Mental health and asthma control during pregnancy: Investigating underlying immune mechanisms

Isabella-Rose Sibly Meredith
BSc, B Health Science (Hons)

Submitted in fulfilment of the requirements for the degree of
M. Philosophy (Medical Science)

School of Paediatrics and Reproductive Health,
Discipline of Obstetrics and Gynaecology,
University of Adelaide
February 2015

Supervisors: Associate Professor Vicki Clifton, Dr Luke Grzeskowiak,
Dr Annette Osei-Kumah
**Declaration**

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

________________________  __________________
Isabella-Rose Meredith                  Date
Table of Contents

List of Figures .......................................................................................................................... 6
List of Tables ............................................................................................................................. 7
Acknowledgements ..................................................................................................................... 8
Abbreviations ............................................................................................................................. 9
Abstract ....................................................................................................................................... 12
Chapter 1: Literature Review .................................................................................................... 13
  1.1. Asthma and Exacerbations ................................................................................................. 13
  1.2. Asthma and Pregnancy ...................................................................................................... 15
  1.3. Mental Health and Asthma ............................................................................................... 15
  1.4. Immune Mechanisms of Asthma and Pregnancy ............................................................. 16
      1.4.1. Immune Cells and Asthma ....................................................................................... 16
        1.4.1.1. T lymphocytes ................................................................................................. 17
        1.4.1.2. Monocytes ....................................................................................................... 20
      1.4.2. Inflammation Subtypes in Asthma ........................................................................... 23
        1.4.2.1. Eosinophilic Inflammation ............................................................................. 23
        1.4.2.2. Neutrophilic Inflammation ............................................................................. 25
      1.4.3. Lung Function in Pregnancies Complicated by Asthma ........................................... 27
  1.5. Immune Mechanisms of Mental Health .......................................................................... 28
      1.5.1. Asthma and Mental Health ..................................................................................... 30
      1.5.2. Anxiety .................................................................................................................. 30
      1.5.3. Depression ............................................................................................................. 32
      1.5.4. Dopamine and Serotonin ....................................................................................... 32
      1.5.5. Asthma, Pregnancy and Mental Health ................................................................... 33
  1.6. Conclusion ....................................................................................................................... 37
  1.7. Research ........................................................................................................................... 37
      1.7.1. Knowledge Gap ..................................................................................................... 37
      1.7.2. Research Questions ............................................................................................... 38
      1.7.3. Hypotheses ............................................................................................................ 38
      1.7.4. Aims ....................................................................................................................... 38
Chapter 2: Methodology .......................................................................................................... 40
  2.1. Part A: Subject Recruitment and Assessment ................................................................. 40
      2.1.1. Subjects .................................................................................................................. 40
      2.1.2. Maternal Data Collection ...................................................................................... 41
      2.1.3. Assessment of Maternal Asthma ............................................................................ 42
      2.1.4. Assessment of Maternal Depression/Anxiety ......................................................... 42
      2.1.5. Sample Collection ................................................................................................. 42

3
List of Figures

Figure 1: Factors influencing asthma control…………………………………………………………………………………14

Figure 2: The interactions of the major immune cells involved in the pathophysiology of asthma .......................17

Figure 3: The interactions of inflammatory cells and cytokines involved in asthma, depression/anxiety and pregnancy……………………………………………………………………………………………………36

Figure 4: Visiting schedule of participants and experimental immune cell analysis.............................................41

Figure 5: The FlowJo gating strategy for sorting total monocytes from Tc cells (A) and distinguishing CD14 Bright, CD16 Bright and intermediate (CD14 Bright + CD16 Bright) monocytes from each other..............................................45

Figure 6: Diagram of chemotaxis assay using a Transwell® insert.................................................................47

Figure 7: No effect of depression/anxiety in pregnancies complicated by asthma on the percentage of women experiencing an exacerbation throughout gestation (A; p=0.362). Frequency of exacerbations was also unchanged with the presence of depression/anxiety in pregnancies complicated by asthma (B; p=0.589)........53

Figure 8: The presence of depression/anxiety in pregnancies complicated by asthma significantly increased the percentage of women experiencing uncontrolled asthma throughout gestation (A; p=0.017). The number of uncontrolled asthma events was also significantly increased with the presence of depression/anxiety in pregnancies complicated by asthma (B; p=0.009)............................................................................................................55

Figure 9: Percentage total monocytes in the peripheral blood mononuclear cells of pregnant women with and without asthma and with and without depression/anxiety at 18 (A) and 30 weeks gestation (B)...............................61

Figure 10: Percentage CD11a expression on CD14 Bright monocytes in pregnant women with and without asthma and with and without depression/anxiety during pregnancy at 30 weeks gestation...............................................63

Figure 11: Effect of uncontrolled asthma on percentage total monocytes at 18 weeks gestation......................64
List of Tables

Table 1: Experimental numbers at 18 weeks and 30 weeks gestation for each group for the flow cytometric analysis of cell surface molecules (FACS) performed on monocytes.................................................................44

Table 2: Experimental numbers at 18 and 30 weeks gestation for each group for the chemotaxis assays performed on the peripheral blood mononuclear cells (PBMCs).................................................................47

Table 3: Participant data at booking visit (12 or 18 weeks gestation)........................................................................49

Table 4: Participant data of asthmatic women at booking visit (12 or 18 weeks gestation).................................51

Table 5: Asthma control data of women throughout pregnancy..................................................................................52

Table 6: Monocyte flow cytometric analysis subgroup data at booking visit (12 or 18 weeks gestation) and asthma control throughout pregnancy........................................................................59

Table 7: Percentages of total, CD14Bright, CD16Bright, CD14+CD16+ and CD14-CD16- monocytes in pregnant women with and without asthma and with and without depression/anxiety at 18 and 30 weeks gestation...........62

Table 8: Peripheral blood mononuclear cell chemotaxis subgroup data at booking visit (12 or 18 weeks gestation) and asthma control throughout pregnancy.................................................................65

Table 9: Migration index of peripheral blood mononuclear cells throughout pregnancy.........................................67
Acknowledgements

I would like to acknowledge the support of my supervisors Vicki Clifton, Luke Grzeskowiak and Annette Osei-Kumah. Vicki, you have been a great support and inspiration over the past two years, thanks for all the time and energy you put into me, I could not have done this without you. Luke thanks for your help and willingness to supervise me late in the day. Your insights and expertise has given me a have really enhanced my research experience. Annette, your help in the laboratory has been crucial to my development as a researcher and so I want to take this opportunity to thank you for all your help and support.

Also to my family and friends who supported, encouraged and calmed me down when things were crazy. I could not have completed this without you all.
Abbreviations

ACQ: Asthma Control Questionnaire
ACTH: Adrenocorticotropic hormone
ANRQ: Antenatal Risk Questionnaire
AQLQ: Juniper Asthma Quality of Life Questionnaire
AVP: Vasopressin
BMI: Body mass index
CD: Cluster of differentiation i.e. CD14
CD14Bright: ‘Classical’ monocytes
CD16Bright: ‘non-classical’ monocytes
CD14*CD16+: ‘Intermediate monocytes
CCR2: Monocyte chemoattractant protein-1 receptor
CeA: Central nuclei of the amygdala
CI: Confidence interval
CRH: Corticotrophin-releasing hormone
DC: Dendritic cell
DPBS: Dulbecco’s phosphate buffed saline
EGF: Epidermal growth factor
EMT: Epithelial-mesenchymal transition
EPDS: Edinburgh Postnatal Depression Score
FACS: Flow cytometric analysis of cell surface molecules
FBS: Foetal bovine serum
FCV: Force vital capacity
FENO: Fractional exhaled nitric oxide
FEV1: Forced expiratory volume in one second
GR: Glucocorticoid receptor
HADS: Hospital Anxiety Depression Scale
HLA: Human leukocyte antigen
HPA axis: Hypothalamic-pituitary-adrenal axis

ICAM: Intercellular Adhesion Molecule

ICS: Inhaled corticosteroids

IFN: Interferon

IgE: Immunoglobulin E

IL: Interleukin

IQR: Interquartile range

IRR: Incidence rate ratio

LABA: Long acting β2 agonists

LMH: Lyell McEwin Hospital

LPS: Lipopolysaccharide

M1: Classically activated macrophage

M2: Alternatively activated macrophage

MCP: Monocyte chemoattractant protein

MeA: Medial nuclei of the amygdala

nfMLP: N-formly-met-leu-phe

NK: Natural killer cells

OCS: Oral corticosteroids

OVA: Ovalbumin

PBMC: Peripheral blood mononuclear cell

RANTES: Regulated And Normal T cell Expressed and Secreted

RR: Relative risk

SGA: Small for gestational age

Tc cells: T cytotoxic cells

TGF: Transforming growth factor

Th cells: T helper cells

TNF: Tumor Necrosis Factor

T reg: Regulatory T cells
VEGF: Vascular endothelial growth factor
Abstract

**Background:** Asthma during pregnancy has been associated with poor pregnancy outcomes such as pre-eclampsia, small for gestational age babies and preterm birth. Depression and anxiety are associated with reduced asthma control in non-pregnant individuals. This study investigated whether depression/anxiety in combination with pregnancies complicated by asthma has a negative effect on asthma control. Potential immune mechanisms that may drive worsening asthma were also investigated.

**Methods:** One hundred and eighty-nine asthmatic women with and without depression/anxiety were followed throughout their pregnancies. Incidences of uncontrolled asthma and exacerbations were measured throughout gestation. At 18 and 30 weeks of gestation, monocyte inflammatory profile was examined using flow cytometric analysis of cell surface molecules (FACS) and peripheral blood mononuclear cell (PBMC) chemotaxis was also examined.

**Results:** The incidence of uncontrolled asthma increased in women with depression/anxiety compared to women without depression/anxiety during pregnancy (unadjusted incidence rate ratio (IRR) 1.739, adjusted IRR 1.633, CI 1.092-2.442, p=0.017). Relative risk of experiencing uncontrolled asthma during pregnancy was also increased with depression/anxiety (unadjusted RR 1.619; adjusted RR 1.538, CI 1.114-2.122, p=0.009). There was no increase in the incidence rate ratio (unadjusted IRR 0.770; adjusted IRR 0.755, CI 0.412-1.382, p=0.362) or relative risk (unadjusted RR 0.867; adjusted RR 0.859, CI 0.496-1.489, p=0.589) of asthma exacerbations during pregnancies complicated by depression/anxiety. Asthma without depression/anxiety was associated with an increase in peripheral blood total monocyte percentage at 18 but not 30 weeks gestation when compared to asthmatic women with depression/anxiety (p=0.027). There were no changes in PBMC chemotaxis at 18 or 30 weeks gestation in pregnant women regardless of the presence of asthma or depression/anxiety.

**Conclusion:** The presence of asthma and depression/anxiety during pregnancy is associated with an increase in uncontrolled asthma, but not a change in exacerbation risk. This increase in uncontrolled asthma in women with depression/anxiety was not a result of alterations in monocyte inflammatory profile or PBMC chemotaxis.
Chapter 1: Literature Review

Asthma is one of the most common chronic diseases to complicate pregnancy with a prevalence of 12% in Australia\(^1\) and 3.7-8.4% in the USA\(^2\). Asthma during pregnancy has been associated with a range of adverse pregnancy outcomes including pre-eclampsia and gestational diabetes\(^1\), small for gestational age (SGA) babies\(^3-6\), preterm delivery\(^4-6\) and stillbirth\(^4.5\). Reducing these adverse pregnancy outcomes is central to research investigating asthma and its control during pregnancy.

1.1. **Asthma and Exacerbations**

Asthma is an inflammatory disease characterised by reversible airway obstruction, airway hyperresponsiveness and the recruitment, adhesion and migration of inflammatory cells to the sub-mucosa. Asthma control can be worsened by several factors including, non-adherence to medication, smoking\(^7\), gastric reflux\(^8\), obesity\(^8\), viral infection\(^9\), poor mental health\(^10-12\) and pregnancy\(^3,13\). Figure 1 demonstrates the ways in which these factors can interact to worsen asthma control. This thesis will focus on the changes in immune function that can result from the interactions of the various factors, and impact negatively on asthma control.
Asthma control is influenced by a number of factors. Pregnancy influences external triggers and self-management/adherence/health literacy in positive and negative ways i.e. women may quit smoking or not adhere to their asthma medication during pregnancy when trying to provide the best pregnancy environment for their baby. Pregnancy also affects mental health and immune function. A woman’s mental health will impact self-management/adherence/health literacy, external triggers and immune function. Both socioeconomic status and obesity influence external triggers, self-management/adherence/health literacy and immune function. Through these various interactions, asthma control is influenced by pregnancy and mental health.

An asthma exacerbation is an acute inflammatory event which results in bronchoconstriction, inflammation and a decrease in lung function. The American Thoracic Society and European Respiratory Society have jointly defined asthma control as the degree of reduction or removal through treatment of the various asthma symptoms and defined asthma exacerbations as events resulting from a change in the previous status of the patient. These exacerbations can be severe (requiring urgent action to prevent a serious outcome, i.e. hospitalisation or death) or moderate (distressing occasions that require a change in treatment, but are not considered severe). An asthma exacerbation can be triggered by a number of factors, including allergens, infection, exercise, cold air, cigarette smoke and pollution.

Asthma control can be monitored using the Juniper Asthma Control Questionnaire (ACQ). This questionnaire is a seven question survey of the frequency of the patient’s asthma symptoms and β2-agonist use in the previous week. Asthma symptoms deemed most important by clinicians for inclusion in the questionnaire were night time symptoms, limitation of normal activities, daytime symptoms,
breathing difficulties and wheezing\textsuperscript{15}. Juniper \textit{et al.} has validated the tool in an asthmatic adult population\textsuperscript{15}. The ACQ is a highly regarded tool for measuring ongoing asthma control in adults in both the clinical and the laboratory setting.

In response to allergic triggers in the lung, allergen specific IgE binds to FcεRI on the surface of mast cells\textsuperscript{16}. This causes a release of histamine and the subsequent recruitment of leukocytes from the bloodstream to the airways\textsuperscript{16}. Interleukin (IL)-4 and IL-13 are responsible for enhancing IgE production; IL-9 and IL-13 play a role in mucus secretion; and IL-4, IL-5 and IL-13 activate eosinophils, mast cells and basophils\textsuperscript{16}. An enhanced IgE production leads to a more frequent response to allergens and results in increased hyperresponsiveness of smooth-muscle cells to contractile agents; the adhesion, migration and activation of inflammatory cells to the submucosa; and the secretion of mucus. This manifests as acute exacerbations and airway remodelling, leading to the exacerbation symptoms of increasing breathlessness, wheezing, coughing and the sensation of tightness in the chest.

The control of asthma is important as uncontrolled asthma leads to permanent tissue damage, tissue remodelling and a loss of lung function. There is a range of asthma medications used to prevent and treat asthma and asthma exacerbations. These range in strength from short-acting β2 agonists, inhaled corticosteroids (ICS), combination ICS and long-acting β2 agonists to oral corticosteroids.

1.2. \textbf{Asthma and Pregnancy}

The increased risk of adverse outcomes in pregnancies complicated by asthma, could be related to reduced asthma control or increased asthma exacerbations during pregnancy. Both of these have been observed to be more frequent during pregnancy\textsuperscript{3}. Reduced control or increased exacerbations could be due to smoking, obesity or an increased risk of viral infection. Medication non-adherence due to perceived harm to the foetus, and other co-morbidities including poor mental health could also play a role in reducing asthma control. Pregnancy-induced changes in immune function to enable tolerance of the genetically disparate foetus may influence the underlying inflammatory pathways associated with asthma. This thesis will focus on the changes in immune function as just one mechanism of many that affects asthma control during pregnancy.

1.3. \textbf{Mental Health and Asthma}

Mental health can be considered as one’s psychological and emotional wellbeing. This can be evaluated in many ways and is influenced by a number of factors, including stress and social circumstances such as socioeconomic status, exposure to community or family violence, air pollution, and isolation or loneliness. It is also affected by disease states such as anxiety, depression, bipolar disorder and schizophrenia.
Mental illness can be a common comorbidity in individuals with asthma. Previous studies have demonstrated that anxiety, depression and panic disorders are more common among people with asthma than in the general population\textsuperscript{10-12}. While mental illness may be either a consequence of or a contributor to asthma, previous studies demonstrate a strong correlation between poor mental health and poor asthma control\textsuperscript{10,11,17,18}. There are several factors which could potentially influence asthma control when the individual also suffers from depression and/or anxiety. These include social factors such as high cost of medications (for both depression/anxiety and asthma), the social stigma associated with depression/anxiety and a lack of understanding of how to self-manage asthma and maintain control (poor health literacy). These could all result in medication non-adherence or poor health seeking behaviours which would naturally reduce asthma control. Diet, obesity\textsuperscript{19} and increased rates of smoking\textsuperscript{7} could also have a negative influence on asthma control in individuals with depression and/or anxiety. There is also the possibility of an immune mechanism, involved in the association of increased rates of depression and/or anxiety with asthma, which may influence asthma control and exacerbations.

1.4. **Immune Mechanisms of Asthma and Pregnancy**

1.4.1. **Immune Cells and Asthma**

The variable airway obstruction and hyperresponsiveness typical in asthma are a result of an increase in the number of inflammatory cells which are either recruited to, or activated at, the airways in response to the release of histamine. The major immune cells associated with asthma as an inflammatory disease are: eosinophils, neutrophils, monocytes, T lymphocytes, dendritic cells, mast cells, basophils, macrophages and epithelial cells (Figure 2). Previous work has demonstrated changes in peripheral blood T lymphocytes and monocytes during pregnancy, therefore this study will focus on the role of these cells during pregnancies complicated by asthma.
Allergens enter the airways and initiate an allergic response via epithelial cells, macrophages and dendritic cells (derived from monocytes) and mast cells. These cells stimulate the release of various chemokines which recruit and activate Th2 and Th17 cells, eosinophils and neutrophils. These inflammatory cells release cytokines which result in the exacerbation symptoms of mucus secretion, bronchoconstriction and airway remodelling.

1.4.1.1. **T lymphocytes**

T lymphocytes can be grouped into T cytotoxic cells (Tc cells), T helper cells (Th cells), and regulatory T cells (T reg cells). Tc cells destroy foreign bodies and secrete chemokines and cytokines to enhance immune defence. Th cells secrete chemokines and cytokines (specific to the various subsets) which activate Tc cells. T reg cells regulate inflammation and immune responses by suppressing the various Th subset responses. Both Tc cells and Th cells are increased in the lungs of asthmatics and an increase in Th2 cell-derived cytokines was traditionally viewed as essential to the development of asthma. This view then lead to the understanding of asthmatic disease as a skewing of the Th1/Th2 cell balance. The recent discovery of a new Th subset, Th17, which produce IL-17, led to a change in the understanding of Th1/Th2 cell balance in asthma and pregnancy. The concept has been expanded and is now considered a balance of Th1/Th2/Th17/T reg.

Differences in the Th cell balance in the presence of asthma have been observed in the circulation. Peripheral blood T lymphocyte profile was examined in asthmatic and non-asthmatic adults using flow cytometry. After antigen stimulation *in vitro*, Th2 cells were increased in female atopic (allergy
driven) asthmatics compared to male atopic asthmatics and controls of both sexes. This increase in Th2 cells in asthmatics compared to healthy controls was also observed after PMA/ionomycin stimulation (simulating bacterial infection). Percentages of Th17 cells as well as Th2 cells were higher in peripheral blood of uncontrolled, mostly moderate to severe persistent, allergic, asthmatics, when compared to healthy controls. Therefore there appears to be a skewing towards Th2 and Th17 in the blood of asthmatics that was not apparent in non-asthmatics.

Differences in immune cell recruitment to the lungs in the presence of asthma have also been observed. T reg cell numbers were quantified in the bronchoalveolar lavage of moderate to severe asthmatics and then compared with those of mild asthmatics. The use of flow cytometry showed a significant increase in T reg cell number in moderate to severe asthmatics compared to mild asthmatics and controls. In light of the fact that T reg cells can suppress the action of the Th2 subset, the association of increased T reg cell numbers with more severe disease may seem counterintuitive. This is possibly the result of a need to regulate the increases in the many different inflammatory cells associated with asthma. A weaker expression of anti-inflammatory cytokine IL-10 by T reg cells in asthmatics has been observed in sputum and serum. This may help explain the increase in pro-inflammatory cytokines that occurs with asthma.

The immune response is a dynamic process, with the cytokines produced by the various Th subsets affecting the differentiation and cytokine production of themselves or other Th subsets. This effect means that the immune response can be impacted in a positive or negative manner. The Th1 cytokines (mainly IFN-γ) inhibit differentiation of Th2 and Th17 cells. IL-4 (a Th2 cytokine) inhibits development of Th1 and Th17 cells. IL-23 is produced by Th17 and helps in maintaining Th17 immune cell pathology. The result of these interactions is that an excess of one cytokine or a dominance of one Th subset can skew the Th1/Th2/Th17/T reg cell balance and contribute to disease or allergy.

