Innate immunity acquires an anti-inflammatory bias during healthy human pregnancy: opportunities to examine links between early life environment and childhood development of allergic disorders

C. Graham[1], R. Chooniedass[2], W.P. Stefura[1], A.B. Becker[1 2 3], M.R. Sears[4], S.E. Turvey[5], P.J. Mandhane[6], P. Subbarao[7], CHILD Study Investigators* [8], K.T. HayGlass[1 2 3]

*[1] Dept. of Immunology, University of Manitoba, Winnipeg, MB, Canada; [2] Dept. of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada; [3] Children's Hospital Research Foundation of Manitoba, Winnipeg MB; [4] Dept. of Medicine, de Groote School of Medicine, McMaster University, Hamilton, ON; [5] Dept. of Pediatrics, Child & Family Research Institute and BC Children's Hospital, University of British Columbia, Vancouver, BC; [6] Dept. of Pediatrics, University of Alberta, Edmonton, AB; [7] Dept of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, ON; [8] CHILD (Canadian Healthy Infant Longitudinal Development Study) investigators**

** CHILD Principal Investigators: www.canadianchildstudy.ca/org.html#ap31*

Address for Correspondence: E-mail: kent.hayglass@umanitoba.ca

Summary

Environmental influences in early life – during the first year of life and perhaps in utero — influence progression to healthy vs. undesirable immune phenotypes, hence clinical outcomes, in childhood. Maternal innate immunity in vivo during healthy pregnancy (the"environment" for the first nine months of a child's life) is not well understood. Here, we begin examination of systemic pro-/anti-inflammatory innate immune status in vivo in 100 healthy women during healthy term pregnancy. The data reveal that increasingly intense, systemic anti-inflammatory phenotypes develop with gestational age. Defining maternal immune signatures during pregnancy provides a foundation to examine relationships between in utero environments and subsequent development of health *vs* chronic inflammatory disorders in infants.

Key words: maternal health, inflammation, innate immunity, cytokines/chemokines, gestational age

Introduction

While much research examines the immune system in diseases of pregnancy, maternal innate immune status in healthy pregnancy is poorly understood. Whether it is largely unchanged, is transiently skewed towards inflammatory phenotypes (potentially enhancing host defense) or is tilted to anti-inflammatory phenotypes (reducing responses to the fetus) is unclear [1 2]. Existing studies are largely small, cross-sectional and limit their focus to a small number of pro-inflammatory biomarkers. Recent international workshops [3-5] identify need for insight into the biology of normal pregnancy, particularly (i) maternal adaptations during pregnancy and (ii) creating biological definitions of optimal pregnancy phenotypes from fetal, maternal, and paternal standpoints. Here, we begin study of innate immune status in

vivo, with focus on possible changes in pro-/anti-inflammatory balance over the course of healthy pregnancy.

Methods

Following local IRB approval and written, informed consent, non-fasting venous blood was obtained from 100 randomly selected women seen between 9.9 and 38.4 weeks gestational age. All experienced uneventful pregnancies resulting in healthy singleton babies. Plasma samples were obtained from whole blood [6]. Clinical assessments at each blood draw excluded women with active URT or GI infections. MesoScale (MSD, Rockville, MD) singleplex assays were used to quantify biomarkers, with median assay variation < 5%. Each point represents an average value of duplicate analyses for an individual pregnant woman. Spearman rank order correlation coefficient (non-parametric) analyses were used to assess associations between in vivo biomarker levels and increasing gestational age (GraphPad Prism La Jolla, CA). Full details on all methodology are at [7].

