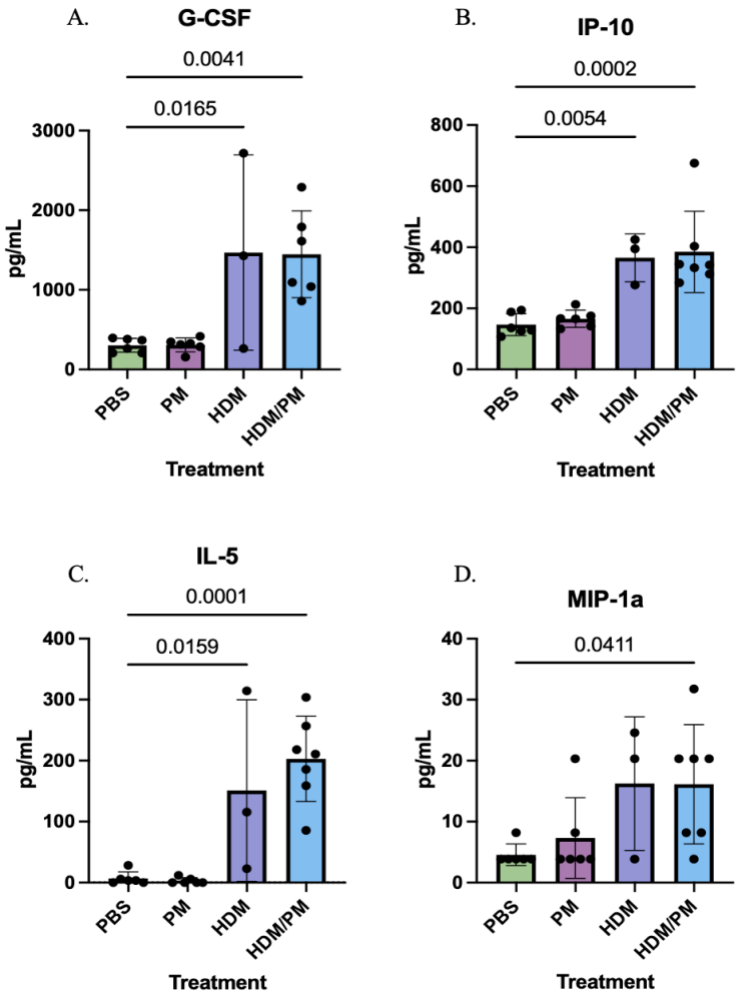

Figure 1*:* Cytokine concentrations in maternal serum of dams exposed to PBS, PM, HDM, or HDM/PM collected on GD15. Serum was collected before placenta and fetal offspring. Cytokine concentrations were assessed using a multiplex bead-based immunoassay. A) G-CSF, B) IP-10, C) IL-5, and D) MIP-1α are represented as pg/mL. Statistical significance determined by one-way ANOVA. PBS dams n=6, PM dams n=6, HDM dams n=3, HDM/PM dams n=7.

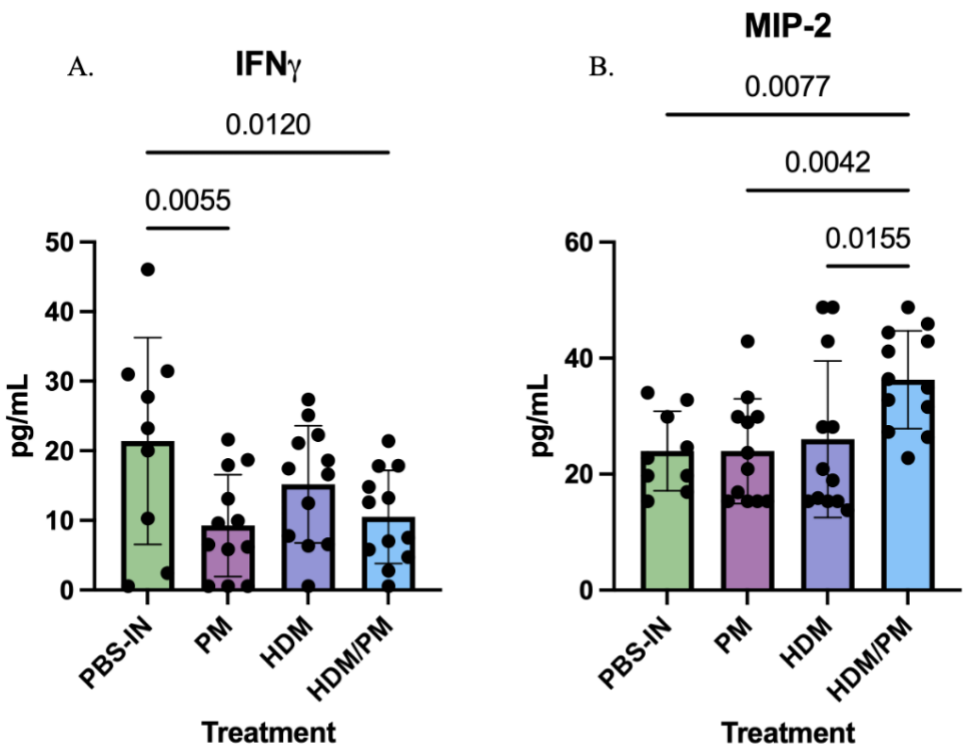

Figure 2: Cytokine concentrations in cortical sections from P15 offspring from dams treated with PBS, PM, HDM, or HDM/PM. Cytokine concentrations were assessed using a multiplex bead-based immunoassay. A) IFNγ, B) MIP-2. Statistical significance determined by one-way ANOVA. PBS n=9, PM n=12, HDM = 12, HDM/PM n=12.