Th2 and Th17 cells express interleukins which regulate the recruitment and activation of other inflammatory cells to the lungs as well as enhance the allergy response. Expressed by Th2 cells, IL-4, IL-5 and IL-13 are responsible for the activation of eosinophils, mast cells and basophils. IL-17 and IL-8, expressed by Th17 cells, are potent activators of neutrophils. IL-4 and IL-13 also enhance IgE production, while IL-9 and IL-13 enhance mucus production. IL-17 also increases survival and proliferation of airway smooth muscle mass. This is a marker of airway remodelling and a good surrogate of asthma progression.

High concentrations of IL-17 were observed in the blood of asthmatics, but it was suggested that this was due to expression by Th2 cells rather than Th17 cells. These IL-17 producing Th2 cells were unique as they still produced IL-4, a Th2 cytokine. It is possible there may be differences in the
asthmatic response due to the ways in which IL-17 is produced. In mice, after allergen challenge there was an influx of Th17 cells to the lungs within the first three hours. Three days later the majority of IL-17 producing cells also expressed IL-4. Histological analysis of mice lungs demonstrated there was an increase in the recruitment of eosinophils, neutrophils, macrophages and lymphocytes after the transfer of antigen-specific IL-17 producing Th2 cells, compared to a transfer of classical Th2 cells, Th17 cells or saline alone. The IL-17 producing Th2 cells resulted in greater peribronchial inflammation, mucin production and goblet cell hyperplasia. This may indicate a two-pronged process of inflammation during an asthma exacerbation:

1. the initial influx of Th17 cells resulting in the acute response and
2. the secondary influx of Th2 cells resulting in a more prolonged inflammation.

Therefore IL-17 (whether expressed by Th17 or Th2) is a cytokine of interest in the pathogenesis of asthma.

Studies have used both peripheral blood immune cells and sputum immune cells to characterise the inflammatory cells associated with asthma. The proportion of peripheral blood IL-4 producing CD4+ (Th2) cells in asthmatics correlated positively with sputum eosinophil counts and exhaled nitric oxide, both of which are important markers of airway inflammation. This validates the use of peripheral blood immune cells to examine immune function in asthmatics as well as induced sputum, bronchial biopsies and other lung measurements. Although these latter techniques may be the gold standard, they are often more expensive, invasive and of greater risk than collecting a peripheral blood sample. Decreased lung function and a hypoxic insult to the foetus can result from obtaining induced sputum during pregnancy. Therefore the peripheral blood sample is especially important when investigating asthma during pregnancy as this is the only safe technique for use in a pregnant population.

Pregnancy induces a very complex immunological situation. Maternal circulating leukocytes experience modifications in cell concentrations, phenotype and function throughout gestation. The pregnancy information in this thesis does not refer to the implantation stage of pregnancy, due to the ways in which inflammation is a necessary and beneficial immune response.

Post-implantation, pregnancy involves a shift away from a pro-inflammatory immune response to an anti-inflammatory immune response in order to suppress the rejection of the genetically disparate foetus. Th1, Th2 and Th17 cells are mediators of inflammation, while T reg cells suppress inflammation. Circulating T reg cells increased throughout pregnancy and suppressed immune responses to the foetus. In newly pregnant women a lack of this increase in T reg cell level was associated with an increased risk of miscarriage. In cases of unexplained recurrent spontaneous abortion, increased Th17 cells were observed in the peripheral blood and decidua. This was inversely related to the number of T reg cells, resulting in a pro-inflammatory state. Progesterone,
produced early in pregnancy by the corpus luteum and then later by the placenta, reduces inflammatory cytokines and blocks allogeneic responses\textsuperscript{40,41}. In these ways the immune status of the mother is modified to reduce maternal response to the foetus.

In pregnancies complicated by asthma, studies have demonstrated there are Th1/Th2/Th17/T reg cell imbalances\textsuperscript{25,34}. Th2 polarization increased throughout pregnancy and greater Th2 cytokine production was associated with worsening of asthma\textsuperscript{42,43}. Th17 producing cells increased in both pregnant and non-pregnant asthmatics\textsuperscript{25}; however the pregnancy-induced increase in T reg cells was not observed in pregnant asthmatic subjects\textsuperscript{25,34}. A higher Th17/T reg cell ratio was also reported in pregnancies with poor outcomes such as preeclampsia\textsuperscript{44}, preterm labour\textsuperscript{45} and spontaneous abortion\textsuperscript{33}. These studies suggest that the Th1/Th2/Th17/T reg cell imbalances inherent in asthma could adversely influence the immune balance during pregnancy, leading to an increased risk of poor pregnancy outcomes with asthma. It could also be that this interaction could worsen asthma control during pregnancy through an additive effect of the pregnancy-induced immune changes on T lymphocyte balance.

This shifting of the immune profile, with the addition of asthma during pregnancy, suggests a reduced immune tolerance to asthmatic allergens. Combined with the increased chemotactic response of peripheral blood mononuclear cells (PBMC; T lymphocytes approximately 45-70%) during pregnancy\textsuperscript{46}, this increase in Th2 and Th17 could be a mechanism for the reduction in asthma control observed with pregnancy.

1.4.1.2. Monocytes

Monocytes are circulating inflammatory cells which, via various chemokines, are released in response to inflammation. They quickly migrate to sites of inflammation (in asthma, the inflammatory site of interest is the lungs) where they then differentiate. Monocytes can differentiate into dendritic cells (DC), as well as classically activated (M1) macrophages and alternatively activated (M2) macrophages. DC play a role in antigen presentation and initiate the immune response in atopic asthma. Both M1 and M2 macrophages play a role in airway inflammation (reviewed by \textsuperscript{47,48}).

Monocytes consist of three different subsets characterised by varying levels of CD14 and CD16 expression. The ‘classical’ monocytes are highly positive for CD14 and negative for CD16 (CD14\textsuperscript{++}CD16\textsuperscript{-}) and are the majority of monocytes in healthy individuals. CD16\textsuperscript{+} monocytes can be either the strongly CD16 positive ‘non-classical’ monocytes (CD14\textsuperscript{-}CD16\textsuperscript{++}) or the ‘intermediate’ (CD14\textsuperscript{+}CD16\textsuperscript{+}) monocytes.

While the CD16 positive monocytes are only 5-15% of the monocyte population, they significantly increase in number in inflammatory conditions\textsuperscript{49}. In non-pregnant asthmatics, co-expression of CD14 and CD16 by the monocytes (intermediate monocytes) was increased compared to non-asthmatics, but
there was no difference in other monocyte membrane markers. There were also no differences associated with the type of asthma (atopic or non-atopic). Obesity is associated with inadequate asthma control and a BMI greater than 30 was associated with an increased percentage of CD14⁺CD16⁺ monocytes. In chronically stressed caregivers to a terminally ill family member, there was a significant increase in CD16 positive monocytes compared to non-stressed members of the community. In the stressed individuals these monocytes were a major source of pro-inflammatory gene expression. These data demonstrated that monocytes expressing CD16 produced more inflammatory cytokines than those that did not express CD16. The increase in pro-inflammatory markers as a result of increased CD16 positive monocytes, could result in greater uncontrolled asthma or increased exacerbations in stressed or obese asthmatics.

Monocytes are the main producers of IL-10. IL-10 is an anti-inflammatory cytokine which down-regulates the production of pro-inflammatory cytokines, including those also produced by monocytes. Thus it is desirable to have adequate IL-10 levels. However, in a mouse model of lung fibrosis, up regulation of IL-10 increased the Th2 response and significantly contributed to silica induced lung fibrosis. This apparent contradiction in the generally recognised anti-inflammatory effects of IL-10 and the inflammatory effects observed in mouse models of allergic lung inflammation and atopic diseases, led Prasse et al. to analyse monocyte subsets in patients with atopic disease. Monocytes were sorted on the basis of IL-10 secretion using flow cytometry. After an in vitro lipopolysaccharide (LPS) challenge, atopic patients had almost twice as many IL10⁺CD14⁺ monocytes in their circulation as healthy controls (13.6 ±1.6% vs. 7.3 ±1.0%, p=0.004). It appears that the increase in IL-10 producing monocyte subset may come at the expense of the IL-12 producing monocytes. The IL-12 producing monocytes were almost halved in atopic patients compared to healthy controls (5.4 ±0.9% vs. 11.4 ±1.8%, p=0.003). IL-12 expression results in differentiation of naïve T lymphocytes into Th1 cells and enhances the activity of Tc cells. A reduction in IL-12 enables the further suppression of the Th1 subset by IL-10. This reduction in IL-12 expression in atopic patients could result in an increase in the Th2 cell population enhancing atopy. There were further changes in differentiation and influence on T cell cytokine production by IL-10 secreting monocytes when compared with IL-12 secreting monocytes. Instead of DC, IL-10 secreting monocytes were more likely to differentiate into M2 macrophages (74.8 ±10.1% vs. 50.8 ±7.6% IL12), and in co-culture with Tc cells more IL-13 was produced (103 ±37 vs. 82 ±38.3 pg/mol, p=0.039). These data demonstrate the pro-inflammatory effects of overexpression of IL-10. These pro-inflammatory effects may be due to IL-10 secreting monocytes' role in preferentially producing M2 macrophages. M2 macrophages are a major source of IL-13, an interleukin involved in increased IgE production, mucus secretion and eosinophil, mast cell and basophil activation. Through their role in cytokine production and macrophage differentiation, monocytes are a key player in the lung inflammation associated with asthma.
A key chemoattractant of monocytes to sites of inflammation is Monocyte Chemoattractant Protein-1 (MCP-1). Expression levels of MCP-1 and its receptor CCR2 change in response to various influences. Obesity (BMI ≥30) is associated with increased monocyte expression of CCR2 and migration in vitro\textsuperscript{51}. An acute exercise stress test increased MCP-1 and cortisol expression\textsuperscript{59}. Interestingly, exercise stress had no effect on monocyte CCR2 expression in vivo, but in vitro the addition of post-exercise serum to the monocytes for 24 hours was associated with increased CCR2 expression\textsuperscript{59}. It was observed that MCP-1 expression increased in 45 women exposed to prolonged psychosocial stress\textsuperscript{60}. In contrast, in a similar population of 42 women exposed to prolonged psychosocial stress, there was no difference in MCP-1 expression\textsuperscript{61}. Factors that influence monocyte chemotaxis in individuals under chronic stress (caregivers to terminally ill cancer patients) were examined and ICAM-1 (adhesion molecule) and IL-6 (chemoattractant) were over-expressed in these people\textsuperscript{52}. Increased MCP-1, CCR2 and ICAM-1 expression suggests that stress may enhance the chemotactic ability of monocytes, though there are some inconsistencies with these findings. This may present a problem for asthmatics as stress could then reduce asthma control.

During normal pregnancy, a progressive up-regulation of CD11a, CD54 and CD64 expression by monocytes was observed without an increase in total monocyte number\textsuperscript{62}. This suggests increased monocyte activation throughout pregnancy, rather than increases in the monocyte population. However, this contradicted the first description of leucocyte blood counts during pregnancy. Monocyte numbers increased in early gestation (3-4 months) before gradually decreasing and by late pregnancy (9\textsuperscript{th} month) were at the levels of non-pregnant women\textsuperscript{63}.

In pregnancies complicated by asthma, total monocyte number increased significantly as pregnancy progressed, with a trend of increasing monocytes in pregnancies without asthma\textsuperscript{46}. Total monocyte number increased from the first trimester (mean 0.5 (0.4–0.7)) to the third trimester (mean 0.6 (0.6–0.9); p=0.03) in women using inhaled glucocorticoids. There was a similar progressive increase in asthmatics not using inhaled glucocorticoids but no significant progressive increase in non-asthmatic pregnant women\textsuperscript{46}. Another study observed these increases in monocyte numbers only in asthmatic women pregnant with a female foetus\textsuperscript{64}. The increase in monocytes may be one of the mechanisms behind the worsening of asthma control during pregnancy. Further work would be needed to clarify these inconsistencies in monocyte number.

A pregnancy-induced suppression of peripheral blood mononuclear cell (PBMC; monocytes approximately 10-30%) chemotaxis has been observed\textsuperscript{46}. A reduced expression of CCR2 (MCP1 receptor) on monocytes during pregnancy\textsuperscript{65} supports this idea of pregnancy induced chemotaxis suppression. Reduced CCR2 expression was considered to be a pregnancy response rather than an effect of systemic inflammation, as pregnant women with systemic lupus had a similar response\textsuperscript{65}. In pregnancies complicated by asthma, this pregnancy-induced suppression of PBMC chemotaxis was not
maintained\textsuperscript{46}. Using plasma from pregnant asthmatics as the chemoattractant resulted in migration levels similar to the plasma of non-pregnant asthmatics\textsuperscript{46}. Using non-asthmatic pregnant plasma resulted in a significant reduction of PBMC chemotaxis\textsuperscript{46}. The migration of PBMCs in response to non-asthmatic pregnant plasma was half the migration of PBMCs in response to asthmatic pregnant plasma\textsuperscript{46}. It appears that asthma overrides the pregnancy-induced suppression of PBMC chemotaxis. This up-regulated immune pathway may play a key role in increased uncontrolled asthma during pregnancy.

Asthma is associated with increases in CD14+CD16+ monocytes and subsequent increases in pro-inflammatory cytokines. Exposure to acute and chronic stress increased chemoattractant and adhesion molecule expression, and led to increased monocyte migration. Pregnancy appears to be associated with a progressive up-regulation of monocytes, while conflicting data suggests an early increase in monocyte number. When asthma complicates pregnancy, circulating monocytes are increased and there is no pregnancy-induce suppression of PBMC chemotaxis. These alterations in monocyte profile and function with asthma, stress and pregnancy, suggest that monocytes may be an immune mechanism of worsening asthma control during pregnancy and will be examined in the current thesis.

1.4.2. Inflammation Subtypes in Asthma

The heterogeneity of asthma is a result of the differing mechanisms of inflammation in the lung. As previously stated, asthma is characterised by increases in eosinophils, neutrophils and other lymphocytes in the lungs, sputum and mucus. Asthma can be driven by atopic or non-atopic mechanisms; manifest in childhood or adulthood; and can be induced by exercise, occupation, infection or environmental triggers. Eosinophils and neutrophils are the major inflammatory cells involved in the airway inflammation associated with asthma. Tc cells and monocytes are also involved in this inflammatory process as previously discussed.

1.4.2.1. Eosinophilic Inflammation

The most common type of inflammation in asthma is eosinophilic inflammation. Early research linked eosinophils to the pathogenesis of asthma by demonstrating a correlation between the levels of peripheral blood eosinophils and patients’ asthma severity and pulmonary function, in asthmatic patients not using inhaled corticosteroids (ICS)\textsuperscript{66}. Increased levels of sputum eosinophils were associated with atopic asthma\textsuperscript{67}. High peripheral blood eosinophil count has been associated with increased IgE and a lower FEV1\textsuperscript{68}. Asthmatics with a high eosinophil count also presented with a more active asthma than those with a lower eosinophil count\textsuperscript{68}.

Eosinophilic inflammation involves the Th2 and Th17 cells. A mouse model revealed (through enforced expression of IL-23 and adoptive transfer of Th17 cells) that eosinophilic airway inflammation, although
still mediated by Th2 cells, was up-regulated by IL-23 and Th17 cells\textsuperscript{68}. This enhancement of eosinophilic inflammation seemed to be specific to the Th17 chemokine, IL-23, as it was observed even without IL-17A\textsuperscript{69}. Therefore imbalances in Th1/Th2/Th17/T reg cells which result in increased Th2 and Th17 cells, would increase eosinophilic inflammation. This could adversely impact on asthma control.

Expression of eotaxin, the specific chemoattractant for eosinophils\textsuperscript{70}, is increased in the bronchial mucosa of asthmatics not using ICS, and corresponds to asthma severity\textsuperscript{71}. Eotaxin is expressed by many cells including the epithelial cells, Tc cells, macrophages and eosinophils\textsuperscript{71}. Exotoxin appears to play a key role in the recruitment of eosinophils to the lungs and the subsequent eosinophil-induced tissue damage and loss of lung function.

Eosinophil-induced tissue damage was examined by measuring epithelial-mesenchymal transition (EMT) of the bronchial epithelial cells. An intra-tracheal transplant of eosinophils derived from mouse bone marrow was administered to mice\textsuperscript{72}. After the eosinophil transplant E-cadherin levels decreased suggesting increased EMT\textsuperscript{72}. \textit{In vitro}, eosinophils from eosinophilic leukaemia were cultured with the human bronchial epithelial cell line BEAS-2B and also induced EMT\textsuperscript{72}. It appears that eosinophilic inflammation in the lungs promotes airway remodelling through bronchial epithelial cell EMT.

Eosinophilic inflammation responds well to ICS through the induction of apoptosis of the eosinophils by glucocorticoids\textsuperscript{73}. Using FKBP51 as a surrogate measure of the glucocorticoid receptor (GR), high blood eosinophil proportions in steroid-naive asthmatics were associated with lower FKBP51 expression\textsuperscript{74}. Reduced FKBP51 implies that expression of GR is decreased in these patients. It is unknown at present whether this reduced GR expression is causative, or is a result of worsening eosinophilic inflammation. However in mice, injection of a GR antagonist reduced asthma-induced eosinophilic inflammation in the lungs\textsuperscript{75}. Eosinophilic inflammation is a complex process and it could be that GR sensitivity and glucocorticoids are key players in its control.

In the first trimester of pregnancy, peripheral blood eosinophil counts significantly increased in pregnancies complicated by asthma compared to normal pregnancy\textsuperscript{64,46}. Throughout pregnancy, a progressive reduction in eosinophil count was observed in pregnancies complicated by asthma compared to pregnancies without asthma\textsuperscript{46}. This reduction in eosinophils would be beneficial for asthma control and it may be the reason why some women report an improvement of their asthma during pregnancy. Reduced asthma control observed in a large percentage of women occurs in the late second trimester\textsuperscript{2}, around the time when eosinophil counts are decreasing. This suggests that there could be a change in inflammation type during pregnancy that could result in reduced asthma control. Given there has been an observed increase in monocytes and decrease in eosinophils as pregnancy progressed in women in asthma, it is possible that worsening asthma is driven by a non-eosinophilic pathway which may include monocytes. For this reason examining changes in the profile and function of
an alternate immune cell could be beneficial in revealing an immune mechanism that may drive worsening asthma control during pregnancy.

1.4.2.2. *Neutrophilic Inflammation*

The major inflammatory cell responsible for non-eosinophilic inflammation observed in up to 50% of asthmatics is the neutrophils\textsuperscript{76,77}. Neutrophilic inflammation is associated with an increased prevalence of chest infections, rhinosinusitis and symptoms of gastroesophageal reflux disease when compared to eosinophilic inflammation\textsuperscript{78}. These are potential triggers of increased neutrophilic inflammation, so it is not surprising that asthmatics with neutrophilic inflammation report increased unscheduled doctor visits for asthma exacerbations compared to asthmatics with eosinophilic inflammation\textsuperscript{78}.

In non-atopic asthmatics, neutrophils play a large part in asthma exacerbations\textsuperscript{68} with these asthmatics having increased numbers of neutrophils in their circulation\textsuperscript{79}. Atopic asthmatics have reduced neutrophilic inflammation and this neutrophil inflammatory response was impaired after an LPS challenge, when compared with healthy controls\textsuperscript{67}. This suggests that in atopic asthma there may be a reduced activation of the neutrophils which may cause increased susceptibility to infections and subsequent asthma exacerbations.

IL-17 and IL-8 (expressed by Th17 cells) play a role in chronic neutrophilic inflammation\textsuperscript{16}. Early work demonstrated that IL-8 was essential for the recruitment and activation of neutrophils\textsuperscript{80}. Peripheral blood neutrophils of non-eosinophilic asthmatics secreted increased levels of IL-8 at rest than neutrophils of eosinophilic asthmatics\textsuperscript{81}. This suggests that not only is IL-8 needed for the activation of neutrophils, but that neutrophilic inflammation has a positive feedback effect. IL-8 recruits and activates neutrophils which then, in the absence of eosinophilic inflammation, secrete greater amounts of IL-8 which further recruits and activates neutrophils. IL-17 also activates and attracts neutrophils as well as having a role in the production of neutrophils\textsuperscript{31}. IL-17 is expressed by both Th17 cells and a specific subset of Th2 cells, at different stages of the asthmatic response to an inhaled allergen\textsuperscript{33}. These two distinct pathways of IL-17 secretion and subsequent action may help to explain the heterogeneity of the inflammation response in severe asthma. Along with Th17 cells, IL-23 induces neutrophilic inflammation in the airways of mice\textsuperscript{69}. These interleukins indicate several possible mechanisms for an increase in neutrophilic inflammation in asthmatics.

Not only are neutrophils less responsive to corticosteroids, the most widely used asthma medication, but these medications also contribute to neutrophilic inflammation. Although glucocorticoids induce apoptosis in eosinophils, they have the opposite effect in neutrophils\textsuperscript{82}. The administration of four different glucocorticoids to isolated neutrophils inhibited apoptosis and enhanced their survival rate in a dose-dependent manner\textsuperscript{62}. Prolonged neutrophil survival with glucocorticoid treatment is one possible mechanism for an increase in neutrophilic inflammation in asthmatics.
Impaired macrophage phagocytosis may also play a role in chronic inflammation. Sputum-derived macrophages from asthmatics with neutrophilic inflammation demonstrated reduced phagocytosis of apoptotic bronchial epithelial cells compared to those from eosinophilic inflammation\textsuperscript{76}. Reduced macrophage phagocytosis may explain the increased inflammation in these individuals as the uncleared apoptotic cells may be cleared via a secondary pro-inflammatory necrosis. Although only bronchial epithelial cells were examined, the reduced macrophage phagocytosis may also affect the clearance of apoptotic neutrophil, resulting in increased neutrophilic inflammation in the lungs.

Other factors can also influence levels of neutrophilic inflammation. Cigarette smoking is associated with increased neutrophilic inflammation\textsuperscript{83}. Percentages of neutrophils in the induced sputum of current smokers increased compared to healthy age matched non-smokers\textsuperscript{83}. This increase in neutrophils was negatively correlated with fractional exhaled nitric oxide (FENO)\textsuperscript{83}. IL-8 secretion by monocytes increased after stimulation with cigarette smoke\textsuperscript{84}. This could be the mechanism responsible for increased neutrophil recruitment and activation with cigarette smoking.

Obesity is also associated with increased neutrophilic inflammation\textsuperscript{85,86}. This may be due to increased systemic inflammation in response to adiposity\textsuperscript{86}. Sputum neutrophil percentage was positively associated with total plasma saturated fatty acids and negatively associated with monounsaturated fatty acids in obese asthmatics\textsuperscript{85}. This was only observed with neutrophil percentage, as there was no effect of obesity on the level of eosinophilic inflammation observed in the disease of asthma\textsuperscript{85}. To examine this further, Fu et al. investigated the spirometry and sputum gene expression of asthmatics with systemic inflammation, as they hypothesised that these patients could have a group of differentially expressed genes compared to asthmatics without systemic inflammation\textsuperscript{86}. IL-8 and IL-8 receptor gene expression significantly increased with systemic inflammation\textsuperscript{86}, providing a possible pathway by which neutrophilic inflammation is increased in obese asthmatics.

In normal pregnancies neutrophils numbers are increased\textsuperscript{87,88}, possibly due to the delay in apoptosis\textsuperscript{89}. Reduced neutrophil activation was observed in the second and third trimesters of normal pregnancy\textsuperscript{87}. Placental factors activate the neutrophils and greater activation was observed in pre-eclamptic pregnancies\textsuperscript{90}. Neutrophils from healthy non-pregnant females were cultured in conditioned medium from either normal or pre-eclamptic placental villious culture. Neutrophil adhesion and CD62L and CD11a expression increased when exposed to pre-eclamptic medium compared to normal pregnancy medium\textsuperscript{80}. Generation of superoxide radicals increased and there was no difference in elastase activity when neutrophils were exposed to either media\textsuperscript{80}. Therefore neutrophilic inflammation during pregnancy appears to enhance neutrophil activation and this is further increased in pregnancies already complicated by inflammation.
Concentrations of peripheral blood neutrophils increased throughout gestation in pregnancies complicated by asthma, along with an increase in monocytes and a decrease in eosinophils. This suggests that worsening asthma during pregnancy may be a non-eosinophilic form driven by neutrophils and monocytes. An increase in neutrophilic inflammation during pregnancy could further worsen asthma control in obese women or those who smoke, as obesity and smoking also increase neutrophilic inflammation.

Based on current findings, there are several pregnancy related immune changes that may worsen asthma control. Th2 polarisation increases throughout pregnancy in all women regardless of asthma status. In combination with this, Th17 cells are increased in pregnant asthmatics with no pregnancy-induced expansion of the T reg cells to regulate this pro-inflammatory pathway. This results in a higher Th17/T reg cell ratio which could worsen asthma control during pregnancy. Other mechanisms are also disrupted which could contribute to a greater pro-inflammatory environment in asthmatic women. In pregnancies complicated by asthma, monocyte and T lymphocyte chemotaxis is not suppressed as it is in normal pregnancy. Monocytes are progressively activated throughout pregnancy and, in pregnancies complicated by asthma, monocyte and neutrophil numbers are increased throughout gestation. Therefore Th17 cells, monocytes and neutrophils may drive a non-eosinophilic form of asthma during pregnancy contributing to worsening asthma during pregnancy.

1.4.3. Lung Function in Pregnancies Complicated by Asthma

Although there are physiological changes during pregnancy that result in a compression on the lungs by the diaphragm, adaptions of the thoracic cage and muscles result in no decrease in total lung capacity with normal pregnancy\textsuperscript{81}. In pregnancies complicated by asthma, lung function, measured by spirometry, decreases compared to pregnancies without asthma\textsuperscript{64}. This decrease in lung function worsens with a female foetus\textsuperscript{64}, as gestation progresses and with asthma severity\textsuperscript{5}. Decreased lung function is associated with an increased risk of exacerbations\textsuperscript{5}. While cells of the innate immune system, such as monocytes and neutrophils, are up-regulated during pregnancy which may exacerbate asthma; changes in cell function in the lungs may also affect asthma control. Airway remodelling including increased epithelial hypertrophy, subepithelial fibrosis and hyperplasia have been observed in the lungs of non-pregnant, mice with a previous infection, during allergen challenge\textsuperscript{92}. Airway remodelling in response to allergens after respiratory infection could be one mechanism which results in worsening lung function observed during human pregnancy, especially as pregnant women are more susceptible to viral infection\textsuperscript{9,93,94}.

There could be a direct influence of pregnancy on lung function via inflammatory changes in the bronchial smooth muscle and airway epithelium. Inflammatory mediator release in bronchial smooth muscle cells has been examined \textit{in vitro} through the addition of plasma from pregnant women with and
without asthma, and from non-pregnant women to a cultured smooth muscle cell line\textsuperscript{43}. IL-6, soluble Intercellular Adhesion Molecule (sICAM)-1 and Regulated And Normal T cell Expressed and Secreted (RANTES, a recruiter of leukocytes to sites of inflammation) production by the bronchial smooth muscle were increased with the addition of plasma from pregnant women compared to non-pregnant women\textsuperscript{43}. These increases in IL-6, sICAM-1 and RANTES production by the bronchial smooth muscle cells were also observed when maternal plasma was used from pregnancies complicated by asthma compared to non-pregnant asthmatic plasma\textsuperscript{43}. This suggests that these changes were independent of asthma status. Therefore pregnancy-related factors may activate asthma-associated mediators in bronchial smooth muscle cells\textsuperscript{43}. These increases in production of chemokines and adhesion molecules by the bronchial smooth muscle in response to pregnancy, could enhance immune cell migration to the lungs. In this way, pregnancy-induced changes in inflammation could directly contribute to the reduction in lung function experienced by pregnant asthmatics.

Airway epithelial cells also express different levels of chemokines and adhesion receptors when exposed to maternal plasma. In the presence of plasma from pregnant asthmatics, airway epithelial cell production of ICAM-1, IL-6, IL-8 and RANTES increased when compared to non-asthmatic pregnant women\textsuperscript{46}. This suggests that it is the additional complication of asthma which is affecting airway epithelial cell chemokine and adhesion receptor production, rather than it being a pregnancy-induced effect. The increased production of ICAM-1, IL-6, IL-8 and RANTES by the airway epithelium was in addition to the pregnancy-induced changes in the underlying bronchial smooth muscle. These increases in chemokines and adhesion receptor expression in the lungs would influence immune cell chemotaxis. This may explain the lack of suppression of PBMC chemotaxis in asthmatic pregnant women compared to non-asthmatic women\textsuperscript{43}. Increased monocyte and neutrophil chemotaxis in response to the increased secretion of pro-inflammatory chemokines and adhesion receptors in the lungs may be one mechanism by which asthma can worsen during pregnancy.

1.5. **Immune Mechanisms of Mental Health**

In the disease state of depression there is increased activity of the hypothalamic–pituitary–adrenal (HPA) axis (reviewed by \textsuperscript{95}). HPA axis activity is governed by corticotrophin-releasing hormone (CRH) and vasopressin (AVP) release from the hypothalamus. These then cause the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary which in turn stimulates the secretion of cortisol from the adrenal cortex. As well as interacting with its receptors in various tissues, cortisol also interacts with the HPA axis resulting in the negative feedback inhibition of CRH, AVP and ACTH secretion\textsuperscript{95,96}. However cortisol also enhances activity of the amygdala. The medial (MeA) and central (CeA) nuclei of the amygdala contribute to activation of the HPA axis in different ways (reviewed in \textsuperscript{97}). Neurons in the MeA respond to ‘emotional’ stressors such as predators, restraint stress and social interaction\textsuperscript{97}. CeA
neurons respond to ‘physiological’ stressors including haemorrhage and immune challenge. Cortisol increases expression of CRH in the CeA and in this way helps to potentiate autonomic responses to chronic stress. Anxiety, stress and depression influence immune function, although there are conflicting data on the mechanisms involved. However alterations in cortisol and the subsequent disruption of normal HPA axis function could be one mechanism by which immune function is affected.

Many depressed patients have increased cortisol levels in their plasma, saliva and urine. These increased levels of cortisol are thought to be a result of the reduction in glucocorticoid receptor (GR) sensitivity observed during depression. The GR is the main cortisol receptor and so helps to regulate HPA axis activity. Reduced GR sensitivity in the peripheral blood mononuclear cells (PBMC) has been observed in depressed patients. However a reduction in GR sensitivity has not been observed in stressed individuals. Monocyte function was examined in individuals with chronic stress (carers of a terminally ill family member) compared to individuals without major stressors in their lives. The chronically stressed had reduced gene expression of the glucocorticoid response elements, although there was no functional difference in glucocorticoid sensitivity of the monocytes, suggesting that stress may not have as great an effect on GR sensitivity as depression does.

Reduced GR sensitivity reduced feedback inhibition and resulted in higher levels of cortisol. Increased cortisol in depressed patients resulted in a greater stimulation of cytokines and chemokines and their receptors, including RANTES, MCP-1, IFNγ, THFα, IL-1β, IL-6 and IL-12, suggesting reduced sensitivity to the anti-inflammatory effects of cortisol. In depressed patients this reduction in cortisol sensitivity may result in alterations in immune function towards a pro-inflammatory phenotype. Evidence for this interaction with stress is inconclusive. In otherwise healthy women exposed to chronic psychosocial stress, increased levels of circulating MCP-1, VEGF and EGF were observed when compared to women without chronic stress. Conversely, another study in a similar group of stressed women observed no differences in these markers. There may be a different stress-related pathway that affects immune function.

Increased levels of circulating IL-6, TNFα and IL-1β with depression and anxiety also influence T lymphocyte and monocyte function. IL-6 prolongs T cell survival in vitro, expands the T lymphocyte population, shifts the Th1/Th2 balance towards Th2 and promotes Th17 differentiation (reviewed in ). In monocytes, IL-6 switches differentiation from dendritic cells to macrophages and increases monocyte migration. TNFα and IL-6 synergistically induce T lymphocyte growth and activation. TNFα impairs T reg cell function and upsets the Th1/Th2/Th17/T reg cell balance. Early experiments demonstrated that IL-1 is a co-stimulator for Th2, but not Th1, proliferation. Later work has shown that IL-1β changes the T lymphocyte balance by increasing the proportion of IL-4 producing Th2 cells and also the IL-17 producing Th17 cells. IL-1β and IL-6 together also block the suppressive effect of the T reg cells on T lymphocytes. These effects of IL-6, TNFα and IL-1β on the inflammatory
cells suggest that changes in expression of these cytokines with depression, anxiety and stress could result in a more pro-inflammatory state.

The increased IL-6, TNFα and IL-1β in depression and anxiety, and the subsequent effects on the T lymphocytes and monocytes, could lead to more frequent uncontrolled asthma in asthmatics with depression or anxiety. The increased Th2 and Th17 cell populations with increased IL-6 and IL-1β and the increased monocyte migration due to increases in IL-6, mirror inflammatory pathways associated with asthma. The increase in TNFα with depression, along with anxiety reduced expression of IL-10, may increase pro-inflammatory cytokines and ultimately affect asthma control.

1.5.1. Asthma and Mental Health

Asthmatics have a greater incidence of anxiety and depression than non-asthmatics. Mental health status can influence an asthmatic’s asthma control, as suffering from anxiety or depression led to an increased risk of poor asthma control. However experiencing an asthma exacerbation increases the asthmatic’s risk of developing depression and the subsequent further increased risk of poor control. To further add to the complexity, it is unclear at this stage which is present first, anxiety and depression or asthma. While some studies show asthmatics developing anxiety and/or depression, other studies observe that a prior mental illness can lead to the development of asthma. Ruel et al. collected longitudinal data within the South Australian adult population and observed that after eight years, those with initial mood and anxiety disorders had an increased risk of developing asthma (OR 1.62; 1.05-2.49). Regardless of whether it is asthma or poor mental health which develops initially, it is clear that this comorbidity leads to an increased risk of poor asthma control and increased exacerbations.

1.5.2. Anxiety

The illness of anxiety and the experience of stress both have many similar and far reaching effects on the immune system. Women who were sexually or physically abused in childhood had enhanced activation of the HPA axis in adulthood. Repeated stress sensitized the HPA axis response to IL-1β, increasing its activation and subsequent cortisol release. An increase in cortisol caused a shift away from Th1 towards Th2 through inhibition of IL-12, INFγ, IFNα and TNFα and up-regulation of IL-4, IL-10 and IL-13 to avoid prolonged Th1 exposure and possible tissue damage. Th2 polarisation is also observed in asthma and this may have implications for maintaining asthma control.

The shift towards Th2 and increases in IL-4 and IL-13 in times of stress affects allergy. Skin prick test responses in adults with allergic rhinitis were examined before and after a public speaking stressor. Immediately after the stress test, wheal sizes for allergens that previously tested positive had increased and many allergens which previously tested as negative were positive. Subjects who reported a
higher baseline anxiety had a greater number of these previously negative tests appear positive after stress\textsuperscript{116}. Therefore it appears that the disease of anxiety and the experience of stress have an immediate effect on the immune pathways, resulting in a more acute experience of allergy.

Stressful experiences, both acute events and chronically stressful situations, are risk factors for subsequent asthma exacerbations in children\textsuperscript{117,118}. Low social support and socioeconomic status are associated with elevated levels of IL-6 and cortisol\textsuperscript{119-123}. Although cortisol has anti-inflammatory properties, in chronically stressed individuals with high levels of cortisol, there was reduced expression of genes encoding the response elements of the glucocorticoid receptor (GR)\textsuperscript{124}. This suggests that cortical signalling was not recognised and instead there was over-expression of pro-inflammatory genes\textsuperscript{124}. Asthmatic children with high levels of acute and chronic stress also exhibit reduced gene expression of GR, suggesting increased susceptibility to inflammation and reduced responsiveness to medication\textsuperscript{124,125}.

In prestressed mice, though there was no difference in the circulating corticosterone level of mice after ovalbumin (OVA) challenge\textsuperscript{75}, changes were observed in the lungs. In the prestressed mice, a greater number of eosinophils and a higher percentage of mucus-containing goblet cells were observed in the lungs, compared to non-stressed mice\textsuperscript{75}. Furthermore, this increase disappeared with the injection of RU-486, a glucocorticoid receptor antagonist\textsuperscript{75}. This suggests that the influx of eosinophils to the lungs and the increase in mucus containing goblet cells in stressed mice after OVA challenge, are most likely mediated by glucocorticoids. A similar pathway in humans might account for the increased risk of asthma exacerbation after a stressful event.

Chronic stress can also influence monocyte behaviour. As mentioned previously, perceived stress levels and depressive symptoms were higher in carers with family members suffering from terminal brain cancer than healthy controls recruited from the community and undergoing no life stressor\textsuperscript{52}. Interestingly, in the monocytes of the chronically stressed carers, genes for pro-inflammatory cytokines IL-1\textbeta and IL-6, adhesion molecule ICAM1, and transcription factors involved in monocyte/macrophage activation were overexpressed\textsuperscript{52}. There were also increases in the non-classical and intermediate monocyte subsets\textsuperscript{52}. These changes result in a more pro-inflammatory state with a greater activation of monocytes, which could potentially, in an individual with asthma, lead to an increased frequency of uncontrolled asthma.

Immune function is affected by both anxiety and the experience of stress. During stress and anxiety cortisol increases, there is a shift towards Th2 and IL-4 and IL-13 increase in allergic individuals. Monocyte behaviour is affected by stress, with increased non-classical and intermediate monocyte subsets and increased gene expression of pro-inflammatory cytokines, adhesion molecules and monocyte activation transcription factors\textsuperscript{52}. This results in a more pro-inflammatory state with a greater
activation of monocytes. Together these data suggest that the anxiety-driven effects on immune function, especially monocytes, could result in increases in uncontrolled asthma.

1.5.3. Depression

Depression sufferers have an increased risk of developing asthma\textsuperscript{112,113}. Asthmatics are at a greater risk of suffering from depression than the general population\textsuperscript{109}, with hospitalisations and asthma severity being key risk factors\textsuperscript{17,111}. In a Swedish study, the Hospital Anxiety Depression Scale (HADS) score was the most useful determinant of night-time symptoms and attacks of breathlessness in a group of adult asthmatics\textsuperscript{10}. Indeed, participants with anxiety and depression had more respiratory symptoms than the asthmatics without anxiety and depression\textsuperscript{10}. The authors did note that, due to the cross-sectional nature of the study, the cause and effect of the relationship was unable to be determined. Another study in Poland also observed that patients with uncontrolled asthma were more likely to suffer from depression and that as the severity of depression increased, the patient's asthma control declined\textsuperscript{11}. Again, this study was cross-sectional and cannot conclusively say which factor is driving the relationship.

To investigate a possible immune mechanism that could link depression and allergic diseases, Jiang et al. conducted a meta-analysis focusing on the profile of allergy cytokines in subjects with and without depression\textsuperscript{109}. Compared to healthy controls, depression sufferers had an increased concentration of IL-1, IL-4 and IL-6 with similar levels of IL-10\textsuperscript{109}. The previously mentioned cortisol-induced predominance of Th2 cells with increased Th17 cells, macrophage differentiation and monocyte migration and reduced T reg cell suppressive effects with increased IL-6 and IL-1β, provide a possible mechanism for the reduction in asthma control with the additional complication of depression or anxiety.

1.5.4. Dopamine and Serotonin

Dopamine and serotonin are released by the hypothalamus and brain stem respectively. They function as neurotransmitters; their effect is widespread and includes mood, reward-based learning, motivation, memory processing and sleep. Dopamine is involved in several aspects of normal brain function, being released upon nerve stimulation. Dopamine levels can be influenced by drugs, mood, food and alcohol (reviewed in \textsuperscript{126}). IL-6, TNFα and IL-1β, secreted by monocytes and Tc cells, reduce available serotonin by increasing serotonin reuptake and the metabolism of tryptophan (a serotonin precursor)\textsuperscript{127,128}. Dopamine and serotonin stimulate the HPA axis and affect immune function. Both dopamine and serotonin secretion is reduced in depression and anxiety.

The release of dopamine and serotonin effects immune function, via different mechanisms. Serotonin was observed to increase IL-1β and IL-10 production and to inhibit TNFα and IL-12 in PBMCs\textsuperscript{129,130}. 
Serotonin also reduced the stimulatory activity of monocyte derived dendritic cells towards allogeneic Tc cells\textsuperscript{131}. Therefore a reduction in serotonin could result in a more pro-inflammatory state with decreased IL-10 and increased THFα and IL-12 secretion by the PBMCs. Dopamine influences the growth and proliferation of lymphocytes. At levels commensurate with uncoping stress, dopamine significantly inhibited IL-2-induced proliferation and cytotoxicity of Tc and Th cells\textsuperscript{132}. Depression and anxiety adversely affect the immune system through the disruption of dopamine and serotonin secretion.

Some of the effects of reduced dopamine and serotonin on the immune system are similar to those observed in asthma. This could have an additive affect in an asthmatic with the additional complication of anxiety or depression. A decrease in IL-10 secretion could result in the production of pro-inflammatory cytokines. This, along with a reduction in IL-2-induced Th cells may upset the Th1/Th2/Th17/T reg inflammatory balance, and so lead to a reduction in asthma control.

\textbf{1.5.5. Asthma, Pregnancy and Mental Health}

There are few studies investigating the influence of depression/anxiety in pregnant asthmatics. Asthma-related quality of life during pregnancy and subsequent asthma control was examined using a standardized version of the Juniper Asthma Quality of Life Questionnaire (AQLQ) and measuring asthma exacerbations\textsuperscript{133}. Women who experienced subsequent asthma exacerbations during pregnancy initially reported a lower quality of life\textsuperscript{133}. For every 1-point increase in AQLQ score for emotion domain, symptoms domain and overall score, there was more than a 25\% decrease in the chance of a future exacerbation\textsuperscript{133}. The relationship between a women’s perceived control of asthma and psychological state, specific to asthma, and the incidence of future exacerbations was examined\textsuperscript{134,135}. Future exacerbation risk was associated with perceived control and anxiety\textsuperscript{135,136}. Quality of life was reduced by higher levels of anxiety and more negative beliefs about the consequences of asthma\textsuperscript{134}. A reduction in quality of life was associated with an increased risk of future asthma exacerbations \textsuperscript{133}. Many pregnant women also considered asthma medications, in particular oral corticosteroids, to be harmful to the foetus\textsuperscript{134}. This may have implications for the continued adherence to medications as well as their psychological state during pregnancy. In order for women to achieve better asthma control while they are pregnant, it would be beneficial to consider a holistic approach such as asthma education, specifically about the risks to their baby of asthma exacerbations versus asthma medications, as well as considering ways to alleviate social stresses and anxiety about asthma.

As mentioned previously, both asthma and pregnancy change immune function. Monocyte and neutrophil numbers are increased while eosinophils are reduced in pregnancies complicated by asthma. Th cells in asthma are skewed towards Th2 and Th17 and this also occurs in depression and anxiety. This suggests a possible immune mechanism behind the reduction in asthma control experienced during pregnancy by many women. Research so far has not focused on how the additional complication
of depression/anxiety may influence the immune mechanisms behind a worsening of asthma control during pregnancy. An increase in cortisol level occurs in response to stress\textsuperscript{114,137}, in the diseases of anxiety\textsuperscript{138} and depression\textsuperscript{137}, and during pregnancy\textsuperscript{139}. A shift towards Th2 with the cortisol increased may worsen asthma control in these situations. Increases in IL-6, TNFα and IL-1β with depression/anxiety also increase Th cell survival, macrophage differentiation and monocyte migration. This may contribute to an exacerbation during pregnancies complicated by asthma. Figure 3 demonstrates the complexity of the immune changes associated with asthma, pregnancy and depression/anxiety and the possible interactions that might contribute to uncontrolled asthma during pregnancy. In the airway, several chemokines (RANTES, IL-6, eotaxin, IL-8, MCP-1) are upregulated and the majority of these are common to both asthma and pregnancy. In the circulation, there are increases in immune cells (monocytes and neutrophils) in both asthma and pregnancy. Circulatory cytokines (IL-1β, IL-17, IL-6, IL-4, and MCP-1) are increased in both asthma and depression/anxiety. Cortisol increases in asthma, pregnancy and depression/anxiety, shifting immune function towards a Th2 phenotype and increasing expression of pro-inflammatory cytokines. It is thought that these increases in inflammatory cytokines and immune cells with asthma and depression/anxiety may interact during pregnancy to worsen asthma control.
Figure 3: During pregnancy IL-6, RANTES and ICAM-1 expression is increased in the airways with increased cortisol and neutrophils in the circulation (B). Eosinophils, IL-17, IL-10 and IL-1β are increased in the circulation and MCP-1 is increased in the airways of asthmatics (A). When asthma complicates pregnancy there are additional increases in IL-6, RANTES and ICAM-1 in the airways as well as increases in IL-8 and eotaxin. Monocytes, neutrophils and cortisol are also increased in the circulation of pregnant asthmatics. In sufferers of depression/anxiety levels of IL-1β, TNFα, IL-6, IL-4, MCP-1 and cortisol are increased in the circulation and GR sensitivity declines (D). All these increases in pro-inflammatory cytokines and inflammatory cells in asthma, pregnancy and depression/anxiety may interact in pregnancies complicated by asthma and depression/anxiety (C) and result in worsening asthma. Adapted from 43,46,60,95,130,141 RANTES= Regulated And Normal T cell Expressed and Secreted, MCP-1= monocyte chemoattractant protein-1, ICAM= intercellular adhesion molecule, IL= interleukin, TNF= tumor necrosis factor, GR= glucocorticoid receptor
1.6. Conclusion

Pregnancy induces many changes in maternal physiology. In the immune system, this involves increased production of inflammatory chemokines by the bronchial smooth muscle and airway epithelium which are enhanced in the presence of asthma. There are also increases in circulating monocytes, neutrophils and pro-inflammatory cytokines and a decrease in eosinophils. Anxiety and depression also change immune function with increases in circulating cortisol and pro-inflammatory cytokines, increases in macrophage differentiation and monocyte migration and a decrease in glucocorticoid receptor sensitivity. In both asthma and depression/anxiety there is a skewing of the Th1/Th2/Th17/T reg cell balance towards Th2 and Th17. The individual immune changes induced by asthma and depression/anxiety may be exacerbated when these conditions are combined as complications of pregnancy, contributing to worsening asthma control during pregnancy.

Current depression/anxiety and asthma research focuses on adult non-pregnant asthmatics. Asthma control during pregnancy is vital in achieving the best possible maternal and foetal outcomes. Asthmatic women with the additional burden of anxiety and/or depression may be at a greater risk of these poor outcomes. Finding a mechanism for this worsening of asthma would be beneficial in the maintenance and treatment of asthma during pregnancy. There are increases in monocytes during pregnancies complicated by asthma, as well as increases in CD16 positive monocytes, MCP-1 and IL-6 with asthma and with depression/anxiety. This specifically suggests that the monocytes are a target to investigate immune mechanisms of uncontrolled asthma in pregnancies complicated by asthma and depression/anxiety.

1.7. Research

1.7.1. Knowledge Gap

Poor asthma control during pregnancy is associated with poor maternal and foetal outcomes. Research should investigate any influence of depression/anxiety on asthma control during pregnancy. Asthma is driven by alterations in immune function; both pregnancy and depression/anxiety change immune function in different ways. There is a lack of research examining how immune changes resulting from asthma, pregnancy and depression/anxiety interact and impact on asthma control. Therefore there is a need to investigate if there are any significant alterations in immune function during pregnancies complicated by both asthma and depression/anxiety.

Monocyte number increases throughout gestation in pregnancies complicated by asthma and pro-inflammatory monocyte subsets are increased in asthma and with stress. Monocyte chemokines MCP-1 and IL-6 are increased at the lungs in pregnancies complicated by asthma and in the circulation of
individuals with depression/anxiety. Increased monocyte migration is associated with depression/anxiety. Together these findings suggest that the monocyte population is an obvious target for investigating a possible immune mechanism that may be driving worsening asthma control during pregnancies complicated by depression/anxiety.

1.7.2. Research Questions

Does the presence of depression/anxiety in pregnancies complicated by asthma have a negative influence on asthma control?

Does the presence of depression/anxiety in pregnancies complicated by asthma alter the inflammatory profile of the monocytes?

Does the presence of depression/anxiety in pregnancies complicated by asthma induce changes in monocyte and T lymphocyte function?

1.7.3. Hypotheses

Pregnancies complicated by depression/anxiety as well as asthma will be associated with a reduction in asthma control (uncontrolled asthma events = Asthma Control Questionnaire score > 1.5) and/or an increase in exacerbations (an unscheduled visit to a doctor, a course of oral corticosteroids, an emergency department presentation or a hospital admission).

In pregnancies complicated by depression/anxiety as well as asthma, there will be an increase in total peripheral blood monocyte percentage, the percentages of the ‘non-classical’ and ‘intermediate’ monocyte (CD16Bright and CD14+CD16+) subsets will increase and the percentage of monocytes expressing adhesion receptors will increase.

Monocytes and T lymphocytes (as peripheral blood mononuclear cells) from pregnancies complicated by both asthma and depression/anxiety will have a greater chemotactic ability than the monocytes and T lymphocytes from pregnancies not complicated by both asthma and depression/anxiety.

1.7.4. Aims

The aims of this study were the following:

1. To investigate the effect of depression/anxiety and asthma during pregnancy on asthma control.

2. To characterise the peripheral blood monocyte profile of pregnancies complicated by asthma and explore the effect that mental health status may have on the inflammatory profile of monocytes
3. To examine the chemotactic ability of peripheral blood monocytes and T lymphocytes of pregnancies complicated by asthma and depression/anxiety.
Chapter 2: Methodology

The purpose of this study was to determine if there were any significant alterations in asthma control in a prospective cohort of pregnant women with and without depression/anxiety. The study also investigated whether the presence of maternal depression/anxiety during pregnancies complicated by asthma altered the inflammatory profile of the monocytes and the chemotactic ability of the peripheral blood mononuclear cells (PBMC).

Experiments were designed to answer the following questions:

Does the presence of depression/anxiety in pregnancies complicated by asthma have a negative influence on asthma control?

Does the presence of depression/anxiety in pregnancies complicated by asthma alter the inflammatory profile of the monocytes?

Does the presence of depression/anxiety in pregnancies complicated by asthma induce changes in monocyte and T lymphocyte function?

Methods will be described as follows:

A) Subject recruitment and assessment

B) Epidemiology

C) Immune cell experiments

2.1. Part A: Subject Recruitment and Assessment

2.1.1. Subjects

This study was part of a larger prospective cohort study undertaken at the Lyell McEwin Hospital (LMH) that assessed the effects of asthma throughout pregnancy on the mother, placenta and baby. The LMH is a tertiary teaching hospital in a disadvantaged area of Adelaide, South Australia. Recruitment and data collection for all mothers and babies began in May 2009 and ended in July 2013. Of the 347 women who were recruited at their first booking visit (either 12 or 18 weeks gestation), 189 were asthmatics, 85 of whom suffered from depression/anxiety and 104 who did not. Forty-one of the 158 non-asthmatics suffered from depression/anxiety and 117 did not.

The project was approved by The Queen Elizabeth Hospital and Lyell McEwin Hospital Human Research Ethics Committee and The University of Adelaide Human Research Ethics Committee. Participants gave informed, written consent.
Selection criteria included English speaking women over 18 years of age with an otherwise healthy pregnancy that were willing to attend extra antenatal appointments. Those who were obese and/or smoked were included for sub analyses.

Exclusion criteria for the immune cell function tests were the presence of any other co-morbidities of pregnancy, excluding asthma, depression/anxiety, obesity and smoking as well as any women attending fewer than two visits.

Subjects had an appointment with a midwife at 12, 18, 30 and 36 weeks of gestation and 6 months post-partum (Figure 4). Gestational age was determined using menstrual dates and corroborated by ultrasound. At each visit women were questioned about their mental health and asthma (if applicable), as well as provided with standard antenatal care. Venous blood was collected at each visit.

2.1.2. Maternal Data Collection

Previous obstetric history, medical, mental and surgical health information and socioeconomic status as well as maternal weight and height were obtained from medical records. Smoking history was recorded throughout gestation, with four classifications of smoking history; those who never smoked, those who...
quit before pregnancy (median 3 years previously), those who quit during pregnancy (median at 5.5 weeks of gestation) and those who were current smokers.

2.1.3. Assessment of Maternal Asthma

Asthma (yes/no) was determined by the midwife asking the questions “Have you ever been told by a doctor that you have asthma?” and “Have you used any asthma medications in the past year like Ventolin or a preventer?”. Maternal asthma control at each visit was assessed with a combination of self-reporting of symptoms, lung function and medication history. Spirometry was used to assess lung function in both asthmatic and non-asthmatic pregnant women. FEV1 and FVC were measured with the percent predicted FEV1 calculated based on reference values and corrected for sex, age and height. Medication use and compliance was assessed, and asthmatics received education about asthma control and an asthma action plan. Ongoing asthma control was assessed using the Asthma Control Questionnaire (ACQ) which assesses self-reported frequency of morning symptoms, night-time symptoms, the degree of limitation of physical activity and the usage of reliever medications in the previous week (Appendix)

2.1.4. Assessment of Maternal Depression/Anxiety

Maternal depression/anxiety was assessed at the booking visit, with women answering yes/no to the question “Do you have depression/anxiety?”. At the same visit women also answered the Antenatal Risk Questionnaire (ANRQ; Appendix) and the Edinburgh Postnatal Depression Score Questionnaire (EPDS; Appendix) as part of their routine antenatal care.

2.1.5. Sample Collection

Blood was collected in 9 ml Lithium Heparin coated tubes. Peripheral blood mononuclear cells (PBMCs) were isolated according to the following procedure. Blood was diluted in 10 ml Dulbecco’s modified Phosphate Buffered Saline (DPBS; Sigma-Aldrich Co, St Louis, MO, USA), layered onto 7 ml Lymphoprep™ (Axis Shield, Oslo, Norway) and centrifuged at 850 x g for 20 minutes at room temperature. After centrifugation, the PBMC band was removed using a Pasteur pipette and resuspended in 20 ml of DPBS. This was centrifuged at 360 x g for 5 minutes at room temperature, the supernatant decanted and the cell pellet resuspended in another 20 ml DPBS. After further centrifugation (360 x g, 5 minutes, room temperature), the cell pellet was resuspended in 1 ml 25% foetal bovine serum (FBS; Sigma-Aldrich Co, St Louis, MO, USA) and 15% dimethyl sulfoxide (DMSO; Sigma-Aldrich Co, St Louis, MO, USA) in RPMI-1640 media (Life Technologies Australia, Mulgrave, Vic, Aus.). Uncounted PBMCs were stored in 2 x 500 μl aliquots at -80°C until required. Cell number was
only recorded before the experimental tests as previous data in the lab had shown that cell number decreased slightly due to the freezing and thawing process. Samples had to be frozen in order to store them until there were sufficient samples to undertake the chemotaxis work. Recording cell number after thawing ensued in a more accurate live cell count for each sample and resulted in a more precise dilution in order to seed all samples at the correct cell density. The viability of pre-frozen PBMC samples had been previously determined in the laboratory through cell culture and LPS stimulation. Although there was some cell death due to the freezing process (30% live to 20% live after a freeze and thaw cycle in a control sample) there were still adequate numbers to perform the experiments. The viability of all PBMCs used in the experiments was obtained using a TC10 Automated Cell Counter which gave a cell count as a percentage of live and dead cells in each sample. The range for all samples was 20 – 30% live cells.

2.2. **Part B: Epidemiology**

One hundred and eighty-nine asthmatic pregnant women were included in the analysis. One hundred and four women did not experience depression/anxiety during their pregnancy and 85 did experience depression/anxiety. The primary exposure was the presence of depression/anxiety with the sub-analysis exposures being a high EPDS score, high ANRQ score, antidepressant use and the various psychosocial stressors identified at the booking visit. The primary study outcomes were experiencing an asthma exacerbation during pregnancy and presenting with uncontrolled asthma (ACQ > 1.5) at any visit during pregnancy. An asthma exacerbation was considered one or more of the following: an unscheduled visit to a general practitioner (GP), a course of oral corticosteroids, an emergency department presentation or a hospital admission.

Smoking status was defined as smokers (current smokers plus those who quit during pregnancy) and non-smokers (those who never smoked and those who quit before pregnancy). BMI was calculated based on maternal height and weight recorded at antenatal booking visit. Parity was defined as either the participant’s first pregnancy (parity=0) or at least the participant’s second pregnancy (parity= 1+).

2.2.1. **Statistics**

Incidences of asthma exacerbations and uncontrolled asthma were compared between the two groups using Poisson regression with robust variance estimates, with resulting Incidence Rate Ratios (IRR) and 95% confidence intervals (CIs).

The prevalence of asthma exacerbations and uncontrolled asthma was compared between the two groups using a generalised linear model (Poisson distribution) with robust variance estimates, with resulting relative risks (RR) and 95% confidence intervals (CIs).
Each of the models was adjusted for potential confounders including maternal smoking, BMI, age, parity, and baseline ICS use. Statistical analyses were performed using statistical package IBM SPSS Statistics 21 (IBM, Armonk, NY, USA)

2.3. **Part C: Immune Cell Experiments**

In the following experiments, the PBMCs of a random sample from the initial cohort comprising of 48 non-asthmatic and 76 asthmatic pregnant women with and without depression/anxiety were used in the flow cytometric analysis of cell surface molecules (FACS) experiments. A random sample comprising 35 non-asthmatic and 81 asthmatic pregnant women were used in the chemotaxis experiments. Seventeen non-asthmatic and 45 asthmatic pregnant women were common to both experiments. Some women had samples tested at both 18 and 30 weeks gestation, while others were only tested on one occasion.

2.3.1. **FACS Analysis of Cell Surface Molecules**

<table>
<thead>
<tr>
<th>Gestation</th>
<th>Study Group</th>
<th>Non-asthmatic</th>
<th>Asthmatic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Depression/Anxiety - No</td>
<td>Depression/Anxiety - Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=11</td>
<td>n=5</td>
</tr>
<tr>
<td>18 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 wk</td>
<td></td>
<td>n=36</td>
<td>n=8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Experimental samples at 18 weeks and 30 weeks gestation for each group for the flow cytometric analysis of cell surface molecules (FACS) performed on monocytes from pregnant women with and without asthma and depression/anxiety.

2.3.1.1. **Staining of Cell Surface Molecules**

For flow cytometry analysis, monocytes from isolated PBMCs were stained with monoclonal antibodies against CD14, CD16, CD11a, CD11b, CD62L and HLADR (BD Biosciences, San Diego, USA; Co-operative Research Centre for Biomarker Translation, Australia). Antibodies were titrated to determine the optimal staining concentration. All incubations were performed in the dark. Staining was performed according to standard surface molecule staining protocol for flow cytometry. Data was acquired using FACS Canto (BD Biosciences) and analysed using FlowJo (FlowJo LLC, Ashland, OR, USA).

Cryopreserved PBMCs (1 x 10^6) were thawed at 37°C in a water bath. 500 μl of RF10 media (RPMI 1640 media with 10% FBS, 1% penicillin-streptomycin and 2% glutamine) was slowly added to the cells and centrifuged at 1300 rpm for 5 minutes. Cells were resuspended in 100 μl of Dulbecco PBS (DPBS) (Sigma Aldrich Co, St Louis, MO, USA). 50 μl of cells was added to 96 well round bottom plates and mixed with 12.5 μl of premixed anti-human labelled CD14-APCCy7, CD16-FITC, HLADR-PerCP,
CD11a-APC, CD11b-PECy7, CD62L-PE (BD Biosciences, San Jose, CA, USA). After incubation on ice for 30 minutes, the cells were washed in DPBS and centrifuged at 1300 rpm for 5 minutes. Labelled cells were detected using FACS Canto in which 30,000 cells were analysed in each gated event using Flow Jo Software. Cells only and individual antibody alone for compensation were included as controls.

2.3.1.2. Flow Cytometry Analysis

A fluorescence compensation method was applied by using unstained cells and single-fluorochrome stained anti-mouse Ig, k CompBeads (cat# 552843, BD Biosciences) for every analysis, to correct the spectral overlap. Acquisition of flow cytometry data was done in FACS Canto. Further data analysis was done using FlowJo software. A FACS Aria (BD Biosciences) was used to sort CD14Bright, CD16Bright and intermediate monocytes. The gating strategy for sorting is shown in figures 5A and 5B.

![FACSDiva Version 6.1.3](image)

**Figure 5:** The FlowJo gating strategy for sorting total monocytes from Tc cells (A) and distinguishing CD14 Bright, CD16 Bright and intermediate (CD14 Bright + CD16 Bright) monocytes from each other.

2.3.1.3. Statistics

The median and interquartile range (IOR) of the percentages of total monocytes, monocyte subtype (CD14Bright, CD16Bright and Intermediates) and adhesion receptor expression were compared between the four groups. The normalities of the data were tested using frequency histograms and observed to be skewed. Therefore the non-parametric Kruskal-Wallis test was used to analyse the data.
Results were adjusted for potential confounders including maternal smoking, BMI, age, parity, and baseline ICS use. Statistical tests were performed using SPSS with p values < 0.05 considered statistically significant.

2.3.2. Chemotaxis

Frozen 500 μl aliquots of PBMCs were thawed at 37°C and diluted with 1 ml RPMI-1640 (1% FBS, 1% Penicillin & Streptomycin (Sigma-Aldrich)). After spinning at 4°C the cell pellet was resuspended in 1 ml media and the cell number counted using a TC10 Automated Cell Counter (Bio-Rad, Hercules, CA, USA). Cells were diluted to 2 x 10⁵ cells/ml for assessment of chemotaxis using a Transwell® Chemotaxis Assay (Corning Inc., NY, USA). Assays consisted of a 24 well plate with 12 Transwell® inserts that separated the upper and lower chambers of the well with a 5 μm porous membrane.

2.3.2.1. Optimisation

In the following optimisation experiments, PBMCs were used which had been isolated from two non-pregnant women, one non-asthmatic and one asthmatic.

2.3.2.1.1. Cell number and Incubation Time

After trialling 5 x 10⁴ cells/well and 1 x 10⁵ cells/well, a greater migratory response was observed using 1 x 10⁵ cell/well (2 x 10⁵ cells/ml). 1 x 10⁵ cells/well were used in all subsequent experiments. Previous work in our laboratory suggested that a 3 hour incubation was most appropriate in assessing PBMC migration. This was confirmed in two separate experiments using 2, 3 and 4 hour and 1.5, 2 and 3 hour incubation times. It was important that the cell migration for these optimisations was in the middle of the migration range. This occurred at 3 hours and would enable the enhanced migration of more active PBMCs to be detected.

2.3.2.1.2. nfMLP vs. MCP-1

N-Formyl-Met-Leu-Phe (nfMLP) (Sigma-Aldrich Co, St Louis, MO, USA) is a ubiquitous chemoattractant. Monocyte Chemoattractant Protein-1 (MCP-1) (Sigma-Aldrich Co, St Louis, MO, USA) is a more specific chemoattractant. The later preferentially attracts monocytes and T lymphocytes, which make up the majority of PBMCs. Various concentrations of nfMLP and MCP-1 were compared to optimise the most appropriate chemoattractant and concentration. It was important that the concentration used was in the middle of the migration range, so that migration at the upper limits of the scale could still be detected in those individuals with more active PBMCs. Concentrations of 1, 5 and 10 ng/ml nfMLP and 10, 50 and 100 ng/ml MCP-1 were trialled. After a 3 hour incubation, 10 ng/ml MCP-1 resulted in cell migration in the middle of the PBMC migration range. This was further confirmed in another experiment using 1, 5 and 10 ng/ml MCP-1.
2.3.2.1.3. **FBS Concentration**

The addition of 1% foetal bovine serum (FBS) to the media was beneficial in keeping the cells alive and active (compared to no FBS), without changing the morphology of the cells (compared to 10% FBS).

2.3.2.2. **Final Protocol**

The treatment (in duplicate) was 10 ng/ml MCP-1 and control was media alone (RPMI-1640, 1% FBS + 1% P&S). 600 μl control or treatment was placed into the lower chamber, membrane inserted and 500 μl (1 x 10⁵ cells) loaded into the upper chamber (Figure 6).

![Diagram of chemotaxis assay using a Transwell® insert](image)

**Figure 6:** Diagram of chemotaxis assay using a Transwell® insert. The lower chamber contained 10 ng/ml MCP-1 and the upper chamber contained 1 x 10⁵ PBMCs. Chambers were separated by a 5 μm pore microporous membrane.

After a 3 hour incubation (37˚C, 5% CO2), migrated cells were counted using the TC10 Automated Cell Counter. Every plate included a quality control sample of PBMCs isolated from a non-asthmatic, non-pregnant female adult.

<table>
<thead>
<tr>
<th>Gestation</th>
<th>Study Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-asthmatic</td>
</tr>
<tr>
<td></td>
<td>Depression/ Anxiety - No</td>
</tr>
<tr>
<td>18 wk</td>
<td>n=14</td>
</tr>
<tr>
<td>30 wk</td>
<td>n=14</td>
</tr>
</tbody>
</table>

**Table 2:** Experimental samples at 18 and 30 weeks gestation for each group for the chemotaxis assays performed on the peripheral blood mononuclear cells (PBMCs) from pregnant women with and without asthma and depression/anxiety.
2.3.2.3. **Statistics**

Chemotactic response was expressed as a migration index, relative to cells with media alone. The resulting median and interquartile range (IQR) was compared between the different groups using the non-parametric Kruskal-Wallis test.

Results were adjusted for potential confounders including maternal smoking, BMI, age, parity, and baseline ICS use. Statistical tests were performed using SPSS with p values <0.05 considered statistically significant.
Chapter 3: Results - Epidemiology

3.1. Part A: Maternal Demographics

One hundred and fifty-eight non-asthmatic and 189 asthmatic pregnant women were included in the analysis. One hundred and seventeen non-asthmatic and 104 asthmatic women did not have depression/anxiety during their pregnancy. Forty-one non-asthmatic and 85 asthmatic women did experience depression/anxiety during their pregnancy. Table 3 describes the maternal demographics of the groups used in this study. Large percentages of the women in all groups (85.5%, 82.9%, 81.8% and 89.4%) had a socioeconomic status ranking of ≤ 1 on a scale of 0 – 4 where 0 is the lowest and denotes considerable social disadvantage.

Women with depression/anxiety had higher total EPDS and ANRQ scores regardless of asthma status than their counterparts without depression/anxiety (p=0.00 for all comparisons). High EPDS and ANRQ scores can be considered a surrogate for symptomatic depression and anxiety. Over three times as many women with depression/anxiety had a high EPDS or ANRQ as those women without depression/anxiety, confirming that our method for assigning depression/anxiety status was valid.

<table>
<thead>
<tr>
<th></th>
<th>Non-Asthmatic</th>
<th>Asthmatic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Depression/</td>
<td>With Depression/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anxity (n=117)</td>
<td>Anxity (n=41)</td>
<td></td>
</tr>
<tr>
<td>Maternal Age: median</td>
<td>26.0 (23.0 – 31.0)</td>
<td>26.0 (23.0 – 29.0)</td>
<td>0.632</td>
</tr>
<tr>
<td>(25% - 75%)</td>
<td>a</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>BMI: median (25% - 75%)</td>
<td>25.35 (22.2 – 30.7)</td>
<td>30.48 (24.0 – 34.7)</td>
<td>0.048</td>
</tr>
<tr>
<td>Parity: n (%):</td>
<td>47 (40.2)a</td>
<td>16 (39.0)a</td>
<td>0.926</td>
</tr>
<tr>
<td></td>
<td>70 (59.8)a</td>
<td>25 (61.0)a</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foetal Sex: n (%)</td>
<td>57 (48.7)a</td>
<td>16 (39.0)a</td>
<td>0.122</td>
</tr>
<tr>
<td>Male</td>
<td>60 (51.3)a</td>
<td>25 (61.0)a</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity: n (%):</td>
<td>108 (92.3)a</td>
<td>36 (87.8)a</td>
<td>0.125</td>
</tr>
<tr>
<td>Caucasian</td>
<td>9 (7.7)a</td>
<td>5 (12.2)a</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2. **Part B: Epidemiology**

Maternal demographics were similar in both asthmatic groups, except in measures of mental health status (Table 4). Women from lower socioeconomic backgrounds were highly represented, with 85 (81.8%) of those asthmatics without depression/anxiety and 76 (89.4%) of those with
depression/anxiety having a socioeconomic status ranking of ≤ 1 on a scale of 0 – 4 (where 0 is the lowest).

### Table 4: Participant data of asthmatic women at booking visit (12 or 18 weeks gestation).

<table>
<thead>
<tr>
<th></th>
<th>Asthmatics (n= 189)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Depression/Anxiety (n=104)</td>
<td>With Depression/Anxiety (n=85)</td>
</tr>
<tr>
<td>Maternal Age: median (25% - 75%)</td>
<td>25.0 (22.0 – 29.0)</td>
<td>25.0 (22.0 – 30.0)</td>
</tr>
<tr>
<td>BMI: median (25% - 75%)</td>
<td>27.5 (23.1 – 32.8)</td>
<td>27.7 (23.2 – 32.1)</td>
</tr>
<tr>
<td>Parity: n (%): 0</td>
<td>45 (43.3)</td>
<td>33 (38.8)</td>
</tr>
<tr>
<td></td>
<td>59 (56.7)</td>
<td>52 (61.2)</td>
</tr>
<tr>
<td>Foetal Sex: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (57.7)</td>
<td>37 (43.5)</td>
</tr>
<tr>
<td>Female</td>
<td>44 (42.3)</td>
<td>48 (56.5)</td>
</tr>
<tr>
<td>Ethnicity: n (%): Caucasian</td>
<td>93 (89.4)</td>
<td>83 (97.6)</td>
</tr>
<tr>
<td>Other</td>
<td>11 (10.6)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>Smoking Status: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>58 (55.8)</td>
<td>34 (40.0)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>18 (17.3)</td>
<td>18 (21.2)</td>
</tr>
<tr>
<td>Quit during pregnancy</td>
<td>8 (7.7)</td>
<td>15 (17.6)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>20 (19.2)</td>
<td>18 (21.2)</td>
</tr>
<tr>
<td>FEV1 L: mean (SD)</td>
<td>2.9 (0.4)</td>
<td>3.2 (0.5)</td>
</tr>
<tr>
<td>FEV1 %: mean (SD)</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>FVC L: mean (SD)</td>
<td>3.6 (0.5)</td>
<td>3.9 (0.6)</td>
</tr>
<tr>
<td>FEV/FVC %: mean (SD)</td>
<td>0.8 (0.1)</td>
<td>0.8 (0.1)</td>
</tr>
<tr>
<td>ICS use before pregnancy n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>72 (69.2)</td>
<td>56 (65.9)</td>
</tr>
<tr>
<td>ICS</td>
<td>9 (8.7)</td>
<td>4 (4.7)</td>
</tr>
<tr>
<td>ICS + LABA</td>
<td>23 (22.1)</td>
<td>25 (29.4)</td>
</tr>
<tr>
<td>Total ANRQ: median (25% - 75%)</td>
<td>11 (7 – 18)</td>
<td>29 (23 – 39)</td>
</tr>
<tr>
<td>High ANRQ: n (%)</td>
<td>17 (17.2)</td>
<td>62 (78.5)</td>
</tr>
<tr>
<td>Total EPDS: median (25% - 75%)</td>
<td>4 (2 – 6)</td>
<td>7 (3 – 10)</td>
</tr>
<tr>
<td>High EPDS: n (%)</td>
<td>4 (4.3)</td>
<td>15 (18.8)</td>
</tr>
<tr>
<td>Antidepressant use: n (%)</td>
<td>0 (0)</td>
<td>18 (21.2)</td>
</tr>
</tbody>
</table>

Table 4: Participant data of asthmatic women at booking visit (12 or 18 weeks gestation). BMI= body mass index, FEV1L= forced expiratory volume in 1 second, FVC= forced vital capacity, ICS= inhaled corticosteroids, LABA= long acting β2 agonists, ANRQ= antenatal risk questionnaire, EPDS= Edinburgh Postnatal Depression Score. Statistical tests: Pearson Chi-Square Test or Mann-Whitney U Test. Significance: p <0.05

### 3.2.1. Asthma Exacerbations

The incidence of overall asthma exacerbations (p=0.377) and individual exacerbation types: GP visits (p=0.443), OCS prescriptions (p=0.694), ED presentations (p=0.328) and hospital admissions (p=0.886); were similar in both groups of women (Table 5).
There was no increase in the incidence rate ratio for asthma exacerbations when the mother had depression/anxiety (unadjusted IRR 0.770; adjusted IRR 0.755, CI 0.412- 1.382, p=0.362; Figure 7A).

There was no increase in the relative risk of an asthma exacerbation during pregnancy with the added complication of depression/anxiety (unadjusted RR 0.867; adjusted RR 0.859, CI 0.496- 1.489, p=0.589; Figure 7B).

<table>
<thead>
<tr>
<th></th>
<th>Asthmatics (n= 189)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Depression/Anxiety (n=104)</td>
<td>With Depression/Anxiety (n=85)</td>
<td>p values</td>
</tr>
<tr>
<td>Uncontrolled Asthma: n (%)</td>
<td>34 (32.7)</td>
<td>45 (52.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>No. of Uncontrolled Asthma Events: median (25%- 75%)</td>
<td>0 (0- 1)</td>
<td>1 (0- 2)</td>
<td>0.005</td>
</tr>
<tr>
<td>Asthma Exacerbation: n (%)</td>
<td>24 (23.1)</td>
<td>17 (20.0)</td>
<td>0.610</td>
</tr>
<tr>
<td>No. of Asthma Exacerbations: median (25%- 75%)</td>
<td>0 (0- 0)</td>
<td>0 (0- 0)</td>
<td>0.377</td>
</tr>
<tr>
<td>GP visit: n (%)</td>
<td>19 (18.3)</td>
<td>12 (14.1)</td>
<td>0.443</td>
</tr>
<tr>
<td>OCS use: n (%)</td>
<td>7 (6.7)</td>
<td>7 (8.2)</td>
<td>0.694</td>
</tr>
<tr>
<td>ED presentation: n (%)</td>
<td>7 (6.7)</td>
<td>3 (3.5)</td>
<td>0.328</td>
</tr>
<tr>
<td>Hospital Admission: n (%)</td>
<td>1 (1.0)</td>
<td>1 (1.2)</td>
<td>0.886</td>
</tr>
<tr>
<td>ICS prescribed during pregnancy, n (%): none ICS</td>
<td>56 (53.8)</td>
<td>46 (54.1)</td>
<td>0.839</td>
</tr>
<tr>
<td></td>
<td>ICS</td>
<td>8 (7.7)</td>
<td>4 (4.7)</td>
</tr>
<tr>
<td></td>
<td>ICS + LABA</td>
<td>8 (7.7)</td>
<td>6 (7.1)</td>
</tr>
<tr>
<td></td>
<td>Already on ICS</td>
<td>32 (30.8)</td>
<td>29 (34.1)</td>
</tr>
</tbody>
</table>

Table 5: Asthma control data of women throughout pregnancy. GP= general practitioner, OCS= oral corticosteroids, ED= emergency department, ICS= inhaled corticosteroids, LABA= long acting β2 agonists. Statistical tests: Pearson Chi-Square Test or Mann-Whitney U Test. Significance: p <0.05
Figure 7: No effect of depression/anxiety in pregnancies complicated by asthma on the percentage of women experiencing an exacerbation throughout gestation (A; p=0.362). Frequency of exacerbations was also unchanged with the presence of depression/anxiety in pregnancies complicated by asthma (B; p=0.589).
3.2.2. Uncontrolled asthma

The incidence of uncontrolled asthma increased in women with depression/anxiety (52.9%) compared to women without depression/anxiety (32.7%; p=0.005; Table 5). This resulted in an increased incidence rate ratio of over 60% for these women (unadjusted IRR 1.739, adjusted IRR 1.633, CI 1.092-2.442, p=0.017; Figure 8A).

Relative risk of experiencing uncontrolled asthma was also increased over 50% when the woman also had depression/anxiety. (unadjusted RR 1.619; adjusted RR 1.538, CI 1.114-2.122, p=0.009; Figure 8B).
Figure 8: The presence of depression/anxiety in pregnancies complicated by asthma significantly increased the percentage of women experiencing uncontrolled asthma throughout gestation (A; \( p=0.017 \)). The number of uncontrolled asthma events was also significantly increased with the presence of depression/anxiety in pregnancies complicated by asthma (B; \( p=0.009 \)).
3.3. **Discussion**

In this study of 189 pregnant asthmatics, we have observed for the first time in pregnancy that asthma control worsens in women with depression/anxiety compared to those without. There is no increased risk or rate of asthma exacerbations regardless of the presence or absence of depression/anxiety. The outcomes of uncontrolled asthma and asthma exacerbations were analysed separately as both have been observed to be more common during pregnancy.\textsuperscript{3,5}

This study has built on previous work by examining asthma control in a population with depression/anxiety. Quality of life, anxiety, perceived control of asthma and perceived risks of asthma medications were examined in pregnant, asthmatic women.\textsuperscript{134} Overall the women had a good quality of life and perceived control of their asthma as well as having low levels of anxiety.\textsuperscript{134} However, the more a woman was affected emotionally by her asthma, the more frequently she experienced uncontrolled asthma. Women overestimated the risk of harm to the foetus of asthma medications, though this did not appear to lead to greater anxiety. Asthma-specific quality of life has also been used previously to examine asthma exacerbations during pregnancy.\textsuperscript{133} An association was observed between an increase in asthma-specific quality of life in early pregnancy and a decrease in the risk of a subsequent asthma exacerbation.\textsuperscript{133} The current study differs from previous work in pregnant women, by comparing asthma control during pregnancy in women with depression or anxiety to women without these complications, rather than the woman’s emotional response or perceived quality of life. This work does support a link between mental health status (be it depression/anxiety, an increased emotional response to asthma or poor quality of life) and uncontrolled asthma during pregnancy. We could not confirm a link between mental health status and asthma exacerbations during pregnancy.

The observation of increased risk of uncontrolled asthma with depression/anxiety during pregnancy was unsurprising as similar observations have been reported in a non-pregnant population. Our study in pregnant asthmatics follows on from previous work in non-pregnant asthmatics demonstrating associations between depression and anxiety and asthma control. Asthmatics with uncontrolled asthma are more likely to suffer from depression than those who are controlled.\textsuperscript{11} As depression severity increases, asthma control declines.\textsuperscript{11} Respiratory symptoms were increased in asthmatics with anxiety and depression compared to those without, with Hospital Anxiety Depression Scale (HADS) score being the most useful determinant of night-time symptoms and attacks of breathlessness.\textsuperscript{10} Similar findings were observed in our study with increased levels of depression/anxiety in asthmatics compared to non-asthmatics (45.0% and 25.9% respectively; \(p=0.000\)) and a higher incidence of uncontrolled asthma in women with depression/anxiety compared to women without this added complication. This work contributes to the current understanding of mental health status and asthma control by broadening the research to include pregnant women. The fact that the association between depression/ anxiety and
poor asthma control still holds in pregnancy suggests that a woman’s mental health status can have an 
overreaching effect on her health during pregnancy through interactions with other complications such 
as asthma. This will have implications for the provision of appropriate antenatal care to these women at 
higher risk of uncontrolled asthma.

Several societal factors could be contributing to this increase in uncontrolled asthma. Medication 
adherence and appropriate self-management are of great importance to good asthma control. Non- 
adherence to asthma medications during pregnancy can result because women do not understand 
when to use medications or what a loss of asthma control is (poor health literacy) or due to fears of 
harming their baby. While not specifically investigated in this study, previous work has demonstrated 
that women overestimate the risks of asthma medications\textsuperscript{134}. In future, it would be interesting to 
examine if women with depression/anxiety are more likely to overestimate the risks of either their 
asthma or their depression/anxiety medication. If this were the case, it may help explain the observed 
increase in uncontrolled asthma in pregnant women with depression/anxiety due to a worsening of their 
asthma or their depression/anxiety.

Both uncontrolled asthma and exacerbations have been observed to be more common during 
pregnancy\textsuperscript{3,5}. Appropriate asthma control throughout pregnancy and not just the avoidance of 
exacerbations could be important for maternal and foetal health. Future work should assess other 
maternal and foetal outcomes (such as pre-eclampsia, preterm birth and small for gestational age 
babies) to examine whether there are any associations with the increase in uncontrolled asthma with 
depression/anxiety and adverse pregnancy outcomes.

While an association between smoking and asthma control during pregnancy has been demonstrated\textsuperscript{7}, 
our cohort had similar rates of smoking regardless of the presence of depression/anxiety. No additional 
effect of smoking on uncontrolled asthma or exacerbations was observed in pregnant asthmatics with 
depression/anxiety, suggesting that a woman’s mental health is more important in determining her risk 
of uncontrolled asthma during pregnancy. This was somewhat surprising, however it could have been 
due to the way in which smoking status was analysed. The number of cigarettes per day was not taken 
into account due to the lack of appropriate power. With greater numbers it would be interesting to see if 
the quantity of cigarettes per day might have an additional adverse effect on asthma control or increase 
the risk of exacerbation.

There is an association between low socioeconomic status and an increase in social stress, and an 
increased risk of asthma exacerbations\textsuperscript{117,118}. Our study recruited from a population of high social 
disadvantage. Both women with and without depression/anxiety predominantly lived in a disadvantaged 
area and were consequently more exposed to social stressors during their pregnancies such as 
insecure housing, financial difficulties, relationship breakdown and unemployment. This increased social
stress may have increased the emotional burden on already vulnerable women and contributed to their increased risk of uncontrolled asthma. This was difficult to examine in the current study as there was not appropriate power for these sub-analyses. It would be interesting in future work to examine the effect of depression/anxiety on asthma control during pregnancy in women from a less disadvantaged area to determine if there is an additional effect of socioeconomic status.

Obesity is associated with poor asthma control and may contribute to the increased risk of uncontrolled asthma with depression/anxiety during pregnancy. However, BMI was similar in asthmatics with and without depression/anxiety and we observed no additional effect of BMI in our analysis. This finding is similar to recent findings in obese (BMI >30) adults observing that although both obesity and depression were associated with worse asthma control, their contributions were independent of each other.

There may also be an immune mechanism behind this worsening of asthma control as a result of the influences of depression/anxiety, asthma and pregnancy itself on immune function. The second part of this study examined whether a possible change in monocyte profile or differences in monocyte and T lymphocyte chemotaxis, may help to explain this reduction in asthma control.
4.1. Part A: Flow cytometric analysis of cell surface molecules (FACS)

4.1.1. Maternal Demographics

The individuals randomly selected for the FACS analysis were representative of the entire cohort (Table 6).

<table>
<thead>
<tr>
<th></th>
<th>Non-asthmatic</th>
<th>Asthmatic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Depression/ Anxiety</td>
<td>With Depression/ Anxiety</td>
</tr>
<tr>
<td></td>
<td>(n=37)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Maternal Age: median (25% - 75%)</td>
<td>27.0 (23.0- 30.0) (^{a})</td>
<td>29.5 (25.0- 32.0) (^{a})</td>
</tr>
<tr>
<td>BMI: median (25% - 75%)</td>
<td>23.9 (22.5- 28.5) (^{a})</td>
<td>32.0 (29.1- 35.0) (^{b})</td>
</tr>
<tr>
<td>Parity: n (%): 0 1+</td>
<td>18 (48.6) (^{a})</td>
<td>3 (30.0) (^{a})</td>
</tr>
<tr>
<td>Male</td>
<td>18 (48.6) (^{a})</td>
<td>4 (40.0) (^{a})</td>
</tr>
<tr>
<td>Female</td>
<td>19 (51.4) (^{a})</td>
<td>7 (70.0) (^{a})</td>
</tr>
<tr>
<td>Foetal Sex: n (%)</td>
<td>36 (97.3) (^{a})</td>
<td>9 (90.0) (^{a})</td>
</tr>
<tr>
<td>Caucasian</td>
<td>1 (2.7) (^{a})</td>
<td>1 (10.0) (^{a})</td>
</tr>
<tr>
<td>Other</td>
<td>36 (97.3) (^{a})</td>
<td>9 (90.0) (^{a})</td>
</tr>
<tr>
<td>Smoking Status n (%): Non-smoker</td>
<td>20 (54.1) (^{a})</td>
<td>5 (50.0) (^{b})</td>
</tr>
<tr>
<td>Former smoker</td>
<td>7 (18.9) (^{a})</td>
<td>1 (10.0) (^{a})</td>
</tr>
<tr>
<td>Quit during pregnancy</td>
<td>3 (8.1) (^{a})</td>
<td>2 (20.0) (^{a})</td>
</tr>
<tr>
<td>Current smoker</td>
<td>7 (18.9) (^{a})</td>
<td>2 (20.0) (^{a})</td>
</tr>
<tr>
<td>FEV1L: mean (SD)</td>
<td>3.0 (0.4) (^{a,b})</td>
<td>3.3 (0.7) (^{a,b})</td>
</tr>
</tbody>
</table>
FEV1 %: mean (SD)
0.9 (0.1)<sup>a</sup>  1.0 (0.2)<sup>a</sup>  0.9 (0.1)<sup>a</sup>  1.0 (0.2)<sup>a</sup>  0.060

FVCL: mean (SD)
3.7 (0.4)<sup>a,b</sup>  3.9 (0.8)<sup>a,b</sup>  3.5 (0.5)<sup>a</sup>  4.1 (0.8)<sup>b</sup>  0.028

FEV/FVC %: mean (SD)
0.8 (0.1)<sup>a</sup>  0.8 (0.1)<sup>a</sup>  0.8 (0.1)<sup>a</sup>  0.8 (0.0)<sup>a</sup>  0.869

ICS use before pregnancy n (%):
None  N/A  N/A  24 (50.0)<sup>a</sup>  11 (50.0)<sup>a</sup>  0.808
ICS  5 (20.8)<sup>a</sup>  3 (1.4)<sup>a</sup>  12 (25.0)<sup>a</sup>  4 (18.2)<sup>a</sup>
ICS + LABA

High ANRQ: n (%)
5 (15.8)<sup>a</sup>  7 (20.0)<sup>b</sup>  7 (14.6)<sup>a</sup>  16 (72.0)<sup>b</sup>  0.000

High EPDS: n (%)
1 (2.6)<sup>a</sup>  1 (10.0)<sup>a</sup>  1 (2.1)<sup>a</sup>  2 (12.0)<sup>a</sup>  0.386

Antidepressant use: n (%)
0 (0)  5 (50.0)<sup>a</sup>  0 (0)  7 (28.0)<sup>a</sup>  0.000

Uncontrolled Asthma: n (%)
N/A  N/A  17 (35.4)<sup>a</sup>  9 (40.0)<sup>a</sup>  0.659

Asthma Exacerbation: n (%)
N/A  N/A  12 (25.0)<sup>a</sup>  5 (20.0)<sup>a</sup>  0.837

Table 6: Monocyte flow cytometric analysis subgroup data at booking visit (12 or 18 weeks gestation) and asthma control throughout pregnancy.

Abbreviations: BMI= body mass index, FEV1L= forced expiratory volume in 1 second, FVC= forced vital capacity, ICS= inhaled corticosteroids, LABA= long acting β2 agonists, ANRQ= antenatal risk questionnaire, EPDS= Edinburgh Postnatal Depression Score. Statistical tests: Pearson Chi-Square Test or Kruskal-Wallis Test. Significance: p <0.05; <sup>a</sup>, <sup>b</sup> denote significantly different values

4.1.2. Percentage of Total Monocytes

At 18 weeks gestation, total monocyte percentage was increased in asthmatic pregnant women without depression/anxiety compared to asthmatic pregnant women with depression/anxiety (p=0.027) and non-asthmatic pregnant women without depression/anxiety (p=0.005; figure 9A). By 30 weeks gestation, these increases were no longer apparent (p=0.788 and 0.328 respectively; figure 9B). There was no difference in total monocyte percentage due to asthma status in those women with depression/anxiety at 18 or 30 weeks gestation (p=0.60 and 0.126 respectively).
Figure 9: Percentage total monocytes in the peripheral blood mononuclear cells of pregnant women with (triangles) and without (circles and squares) asthma and with (red and purple) and without (blue and green) depression/anxiety. Percentage total monocytes is significantly increased at 18 weeks gestation (A) in asthmatic pregnant women without depression/anxiety compared. There were no changes in percentage total monocytes at 30 weeks gestation (B). Significance: p <0.05.
4.1.3. CD14Bright, CD16Bright, CD14+CD16+ and CD14+CD16− Monocytes

There were no significant differences in levels of CD14 Bright, CD16 Bright, CD14+CD16+ and CD14+CD16− monocytes in any of the groups regardless of asthma status or the presence of depression/anxiety. This lack of difference was observed at 18 and 30 week of gestation (Table 7). Therefore the presence of depression/anxiety during pregnancies complicated by asthma does not change the expression of CD14 and CD16 on monocytes.

<table>
<thead>
<tr>
<th></th>
<th>Non-asthmatic Without Depression/ Anxiety (n=37)</th>
<th>With Depression/ Anxiety (n=10)</th>
<th>Asthmatic Without Depression/ Anxiety (n=48)</th>
<th>With Depression/ Anxiety (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18wk % Total Monocytes</td>
<td>5.10 (4.83- 7.54)a</td>
<td>6.59 (6.31- 9.64)a&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.67 (6.65- 13.1)b</td>
<td>6.62 (4.29- 7.73)a</td>
<td>0.050</td>
</tr>
<tr>
<td>CD14 Bright</td>
<td>64.5 (52.7- 80.3)a</td>
<td>61.1 (57.9- 63.0)a</td>
<td>61.3 (50.1- 72.2)a</td>
<td>62.4 (52.0- 65.0)a</td>
<td>0.820</td>
</tr>
<tr>
<td>CD16 Bright</td>
<td>5.0 (4.2- 6.5)a</td>
<td>7.8 (7.3- 8.7)a</td>
<td>6.3 (4.1- 7.4)a</td>
<td>7.1 (6.5- 10.0)a</td>
<td>0.153</td>
</tr>
<tr>
<td>CD14+CD16+</td>
<td>5.4 (4.6- 6.8)a</td>
<td>6.9 (5.0- 7.4)a</td>
<td>5.1 (4.0- 6.9)a</td>
<td>6.2 (4.1- 10.8)a</td>
<td>0.711</td>
</tr>
<tr>
<td>CD14+CD16−</td>
<td>14.6 (9.1- 27.2)a</td>
<td>22.3 (14.9- 23.0)a</td>
<td>18.2 (15.1- 25.7)a</td>
<td>16.5 (14.1- 22.4)a</td>
<td>0.786</td>
</tr>
<tr>
<td>30wk % Total Monocytes</td>
<td>7.7 (5.2- 11.5)a</td>
<td>10.9 (6.7- 14.8)a</td>
<td>7.1 (4.5- 11.1)a</td>
<td>9.0 (4.0- 11.3)a</td>
<td>0.308</td>
</tr>
<tr>
<td>CD14 Bright</td>
<td>57.8 (48.3- 64.6)a</td>
<td>65.7 (55.6- 71.2)a</td>
<td>60. (46.9- 66.9)a</td>
<td>54.3 (44.8- 66.8)a</td>
<td>0.451</td>
</tr>
<tr>
<td>CD16 Bright</td>
<td>4.6 (3.1- 6.1)a</td>
<td>7.1 (5.5- 7.7)a</td>
<td>4.0 (2.6- 6.5)a</td>
<td>4.4 (2.8- 8.5)a</td>
<td>0.220</td>
</tr>
<tr>
<td>CD14+CD16+</td>
<td>6.0 (3.4- 8.1)a</td>
<td>4.6 (2.3- 7.0)a</td>
<td>4.5 (2.8- 6.5)a</td>
<td>4.0 (2.9- 6.6)a</td>
<td>0.509</td>
</tr>
<tr>
<td>CD14+CD16−</td>
<td>27.6 (17.6- 34.3)a</td>
<td>19.5 (14.5- 30.3)a</td>
<td>23.0 (17.1- 38.6)a</td>
<td>27.4 (21.5- 36.1)a</td>
<td>0.506</td>
</tr>
</tbody>
</table>

Table 7: Percentages of total, CD14Bright, CD16Bright, CD14+CD16+ and CD14+CD16- monocytes in pregnant women with and without asthma and with and without depression/anxiety at 18 and 30 weeks gestation. Data are median (IQR), Mann-Whitney U Test. Significance: p <0.05; <sup>a</sup>, <sup>b</sup> denote significantly different values

4.1.4. Adhesion Receptor and HLA-DR Expression

The only difference in expression of CD11a, CD11b, CD62L and HLA-DR on the different types of monocytes (CD14 Bright, CD16 Bright, CD14+CD16+ and CD14+CD16−) was observed at 30 weeks gestation. Non-asthmatic women with depression/anxiety had a reduced percentage of CD11a receptors on their CD14 Bright monocytes (p=0.035; Figure 10). This is most likely due to the median percentage CD11a of these women being 0%, while other women in this group have percentages of up to 60%. Therefore most of the non-asthmatic women with depression/anxiety had no CD11a on their CD14 Bright monocytes (although there was no difference in CD14 Bright levels).

Other than the aforementioned decrease in CD11a on CD14 Bright monocytes at 30 weeks gestation, there were no differences in any of the other receptors tested at either 18 or 30 weeks of gestation.
There were no effects of smoking, BMI, foetal sex, maternal age or parity on monocyte percentages or receptor expression (data not shown).

4.1.5. Effect of Uncontrolled Asthma and Asthma Exacerbations

In women with depression/anxiety an increase in total monocyte percentage at 18 weeks gestation was observed in those with uncontrolled asthma compared to those with good asthma control (p = 0.019, Figure 11). It is the presence of depression/anxiety which appears to reduce total monocyte percentage rather than uncontrolled asthma increasing monocytes. In women with controlled asthma, those with depression/anxiety had a significant reduction in total monocyte percentage compared to those without depression/anxiety (p=0.001). In asthmatics without depression/anxiety there is no difference in total monocyte percentage at 18 weeks regardless of asthma control status. When the total monocyte percentage of controlled asthmatics without depression/anxiety was compared to non-asthmatics, also without depression/anxiety, they were observed to be significantly increased (p=0.004).
Figure 11: Effect of uncontrolled asthma on percentage total monocytes at 18 weeks gestation in non-asthmatics without (blue) or with (red) depression/anxiety compared to controlled asthmatics without (green) or with (purple) depression/anxiety and uncontrolled asthmatics without (orange) or with (black) depression/anxiety. Statistical significance: p <0.05.

There were no other differences in monocyte markers at 18 or 30 weeks gestation.

There were no differences in total monocyte percentage, the percentages of the various monocytes types (CD14 Bright, CD16 Bright, CD14^+CD16^+ and CD14^-CD16^-) and the monocyte adhesion receptors at 18 or 30 weeks gestation when data was analysed according to the presence or absence of asthma exacerbations during pregnancy.

There was no confounding effect of smoking, BMI, foetal sex, maternal age or parity on monocyte receptor expression when data was analysed in light of uncontrolled asthma or asthma exacerbations (data not shown).
### 4.2. Part B: PBMC Chemotaxis

#### 4.2.1. Maternal Demographics

The individuals chosen for the chemotaxis experiments were representative of the entire cohort (Table 8).

<table>
<thead>
<tr>
<th>Non-asthmatic Without Depression/Anxiety (n=21)</th>
<th>Non-asthmatic With Depression/Anxiety (n=13)</th>
<th>Asthmatic Without Depression/Anxiety (n=47)</th>
<th>Asthmatic With Depression/Anxiety (n=32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age: median (25% - 75%)</td>
<td></td>
<td></td>
<td></td>
<td>0.861</td>
</tr>
<tr>
<td>24.0 (21.0 - 27.0)</td>
<td>25.0 (22.0 - 29.0)</td>
<td>25.0 (22.0 - 29.0)</td>
<td>25.0 (21.0 - 30.0)</td>
<td></td>
</tr>
<tr>
<td>BMI: median (25% - 75%)</td>
<td></td>
<td></td>
<td></td>
<td>0.027</td>
</tr>
<tr>
<td>25.3 (21.7 - 29.6)</td>
<td>32.3 (26.2 - 40.0)</td>
<td>28.6 (23.2 - 33.6)</td>
<td>25.0 (22.7 - 30.7)</td>
<td></td>
</tr>
<tr>
<td>Parity: n (%): 0 1+</td>
<td></td>
<td></td>
<td></td>
<td>0.667</td>
</tr>
<tr>
<td>9 (42.9)</td>
<td>5 (38.5)</td>
<td>17 (36.2)</td>
<td>16 (50.0)</td>
<td></td>
</tr>
<tr>
<td>12 (57.1)</td>
<td>8 (61.5)</td>
<td>30 (63.8)</td>
<td>16 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Foetal Sex: n (%) Male</td>
<td></td>
<td></td>
<td></td>
<td>0.881</td>
</tr>
<tr>
<td>10 (47.6)</td>
<td>5 (38.5)</td>
<td>24 (51.1)</td>
<td>15 (46.9)</td>
<td></td>
</tr>
<tr>
<td>11 (52.4)</td>
<td>8 (61.5)</td>
<td>23 (48.9)</td>
<td>17 (53.1)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity: n (%): Caucasian Other</td>
<td></td>
<td></td>
<td></td>
<td>0.535</td>
</tr>
<tr>
<td>19 (90.5)</td>
<td>11 (84.6)</td>
<td>42 (89.4)</td>
<td>31 (96.9)</td>
<td></td>
</tr>
<tr>
<td>2 (9.5)</td>
<td>2 (15.4)</td>
<td>5 (10.6)</td>
<td>1 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Smoking Status: n (%) Non-smoker</td>
<td></td>
<td></td>
<td></td>
<td>0.179</td>
</tr>
<tr>
<td>13 (61.9)</td>
<td>3 (23.1)</td>
<td>26 (55.3)</td>
<td>12 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (9.5)</td>
<td>2 (15.4)</td>
<td>8 (17.0)</td>
<td>6 (18.8)</td>
<td></td>
</tr>
<tr>
<td>Quit during pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (9.5)</td>
<td>4 (30.8)</td>
<td>5 (10.6)</td>
<td>10 (31.3)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (19.0)</td>
<td>4 (30.8)</td>
<td>8 (17.0)</td>
<td>4 (12.5)</td>
<td></td>
</tr>
<tr>
<td>FEV1L: mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td>0.097</td>
</tr>
<tr>
<td>3.0 (0.5)</td>
<td>3.2 (0.4)</td>
<td>2.9 (0.4)</td>
<td>3.2 (0.6)</td>
<td></td>
</tr>
<tr>
<td>FEV1 %: mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td>0.323</td>
</tr>
<tr>
<td>0.9 (0.1)</td>
<td>1.0 (0.1)</td>
<td>0.9 (0.1)</td>
<td>0.9 (0.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>FVCL:</strong></td>
<td>3.6 (0.6)</td>
<td>3.8 (0.3)</td>
<td>3.5 (0.4)</td>
<td>3.9 (0.5)</td>
</tr>
<tr>
<td><strong>FEV/FVC %:</strong></td>
<td>0.8 (0.1)</td>
<td>0.9 (0.1)</td>
<td>0.8 (0.1)</td>
<td>0.8 (0.1)</td>
</tr>
<tr>
<td>ICS use before pregnancy</td>
<td>None</td>
<td>ICS</td>
<td>ICS + LABA</td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td>N/A</td>
<td>25 (53.2)</td>
<td>19 (59.4)</td>
<td>6 (12.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8 (17.0)</td>
</tr>
<tr>
<td>High ANRQ: n (%)</td>
<td>3 (15.8)</td>
<td>10 (70.0)</td>
<td>12 (25.5)</td>
<td>25 (78.1)</td>
</tr>
<tr>
<td>High EPDS: n (%)</td>
<td>1 (2.6)</td>
<td>0 (0.0)</td>
<td>1 (2.1)</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>Antidepressant use: n (%)</td>
<td>0 (0)</td>
<td>6 (50.0)</td>
<td>0 (0)</td>
<td>8 (25.0)</td>
</tr>
<tr>
<td>Uncontrolled Asthma: n (%)</td>
<td>N/A</td>
<td>N/A</td>
<td>15 (31.9)</td>
<td>16 (50.0)</td>
</tr>
<tr>
<td>Asthma Exacerbation: n (%)</td>
<td>N/A</td>
<td>N/A</td>
<td>12 (25.5)</td>
<td>7 (21.9)</td>
</tr>
</tbody>
</table>

Table 8: Peripheral blood mononuclear cell chemotaxis subgroup data at booking visit (12 or 18 weeks gestation) and asthma control throughout pregnancy. BMI= body mass index, FEV1L= forced expiratory volume in 1 second, FVC= forced vital capacity, ICS= inhaled corticosteroids, LABA= long acting β2 agonists, ANRQ= antenatal risk questionnaire, EPDS= Edinburgh Postnatal Depression Score. Statistical tests: Pearson Chi-Square Test or Kruskal-Wallis Test. Significance: p <0.05; a, b denote significantly different values.
4.2.2. PBMC Chemotaxis

There were no differences in migration index due to asthma status or the presence or absence of depression/anxiety at each individual time point (Table 9).

<table>
<thead>
<tr>
<th></th>
<th>Non-Asthmatic</th>
<th>Asthmatic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without</td>
<td>Without</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depression/</td>
<td>Depression/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anxiety (n= 21)</td>
<td>Anxiety (n= 47)</td>
<td></td>
</tr>
<tr>
<td>18 weeks</td>
<td>0.9 (0.7- 1.1)</td>
<td>1.0 (0.8- 1.4)</td>
<td>0.171</td>
</tr>
<tr>
<td>30 weeks</td>
<td>1.1 (0.8- 1.4)</td>
<td>0.9 (0.8- 1.2)</td>
<td>0.066</td>
</tr>
</tbody>
</table>

Table 9: Migration index of peripheral blood mononuclear cells throughout pregnancy. Data are median (IQR), Kruskal-Wallis Test. Significance: p <0.05.

No changes in migration index were observed when women with uncontrolled asthma were compared to those with good asthma control at 18 (p=0.169) and 30 (p=0.179) weeks gestation. Migration index was unchanged when women who experienced an asthma exacerbation during pregnancy were compared to those who did not at 18 (p=0.196) and 30 (p=0.160) weeks gestation (data not shown).

No effect was observed due to smoking, BMI, initial ICS use, high ANRQ score or foetal sex (data not shown).

4.3. Discussion

4.3.1. FACS Analysis

Asthma without depression/anxiety was associated with a small increase in monocyte percentage at 18 weeks but not 30 weeks of pregnancy. This contradicted previous research that observed increased monocytes throughout gestation in pregnancies complicated by asthma\textsuperscript{46}. This was observed in women who were and were not using inhaled glucocorticoids\textsuperscript{46}. When the current asthmatic group was reanalysed according to their use of inhaled corticosteroids (ICS), there was no significant differences in total monocyte percentages at 18 or 30 weeks of pregnancy (p= 0.215 and 0.742) or any increase in monocyte number throughout pregnancy. One limitation of this study was that daily glucocorticoid dose was not calculated as was undertaken by Osei-Kumah et al. Instead, as the focus was on the effect of depression/anxiety on monocyte characteristics, we used the pre-pregnancy ICS use as a medication approximation. A more complete study might consider the effect of the ICS dose. This was unable to be performed in this study as the stratification for depression/anxiety and ICS dose resulted in inadequate power.
In asthmatic women, not having depression/anxiety during pregnancy was associated with an increase in total monocyte percentage at 18 weeks gestation. This was no longer apparent at 30 weeks gestation. This increase in monocyte percentage might be expected to enhance inflammatory pathways associated with asthma and lead to a loss of asthma control in women without depression/anxiety. However, uncontrolled asthma increased in women with depression/anxiety compared to women without depression/anxiety. It was thought that this contradiction might be a result of the heterogeneity of asthma. Increased monocyte percentage at 18 weeks gestation might worsen asthma control in asthmatic women without depression/anxiety, but when depression/anxiety further complicates pregnancy, a different mechanism could be activated leading to a greater loss of control.

The presence of depression/anxiety during pregnancies complicated by asthma did not change monocyte expression of CD14 and CD16. Although early work had demonstrated an increase in CD14+CD16+ monocytes in asthmatics compared to non-asthmatics, this was not observed in our study even when discounting any influence of depression/anxiety. However there were a couple of key differences between this study and the earlier work. Some women in our study were using ICS, whereas in River et al.’s study, the asthmatics had not been using ICS in the previous month. ICS use reduces inflammation and may cause the monocytes in these asthmatics to express CD14 and CD16 in a similar manner to monocytes of non-asthmatics. The major difference was that our study involved pregnant women, specifically 48 non-asthmatic and 73 asthmatic pregnant women compared to the 9 non-asthmatic and 11 asthmatic adults used previously. A number of studies have observed differences in monocyte activation and number with pregnancy so it does not seem unreasonable to expect monocytes in pregnant women to behave differently to non-pregnant adults.

A decrease in CD11a on CD14 Bright monocytes at 30 weeks gestation was observed in non-asthmatic women with depression/anxiety (n=10). There were no changes at 18 weeks. This is in contrast to another study that observed a progressive up-regulation of CD11a on CD14+ monocytes throughout pregnancy. This raises the possibility that depression/anxiety in the pregnant individual down-regulates CD11a expression on CD14+ monocytes. This conclusion may be a little premature, as there were no changes in CD11a in our non-asthmatic women without depression/anxiety from 18 to 30 weeks, so we were unable to replicate Luppi et al.’s initial finding in a non-asthmatic pregnant population.

In addition, the reduced CD11a expression on CD14 Bright monocytes in non-asthmatics with depression/anxiety, although initially exciting, is due to individuals with 0 or 0.1% CD11a expression. This result should be interpreted with caution as 0 and 0.1% CD11a expression occurs in some individuals in all groups. Non-asthmatics with depression/anxiety are a much smaller group (11.8% of the total cohort; depression/anxiety being less common in our controls than asthmatics) therefore this significant reduction in expression could be due to random selection bias rather than true result. The
spread in these non-asthmatics does appear similar to asthmatics with depression/anxiety. Perhaps with greater numbers the median would be more similar to that of the asthmatics.

Other factors may influence monocyte number, type and receptor expression. Although the body of work is small, increases in monocyte numbers have been observed in women pregnant with a female obesity also appears to increase the percentage of CD14+CD16+ monocytes. In our study these findings were unable to be replicated. Both foetal sex and BMI had no effect on monocyte number or the various receptors expressed on the monocytes. The effects of smoking, maternal age and parity were also examined and there were no effects on monocyte count or receptor expression.

The changes in monocyte number at 18 weeks gestation when women were assessed according to uncontrolled asthma during pregnancy, appear somewhat conflicting. We observed increased monocytes with depression/anxiety in uncontrolled asthma, but decreased monocytes with depression/anxiety in controlled asthma. Total monocyte percentages were similar in asthmatics without depression/anxiety regardless of asthma control. However, we observed increased monocytes in controlled asthmatics compared to non-asthmatics when both had no depression/anxiety. From this it appears that asthma control does not affect monocyte numbers but the addition of depression/anxiety to asthma has opposing effects on monocytes number according to asthma control status. At this stage it is unclear as to what effect these differences may have on asthma control, considering that by week 30 of pregnancy, these changes were no longer observed. It would be interesting to investigate this further, perhaps with more follow up around the timing of asthma events and the changes in monocyte profile.

In keeping with our first finding that depression/anxiety did not affect the incidence of asthma exacerbations, there was no difference in monocyte number or markers at either 18 or 30 weeks gestation when women were assessed according to the presence or absence of asthma exacerbations during pregnancy.

4.3.2. PBMC Chemotaxis

No increase in T lymphocyte and monocyte (the PBMCs) migration was observed with the complication of asthma during pregnancy. Previous work had observed a suppression of PBMC chemotaxis with pregnancy and a lack of this suppression in asthmatic pregnant women. Instead plasma from asthmatic pregnant women resulted in a PBMC chemotaxis response similar to that in response to non-pregnant plasma. We were unable to replicate a lack of suppression of chemotaxis in the PBMCs from pregnant asthmatics. A major difference in the current study and Osei-Kumah et al.’s work was the chemoattractant used in the assay. As has been used previously, we used monocyte chemoattractant protein-1 (MCP-1) while Osei-Kumah et al. used maternal plasma, thereby more completely recreating the maternal environment. Our work examined the chemotactic response of monocytes and T
lymphocytes from pregnancies with different complications to a specific chemoattractant (MCP-1) and this may not fully replicate the in vivo experience of the cells. Increases in chemokines (due to stress, depression, anxiety, obesity, smoking and the like) may change the chemokine milieu in individuals and this is not controlled for in our study. Future work using maternal plasma from pregnancies with the complications of asthma and depression/anxiety would control for any changes in the chemokine milieu due the presence of asthma or depression/anxiety.

There was also no increase in chemotactic ability of the PBMCs with the addition of depression/anxiety to pregnancies complicated by asthma as originally hypothesised. This could also be because using MCP-1 at the one concentration for all groups, may not adequately replicate the chemokine milieu in vivo. Other work has demonstrated increases in ICAM-1 and IL-6 expression in non-asthmatic, non-pregnant individuals under stress. MCP-1 levels were increased in women exposed to prolonged psychosocial stress although this should be interpreted with caution as, in a similar study, no differences in MCP-1 levels were observed. It could be that these in vivo increases in MCP-1 and ICAM-1 result in increased PBMC migration and subsequent uncontrolled asthma, however our experimental design did not examine in vivo levels of MCP-1 or ICAM-1.

Obesity affected 34.8% of women in this study and there was some expectation that obesity might have an effect on PBMC chemotaxis, as a previously increased monocyte expression of CCR2 (MCP-1 receptor) and in vitro migration in obese women was observed. This did not occur in our study. The previous work involved non-pregnant women and although their obese measurement was the same as ours (BMI ≥ 30.0) our participants were pregnant. This may have resulted in some participants being inappropriately classed as obese due to their pregnancy weight gain and may have affected our results. Alternatively, it may be that pregnancy has a greater effect on PBMC chemotaxis than obesity and therefore masked any possible effects due to obesity.

Both the FACS and chemotaxis work in these experiments show that monocytes and T lymphocytes are not associated with increased uncontrolled asthma during pregnancies complicated by asthma and depression/anxiety. This suggests that these cells may not be central to mechanisms associated with uncontrolled asthma or exacerbations during pregnancy. Further studies need to be conducted with greater numbers of non-asthmatics with depression/anxiety and possibly using a monocyte enriched PBMC population to investigate the chemotactic capabilities of monocytes alone.
Chapter 5: Discussion

5.1. Discussion

The presence of depression/anxiety during pregnancies complicated by asthma is associated with an increase in uncontrolled asthma, but not a change in exacerbation risk. This increase in loss of control in women with depression/anxiety was not a result of alterations in monocyte percentage, type or adhesion receptor expression or changes in PBMC chemotaxis.

Our work rules out a change in monocyte receptor expression, an expansion of the monocyte population or an increase in T lymphocyte and monocyte chemotaxis as an explanation of the increase in uncontrolled asthma observed in pregnancies already complicated by asthma and depression/anxiety. As mentioned earlier, there could be a range of societal factors (medication non-adherence, smoking, poor health literacy, obesity, socioeconomic status) that could account for our result. Changes in other immune cells and inflammatory processes could also play a role in the increased risk of uncontrolled asthma with depression/anxiety during pregnancy.

An increase in susceptibility to viral infection with stress and anxiety could also trigger uncontrolled asthma during pregnancy. When influenza virus was administered to adults, those who tested higher on a perceived stress scale reported more severe flu symptoms\textsuperscript{142}. The greater symptom severity was not just a product of the subjects' perception of illness, the underlying immune response was also changed. Mucus weights were increased along with IL-6 concentrations in nasal lavage\textsuperscript{142}. Therefore highly stressed individuals are more susceptible to infection and it could be this increased susceptibility which may have triggered the increased uncontrolled asthma in our women with depression/anxiety.

This study used peripheral blood immune cells. This is the safest immune cell sample collection in a pregnant population. It could be that the increases in monocyte percentages that we expected to observe were not measurable in circulating immune cells but changes occurred locally in immune cells present in the lung. In mice, cell changes in the lungs have been associated with increased allergic airway disease. IL-4 induced development of allergic inflammation and hyperplasia in the lungs during allergen challenge has been demonstrated after early-life viral infection\textsuperscript{92}. In the same model IL-25 induced subepithelial fibrosis and epithelial hypertrophy\textsuperscript{92}. Early-life Chlamydia respiratory infection in mice, increased Tc cells, macrophages, neutrophils and dendritic cells in the lungs compared to sham infection\textsuperscript{143}. This influx of inflammatory cells in the lungs promoted airway hyperresponsiveness and increased the severity of allergic airway disease in later life\textsuperscript{143}. A link between respiratory infections early in life and subsequent respiratory disease may be tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). TRAIL is expressed by most inflammatory cells and regulates both inflammation and
apoptosis. In mice, it was demonstrated to be vital in promoting infection-induced inflammation and mucus hypersecretion as well as the subsequent lung function impairment\(^\text{144}\). Allergic inflammation and airway remodelling contribute to worsening lung function and could result in an increase in uncontrolled asthma in humans via a similar pathway.

Induced sputum or a lung biopsy would clarify the level of inflammatory cell migration to the lungs. These procedures are not ethically possible in pregnant women. Induced sputum can result in a decrease in lung function which in turn could result in a hypoxic insult to the foetus. Therefore these procedures are unethical in pregnant women and so only peripheral blood immune cells are accessible in this population. Alternatively, the lack of changes in circulating monocyte inflammatory profile could mean that a different cell population is responsible for the increased uncontrolled asthma we observed.

Worsening asthma during pregnancies complicated by asthma and depression/anxiety could be driven by the neutrophil population. Neutrophils increase during pregnancies complicated by asthma and this could result in an increase in pro-inflammatory cytokine production or increased neutrophil chemotaxis. IL-6, which is increased during depression/anxiety as well as asthma and pregnancy, increases neutrophil migration towards IL-8\(^\text{145}\) (which itself is increased in depression\(^\text{146}\)). Neutrophils are also less responsive to corticosteroids and corticosteroids are protective against neutrophil apoptosis. If the underlying inflammation type in women with asthma and depression/anxiety is changed from eosinophilic to neutrophilic inflammation during pregnancy, this could contribute to the increase in uncontrolled asthma through a reduction in medication efficacy.

Differences in glucocorticoid receptor (GR) sensitivity could also be a plausible immune mechanism of worsening asthma during pregnancies complicated by asthma and depression/anxiety. Glucocorticoids bind to the GR to suppress the expression of various pro-inflammatory cytokines, inhibit the recruitment of neutrophils and monocytes\(^\text{147}\) and induce apoptosis in lymphocytes\(^\text{148}\). Although there is only one gene encoding the GR, several different isoforms exist with differing sensitivities. There are two main isoforms, GR\(\alpha\) and GR\(\beta\)\(^\text{149}\) (GR\(\alpha\) being more active than GR\(\beta\)) with an additional eight isoforms\(^\text{150}\). The GR-C3 isoform has an enhanced response and greater anti-inflammatory action compared to the other isoforms, while GR-D is the least sensitive\(^\text{151}\).

The ratio of GR\(\alpha\)/GR\(\beta\) in PBMCs of stable and unstable asthmatics on high and low doses of glucocorticoids is similar\(^\text{152}\). GR\(\beta\), the isoform associated with glucocorticoid resistance, is up-regulated by IL-17 and -23 in PBMCs\(^\text{153}\). In adults with depression, monocyte GR\(\alpha\) gene expression was down-regulated in those with a higher depression score\(^\text{146}\). In contrast, serum levels of IL-6, IL-8, MCP-1 and VEGF were significantly increased in individuals with a higher depression score\(^\text{146}\). In fact, hyperactivity of the HPA axis as occurs in depression and anxiety, is a marker of glucocorticoid resistance\(^\text{96}\). The GR ratio has not yet been examined in pregnant asthmatics with the additional complication of
depression/anxiety. It may be that the combination of pregnancy and depression/anxiety increases the level of GRβ and/or GR-D in asthmatics. This would increase glucocorticoid resistance and could lead to a loss of asthma control during pregnancy, even with appropriate asthma medication. More research is needed to determine if these are possible mechanisms by which uncontrolled asthma is increased in pregnant asthmatics with depression/anxiety.

Our research will have implications for the antenatal care of asthmatic women with depression/anxiety as a more holistic approach may need to be taken. This would need to incorporate the traditional aspects of asthma management: an asthma action plan and asthma education (encouraging medication adherence, smoking cessation and correct inhaler technique), with depression and anxiety specific treatment. This might include counselling and medication reviews as well as more frequent appointments with a respiratory physician.

5.2. Strengths and Limitations

This study is the first to specifically examine the effect of the presence of depression/anxiety on asthma control in pregnancies complicated by asthma. We observed a 63.3% increased incidence of uncontrolled asthma with the additional complication of depression/anxiety. This resulted in women with asthma and depression/anxiety having a 53.8% increased relative risk of experiencing uncontrolled asthma. Furthermore, we investigated a possible immune mechanism for this worsening of asthma, being the first to examine changes in monocyte characteristics and changes in PBMC chemotactic capabilities during pregnancies complicated by asthma and depression/anxiety. As such, this study suggests that there are is an increase in total monocyte percentage at 18 weeks gestation in asthmatic women compared to non-asthmatic women, that is not observed when asthmatic women also suffer from depression/anxiety. This increase is observed whether the asthma is controlled or uncontrolled. No changes in PBMC chemotaxis were observed with the additional complication of depression/anxiety during pregnancies already complicated by asthma.

This study was limited by the way in which depression and anxiety were defined. There was no doctor diagnosis, instead it was self-reported with participants who answered yes to the question “Do you have depression/anxiety?” being considered to have depression/anxiety. As there is still stigma associated with mental health issues there is the slight possibility that some women did not disclose depression/anxiety and have been misclassified. However, as this question was asked by a nurse in the clinical setting of the patient’s first antenatal visit we have confidence that women did disclose appropriately. Case notes were also reviewed for verification of the report from the patient. The Edinburgh Postnatal Depression Score (EPDS) and the Antenatal Risk Questionnaire (ANRQ), while not being diagnostic tools, did give a good indication of the depression and anxiety risk profiles of the
women, and corresponded to their depression/anxiety profile. Of the women with depression/anxiety, 18.8% had a high EPDS and 78.5% had a high ANRQ compared to 4.3% and 17.2% respectively, of the women without depression/anxiety. An interesting addition to the study which could be carried out in future work, would be to collect salivary cortisol measurements at each visit. This would enable an objective measurement of stress at the same time as asthma control was assessed and blood collected.

A major limitation of this study was the way in which the outcomes were defined. An asthma exacerbation was considered a self-reported, unscheduled doctor’s visit, a course of oral corticosteroids, an emergency department presentation or a hospital admission. By its very nature, for an exacerbation to occur and be reported as such, the patient needed to take some action. This can be subject to bias if for some reason the patient does not take the appropriate action for example through a lack of understanding of asthma control (i.e. poor health literacy), lack of money to pay for medications or a lack of access to a doctor. Therefore the actual number of exacerbations may be under-reported and this may be affecting the results, such that a woman who is not considered to have exacerbated during pregnancy may have actually had an exacerbation. However these measurements for an exacerbation are internationally recognised and considered appropriate.

Uncontrolled asthma was defined as an Asthma Control Questionnaire score of greater than 1.5. This was administered at every visit but also relied on self-reported symptoms as well as spirometry. The timing of the visit may introduce some bias into our results as the women were assessed at 12, 18, 30 and 36 weeks of gestation. There is the possibility that women were experiencing uncontrolled asthma in between visits and this would not have been considered unless they visited their doctor and reported it at the next visit. One way to improve the accuracy of these outcomes, might be to include telephone follow ups fortnightly. This would have the two-fold benefit of increasing the accuracy of exacerbation reporting and help to improve the women’s asthma and pregnancy health literacy, thereby increasing their inclination to visit their doctor when their asthma is uncontrolled. Of course while this would remove any doubt about the exacerbations and uncontrolled asthma that women are experiencing during pregnancy, it does have to be weighed against the costs to this scenario, both in terms of time and monetary costs for the researcher and the inconvenience cost to the participant.

The timing of uncontrolled asthma and asthma exacerbations did affect the immune cell experiments. Some individuals experienced these events earlier in their pregnancy and some had later events. As a result, the immune cell experiments had a mixture of pre- and post-exacerbation blood samples. As the focus of the current study was investigating the effect of depression/anxiety on asthma control during pregnancy and any changes in monocyte profile and PBMC chemotaxis, when wanting to examine further the possible effect of uncontrolled asthma or exacerbations, there were insufficient experimental numbers to properly stratify to this level. Analysis of monocyte markers and PBMC chemotaxis at 18
and 30 weeks gestation in pre- and post-exacerbation individuals resulted in no differences in markers or migration index. However, this could not adjust for depression/anxiety.

One limitation of the study is that there was no characterisation of the type of asthma (atopic/ non-atopic) or inflammation subtype (eosinophilic/ neutrophilic) at the first visit. Both the asthma and inflammation type will affect medication responsiveness and asthma control. Unfortunately these effects cannot be adjusted for in the current analysis. It would be interesting to investigate this further to observe if the increase in uncontrolled asthma in pregnant women with depression/anxiety is due to a particular inflammation subtype. This would also help to clarify which immune pathways may be involved in this uncontrolled asthma, and may help with maintaining control during pregnancy through the identification of a marker of uncontrolled asthma or result in a treatment opportunity.

5.3. Further work

The immune cell function part of this study was cross-sectional in nature and as such can only give insights into what is happening at the immune cell level at one time point. To give a more complete picture about what is happening to the immune cells throughout pregnancy and what influence an early exacerbation may have on later asthma control, it would be useful to undertake a longitudinal study on these women. This may also include monocyte cytokine expression in response to inflammatory stimuli such as bacterial and viral infection. It may be that there is increased production of monocyte cytokines in pregnancies complicated by asthma and depression/anxiety. This may then lead to uncontrolled asthma through subsequent enhanced chemotaxis of inflammatory cells after infection.

As we did not observe major changes in monocyte profile or chemotaxis that would be an adequate immune mechanism of worsening asthma during pregnancy, another area of interest might be the neutrophils. Neutrophilic inflammation is one of the major inflammation subtypes in the disease of asthma and previous work has demonstrated an increase in neutrophils during pregnancies complicated by asthma. Although it was beyond the scope of this study, it would be interesting to examine if there are any changes in neutrophil number, chemotaxis and cytokine production during pregnancies complicated by asthma and depression/anxiety.

Glucocorticoid receptor (GR) sensitivity plays a large role in asthma control, especially as the increased cortisol observed in depression and anxiety can lead to GR insensitivity. This work did not investigate the various GR isoforms and examine which are more prevalent in pregnant asthmatics with depression/anxiety. It would be useful to investigate this in another study as the increased uncontrolled asthma with depression/anxiety observed in these pregnant women could be due to reduced mediation efficacy rather than non-adherence. At this stage it is impossible to determine which is the leading cause. While it is always important to educate pregnant women about medication use and adherence,
identifying the major GR isoforms in women with uncontrolled asthma would help to provide some clarity.

5.4. Conclusion

Women with depression/anxiety during pregnancies complicated by asthma are at an increased risk of uncontrolled asthma. This uncontrolled asthma could lead to an increased risk of poor pregnancy outcomes such as pre-eclampsia, preterm birth or small for gestational age babies. Asthma control during pregnancy may be improved by asthma education, administration of the influenza vaccine, the encouragement of smoking cessation, the encouragement of medication adherence (both asthma and depression/anxiety) and potentially professional counselling.

There could also be an underlying immune mechanism resulting in uncontrolled asthma in women who already take all appropriate precautions. This study suggests that a change in the monocyte inflammatory profile or an increase in peripheral blood mononuclear cell chemotaxis is not the potential cause of uncontrolled asthma in these women. Future work should focus on the expression patterns of the various glucocorticoid receptor isoforms or the function of the neutrophil population.
References

7. Murphy VE, Clifton VL, Gibson PG. The effect of cigarette smoking on asthma control during exacerbations in pregnant women. *Thorax* 2010; 65(8): 739-44.
27. Yalcin AD, Bisgin A, Gorczynski RM. IL-8, IL-10, TGF-beta, and GCSF levels were increased in severe persistent asthma patients with the anti-IgE treatment. Mediators of inflammation 2012; 2012: 720976.
30. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nature medicine 2007; 13(2): 139-45.
Peripheral monocyte expression of the chemokine receptors CCR2, CCR5 and CXCR3 is 65.

1317

female fetal growth.

monocyte 64.

leucocyte blood counts and lymphocyte responses in pregnancy: a marked early increase of 63.

2002; 62.

are progressively activated in the circulation of pregnant women.

61.

2008; 60.

and growth factors

59.

New, NY) 69.

TH1 CD4+ T cells through IL

58.

Journal of allergy and clinical immunology

57.

physiology Lung cellular and molecular physiology

au

56.

produced by monocytes.

55.

immunology

Differentiation of human dendritic cells from monocytes in vitro.

54.

monocytes are a major source of TNF.

53.

immunity

52.

glucocorticoid signaling in mon

51.


50.


49.

Allergy

monocytes/macrophages  and Il

48.


47.


46.


45.


44.


43.


42.


41.


40.


39.


38.


37.

Peripheral monocyte expression of the chemokine receptors CCR2, CCR5 and CXCR3 is
78. Simpson JL, Baines KJ, Ryan N, Gibson PG. Neutrophilic asthma is characterised by increased rhinosinusitis with sleep disturbance and GERD. *Asian Pacific journal of allergy and immunology / launched by the Allergy and Immunology Society of Thailand* 2014; 32(1): 66-74.
110. Favreau H, Bacon SL, Labrecque M, Lavoie KL. Prospective impact of panic disorder and panic-anxiety on asthma control, health service use, and quality of life in adult patients with asthma over a 4-year follow-up. *Psychosomatic medicine* 2014; 76(2): 147-55.


141. Wong GWK, Ho CY, Ko FWS, et al. Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFNγ, IL-4, IL-10 and IL-13) in patients with allergic asthma. *Clin Exp Immunol* 2001; 125: 177-83.


Appendix

Participant information sheet and informed consent

WOULD YOU LIKE TO PARTICIPATE IN A STUDY OF ASTHMA AND PREGNANCY?

PLEASE READ OUR INFORMATION SHEET

INVESTIGATORS
A/Prof Vicki Clifton Paediatrics and Reproductive Health Phone: 08 8182 9337
Dr Nicolette Hodyl, Paediatrics and Reproductive Health
Dr Annette Osei Kumah Paediatrics and Reproductive Health
Dr Michael Stark Paediatrics and Reproductive Health
Prof Gustaaf Dekker Obstetrics and Gynaecology, Lyell McEwin Hospital
Prof Brian Smith Respiratory Medicine, Lyell McEwin Hospital
Ms Karen Rivers, Lyell McEwin Hospital

Short Title: Effect of asthma during pregnancy on maternal health, placental function, fetal growth and childhood development

Patient Initials................................................. Patient
Number......................................................

What is the purpose of the study?

We are investigating the effects of asthma on the baby’s growth and placental function. Pregnancies complicated by severe asthma may be associated with preterm delivery and low birthweight babies. This study will determine how the mother’s asthma changes during pregnancy and whether there are any ways we can detect changes in the mother before her asthma worsens. This study will monitor the baby’s growth throughout pregnancy in women who have mild, moderate or severe asthma and determine if there are any changes in growth associated with asthma. We also want to look at the placenta (the afterbirth) and examine if there are any changes in how the placenta functions in women with asthma. We will also collect cord blood from the placenta and examine the effect of the blood on the activity of different cells. This study will increase our understanding of pregnancy and asthma and
may improve the treatment of asthma during pregnancy. The samples we collect from the placenta may be used for future studies of asthma and pregnancy.

What does the study involve?

1. 20 ml blood samples on 3 occasions at around 18, 30 and 36 weeks of pregnancy
2. 3 ultrasounds at 18, 30 and 36 weeks
3. collection of a sample of urine at 12, 18 and 30 weeks of pregnancy
4. collection of your saliva at 18 and 36 weeks gestation
5. completing a dietary questionnaire at 18 weeks of pregnancy, and a 24 hour recall of dietary intake at 30 and 36 weeks of pregnancy
6. an assessment of your asthma at 18, 30 and 36 weeks of your pregnancy, during an exacerbation of your asthma if you have one, and at 6 months after delivery, including collection of a sample of exhaled breath and a measurement of the amount of oxygen in your blood (pulse oximetry)
7. the donation of your placenta after your delivery and collection of cord blood
8. A skin prick test to determine your allergies and cheek cell swab to examine asthma-related genes at 6 months after delivery

<table>
<thead>
<tr>
<th>Event</th>
<th>12 weeks</th>
<th>18 weeks</th>
<th>30 weeks</th>
<th>36 weeks</th>
<th>Exacerbation</th>
<th>Delivery</th>
<th>6 months after delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood sample</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary Questionnaire</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hour recall of dietary intake</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Sample</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma Assessment (Breathing tests &amp; pulse)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Test Type</td>
<td>18</td>
<td>30</td>
<td>36</td>
<td>40</td>
<td>44</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Exhaled breath sample</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placenta &amp; cord blood collection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergy skin prick test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

**First Visit**

At your first antenatal visit you usually give a blood sample and some extra blood will be taken with your permission for this study. The respiratory nurse will talk to you about what you take for your asthma and how often you get sick. We will look at your lung capacity using breathing tests and we will ask you to record your peak flow everyday for 2 weeks in a diary card. When you are sick with asthma we will ask you to record this in your diary.

At your first antenatal visit, we will give you a dietary questionnaire (the food frequency questionnaire) which usually takes about 30 minutes to complete.

**Urine Collections**

We would like to collect a urine sample at 18, 30 and 36 weeks of pregnancy. As part of your regular antenatal care you will give a urine sample. Once this has been tested by the antenatal clinic we would store this sample to measure some hormones and vitamins.

**Blood Samples**

At 18, 30 weeks and 36 weeks we will take another blood sample. These samples are not normally taken during your pregnancy. The blood sample may hurt a little and there could be bruising, dizziness and/or fainting, but this doesn’t happen very often.

**Saliva**

We would like to monitor how your asthma treatments affect your body and we can do this in saliva. We will collect saliva before your 18 and 30 week visits to the clinic. You will be given instructions on how to collect your saliva by the research nurse and we will remind you to collect your saliva on the day before the visit to the antenatal clinic by sending a text message.
Ultrasounds

At around 18, 30 and 36 weeks of your pregnancy we will monitor your baby's growth using an ultrasound. One study has reported that ultrasound may be associated with a 30% increase in left-handedness which equates to 3 children in every 100 births. No harm has been demonstrated from ultrasound.

Asthma Assessments (Breathing tests)

At around 18, 30 and 36 weeks of your pregnancy the respiratory nurse will conduct breathing tests to examine your lung function during pregnancy so we can compare it to women without asthma. This will involve breathing into a tube for a few seconds. It may involve doing this several times to get the right measurement. These breathing tests are not harmful and will not cause any discomfort to you or your baby. During these breathing tests, we may collect a sample of your exhaled breath which would be used to measure markers of inflammation in the laboratory. We will also use a non-invasive technique called pulse oximetry to measure the amount of oxygen in your blood. This test involves placing a probe on your index finger and is not harmful and should not cause you any discomfort. At these visits, the respiratory nurse will also ask you some questions about your dietary intake in the previous 24 hours. This usually takes about 5 to 10 minutes. You will be given a diary card to fill out to detail your asthma symptoms and the drugs you take during your pregnancy. We will also give you a peak flow meter to measure your lung function. We may contact you by telephone to check your progress with the study diary.

Extra visits if you have an exacerbation of your asthma

If you are having an exacerbation of your asthma, we would like you to come into the hospital for an extra visit that would involve having a 20 ml blood sample taken and having the same breathing tests performed to examine your lung function and for the collection of a sample of exhaled breath. In addition, we would like to repeat the measurement of the amount of oxygen in your blood and collect a nasal swab to see if you have a virus which might be contributing to your exacerbation. We would also like you to complete a common cold questionnaire. Please contact one of the research nurses of on 8182 9337 to arrange a time to come in for the extra visit if you are sick.

Placenta collection

After your baby is delivered, if you agree, we will collect your placenta (the afterbirth) and determine whether there are any changes in how it functions. The placenta is delivered after the baby and our collection of it will not harm mother or baby. We will collect blood from the placental cord and use it in the laboratory to examine the effect blood from a male or female fetus has on the activity of different cells. Some of the samples of blood, urine, exhaled breath, cord blood and placenta will be stored and used in the future for further studies of asthma and pregnancy. The samples of blood, urine, exhaled breath and placenta may also be used by students in the future as part of their research projects. All of the samples collected will be de-identified so that your privacy is maintained.
These samples will not be used for cloning or stem cell research.

If you agree to donate your placenta and cord blood for this study, there will be a note on your medical record to tell the midwife how to contact A/Prof Clifton to collect it. If you are able to remind the midwife after the birth of your baby we would be most appreciative.

After the delivery of your baby, we would like to access your medical records from the John Hunter Hospital to obtain some information about you and your baby which is routinely collected during your antenatal visits and the birth of your baby. This information will allow us to determine if there is any association between your asthma and the outcome of your pregnancy.

This information collected from your medial records will be entered into a database by the clinical staff involved in the project and will be accessible to the necessary investigators only. Research students involved with this project will not be able to access any identifiable information about you, and all information will be de-identified for the purposes of data analysis, in all publications of the work and for all student projects.

Visit at 6 months after delivery of your baby
About 6 months after your delivery, we would like to take a sample of your exhaled breath and reassess your lung function using the same breathing tests performed by the respiratory nurse during the pregnancy visits as well as repeat the measurement of the amount of oxygen in your blood. If you have not already had it done, we will perform an allergy skin test. In this test, a drop of allergy extract will be placed on the forearm and tiny pricks will be made on the skin. The allergens tested will be fungi (aspergillus fumigatus and alternaria tenius), penicillium, dust mite, cockroach, cat hair, dog hair, feather mix and grass mix. If you are allergic to any of these, a small itchy lump will occur. This lump only lasts for about an hour and you will be given some cream to relieve the itch. We will ask you at this visit if you would like to bring your baby in for the childhood development study.

What if I have an asthma attack during my pregnancy?
If you are sick with asthma during your pregnancy, it is important that you follow your action plan and seek medical help from your usual doctor. If it is possible, we would like you to come into the hospital for an extra visit that would involve having a 20 ml blood sample taken, completing a common cold questionnaire, collecting a nasal swab and exhaled breath and having some breathing tests to examine your lung function. Please contact Ms Karen Rivers on 8182 9337 to arrange a time to come in for the extra visit if you are sick.

What do I need to tell the doctor before I participate?
We would like to know if you have any other illnesses other than asthma such as diabetes or hypertension. We would also like to know if you smoke and how many you smoke each day. Please tell us if you take any other medicines including herbal medicines or medicines bought from the supermarket, chemist or health food shop.
Is the information collected confidential?

Any information we collect about your pregnancy and asthma will be kept confidential and you will not be identifiable in any reports of the study.

What if I change my mind?

Taking part in this study is completely voluntary and if you participate you are free to withdraw from the study at any time without giving a reason. Decisions you make regarding participation will not affect your access to care and services you would normally receive. If you choose to withdraw from the study just let the doctor or nurse know at your next visit.

Can I see the results of this study?

You can receive the results of this study by contacting A/Prof Vicki Clifton on 8182 9337 and leaving your name and address. Study results will then be posted to your home address.

Thank you for your interest in this study,

........................................

A/Prof Vicki L Clifton, Chief Investigator
Director Clinical Research
Lyell McEwin Hospital

What if I have a complaint about the study?

This research has been approved by the University of Adelaide Research Ethics Committee and Lyell McEwin Hospital Human Research Ethics Committee. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher or asthma nurse.
Patient Demographics

ASTHMA & PREGNANCY STUDY RECRUITMENT

Today’s Date: _______________ Recruited by: _______________
MRN:________________________ Name:________________________
Street Address: ________________________________________________
Suburb:________________________ Post Code:_________
Phone Home:_____________ Phone Work/Other:_____________________

Date of Birth:_______/______/_____
Current gestational age (weeks): _______________ EDC: _______________

ASTHMA / CONTROL (please circle)

Current asthma status (please complete asthma control questionnaire if time – over page) __________________________

Do they need immediate review by asthma educator? ______________________

If yes, please contact Philippa Talbot (ext: 55635, page 5662 ) or Trish Young (ext: 14963, page 6349)

Current asthma medication _______________________________________________
Other information ________________________________________________________

Current Smoker? Yes/No Cigarettes/day (if yes) ______________________

Other illnesses: ________________________________________________________
_____________________________________________________________________

High blood pressure? Yes/No
18 week appointment (Date & time): _____________________________

30 week appointment (Date & time): _____________________________

36 week appointment (Date & time): _____________________________

Are they delivering at John Hunter Hospital? ________________________________

Have they participated in the Asthma study before? __________________________

Phase II ☐ Phase I ☐

<22 weeks gestation and aware of time commitment >22 weeks gestation or unable to commit to time

Scans can be booked for any time of day Please book scans for after 10:45 am
# Asthma Control Questionnaire

Circle the number of the response that best described how you have been during the past week

<table>
<thead>
<tr>
<th>Question</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. On average, during the past week, how often were you woken by your asthma during the night?</td>
<td>0 Never 1 Hardly ever 2 A few minutes 3 Several times 4 Many times 5 A great many times 6 Unable to sleep because of asthma</td>
</tr>
<tr>
<td>2. On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?</td>
<td>0 No symptoms 1 Very mild symptoms 2 Mild symptoms 3 Moderate symptoms 4 Quite severe symptoms 5 Severe symptoms 6 Very severe symptoms</td>
</tr>
<tr>
<td>3. In general, during the past week, how limited were you in your activities because of your asthma?</td>
<td>0 Not limited at all 1 Very slightly limited 2 Slightly limited 3 Moderately limited 4 Very limited 5 Extremely limited 6 Totally limited</td>
</tr>
<tr>
<td>4. In general, during the past week, how much shortness of breath did you experience because of your asthma?</td>
<td>0 None 1 A very little 2 A little 3 A moderate amount 4 Quite a lot 5 A great deal</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>5. In general, during the past week, how much of the time did you wheeze?</strong></td>
<td><strong>6 A very great deal</strong></td>
</tr>
<tr>
<td>0</td>
<td>Not at all</td>
</tr>
<tr>
<td>1</td>
<td>Hardly any of the time</td>
</tr>
<tr>
<td>2</td>
<td>A little of the time</td>
</tr>
<tr>
<td>3</td>
<td>A moderate amount of the time</td>
</tr>
<tr>
<td>4</td>
<td>A lot of the time</td>
</tr>
<tr>
<td>5</td>
<td>Most of the time</td>
</tr>
<tr>
<td>6</td>
<td>All of the time</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>6. On average, during the past week, how many puffs of short-acting bronchodilator (e.g. Ventolin) have you used each day?</strong></th>
<th><strong>&lt; 2 puffs per week</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 puffs per week</td>
<td>1-2 puffs most days</td>
</tr>
<tr>
<td>3-4 puffs most days</td>
<td>5-8 puffs most days</td>
</tr>
<tr>
<td>9-12 puffs most days</td>
<td>13-16 puffs most days</td>
</tr>
<tr>
<td>More than 16 puffs most days</td>
<td></td>
</tr>
</tbody>
</table>

| **Total Score** | **___ (Divide by 6)** |
Antenatal Risk Questionnaire (ANRQ)

Name: __________________________ Today’s Date: _____/_____/______

Weeks Pregnant: __________ Due date: _____/_____/______

Phone (h) ___________________ (w) ___________________ (m) ___________________

TOTAL

1. When you were growing up, did you feel your mother was emotionally supportive of you? (If you had no mother circle 6). (very much) 1 2 3 4 5 (not at all) 6

2. a) Have you ever had 2 weeks or more when you felt particularly worried, miserable or depressed? Yes  No

b) Do you have any other history of mental health problems? Yes ☐ No ☐

e.g eating disorders, psychosis, bipolar disorder, schizophrenia. Please specify: __________________________

If Yes to 2a or 2b, did this:

c) Seriously interfere with your work and your relationships with friends and family? (very much) 1 2 3 4 5 (not at all) 6

d) Lead you to seek professional help? Yes ☐ No ☐

Did you see a: Psychiatrist ☐ Psychologist/Counsellor ☐ GP ☐ __________ (Name of professional)

e) Did you take tablets/herbal medicine? No ☐ Yes ☐ Please specify: __________________________

3. Is your relationship with your partner an emotionally supportive one? (If you have no partner circle 6)

(very much) 1 2 3 4 5 (not at all) 6

4. a) Have you had any stresses, changes or losses in the last 12 months (e.g separation, domestic violence, unemployment, bereavement ?) Yes ☐ No ☐

Please list: __________________________

b) How distressed were you by these stresses, changes or losses?

5. Would you generally consider yourself a worrier?

(very much) 1 2 3 4 5 (not at all)

6. In general, do you become upset if you do not have order in your life (e.g. regular time table, a tidy house)?

(very much) 1 2 3 4 5 (not at all)
7. Do you feel you have people you can depend on for support with your baby?
   (very much) 1 2 3 4 5 (not at all)

8. Were you emotionally abused when you were growing up? Yes ☐ No ☐

9. Have you ever been sexually or physically abused? Yes ☐ No ☐

If you would like to seek some help with any of these issues please discuss this with your midwife or doctor.

This is part of your Antenatal Booking Evaluation and will guide us as to what services we can offer you during your pregnancy. It is confidential information and will remain in your file.

PLEASE COMPLETE ALL ITEMS. Circle numbers 1-6 or tick YES/NO
Edinburgh Postnatal Depression Score

Today's Date:   /   /     Weeks pregnant:   or weeks postnatal: 
Given Name(s):     Surname:

INSTRUCTIONS:
Please colour in one circle for each question that is the closest to how you have felt in the PAST SEVEN DAYS.

1. I have been able to laugh and see the funny side of things:
   ○ As much as I always could
   ○ Not quite as much now
   ○ Definitely not so much now
   ○ Not at all

2. I have looked forward with enjoyment to things:
   ○ As much as I ever did
   ○ Rather less than I used to
   ○ Definitely less than I used to
   ○ Hardly at all

3. I have blamed myself unnecessarily when things went wrong:
   ○ Yes, most of the time
   ○ Yes, some of the time
   ○ Not very often
   ○ No, never

4. I have been anxious or worried for no good reason:
   ○ No, not at all
   ○ Hardly ever
   ○ Yes, sometimes
   ○ Yes, very often

5. I have felt scared or panicky for no very good reason:
   ○ No, not at all
   ○ Hardly ever
   ○ Yes, sometimes
   ○ Yes, very often

6. Things have been getting on top of me:
   ○ Yes, most of the time I haven’t been able to cope at all
   ○ Yes, sometimes I haven’t been coping as well as usual
   ○ No, most of the time I have coped quite well
   ○ No, I have been coping as well as ever

7. I have been so unhappy that I have had difficulty sleeping:
   ○ Yes, most of the time
   ○ Yes, sometimes
   ○ Not very often
   ○ No, not at all
8. I have felt sad or miserable:
- Yes, most of the time
- Yes, quite often
- Not very often
- No, not at all

9. I have been so unhappy that I have been crying:
- Yes, most of the time
- Yes, quite often
- Only occasionally
- No, never

10. The thought of harming myself has occurred to me:
- Yes, quite a lot
- Yes, sometimes
- No, not much
- No, not at all

NB: If you have had ANY thoughts of harming yourself, please tell your GP or your midwife