Results and Discussion

A panel of pro-inflammatory plasma biomarkers were examined in 100 asymptomatic pregnant women of different gestational ages (Fig. 1A). Constitutive low level expression of multiple pro-inflammatory cytokines was evident in most pregnant women (and healthy non-pregnant women, data not shown). Their intensity did not increase with term, nor correlate with gestational age. Thus, gestational age did not influence systemic basal pro-inflammatory cytokine levels in healthy pregnancy. Conclusions about in vivo status are often drawn from analysis of inflammatory biomarker expression (i.e. IL-6, TNFα, CCL2) with minimal or no assessment of the endogenous controls that shape the net inflammatory response [8-12]. Figure 1B demonstrates that such molecules are readily quantified in pregnant (and non-pregnant, data not shown) individuals. Importantly, unlike pro-inflammatory biomarkers, systemic anti-inflammatory molecules rose significantly over the course of healthy pregnancy. In the population studied, these increases ranged from two to four fold between late first trimester and term.

Thus, in this pilot analysis of 100 healthy women experiencing uncomplicated term pregnancies, an increasingly intense shift in vivo was seen to anti-inflammatory phenotypes with increasing gestational age. With increasing importance attributed to early life conditioning in determining subsequent childhood outcomes [13-16], extension of these data to a substantially larger cohort will create opportunities to stratify individuals into (i) children who exhibit early life (i.e. 3-5 years of age) clinically evident allergic disorders *vs* (ii) those without clinical symptoms and signs of chronic inflammatory disorders such as atopic dermatitis, allergic rhinitis, food allergies and/or asthma.

An important caveat is that basal innate immune status in vivo was examined. The impact of infection and more subtle environmental influences will be important to investigate in examining the putative importance of the in utero environment in later development of clinical phenotypes. Similarly, future examination of maternal responses to defined innate stimuli in vitro can provide insight into the environment in which the allergic, or non-allergic, child developed.

In summary, improved understanding of maternal status during healthy pregnancy provides insight into maternal health and lays the groundwork for future comparisons of the *in vivo* environment of infants who go on to develop allergic disorders during childhood relative to those who, despite chronic environmental exposure to allergens, mount protective immune responses and do not develop pediatric allergic diseases.

References

1 Aagaard-Tillery KM, Silver R, Dalton J. *Immunology of normal pregnancy.* Semin Fetal Neonatal Med 2006;11:279-95.

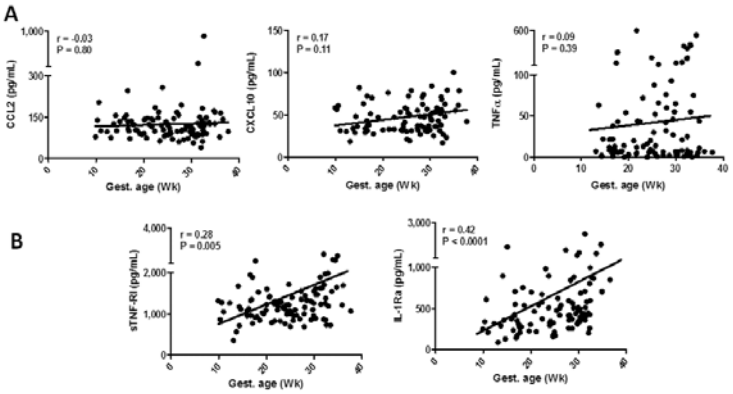

Figure 1. Plasma pro-inflammatory (A) and anti-inflammatory; (B) mediator levels in 100 healthy pregnant women by gestational age. Spearman's r and p shown.

2 Huang E, Wells CA. *The ground state of innate immune responsiveness is determined at the interface of genetic, epigenetic, and environmental influences.* J Immunol 2014;193:13-9.

3 Catalano PM, Williams MA, Wise PH, et al., Eds. *Scientific vision workshop on pregnancy and pregnancy outcomes: White Paper*. Bethesda, MA: Eunice Kennedy Shriver National Institute of Child Health and Human Development 2011.

4 Foulkes MA, Grady C, Spong CY,et al. *Clinical research enrolling pregnant women: a workshop summary.* J Womens Health (Larchmt) 2011;20:1429-32.

5 Beeler JA, Lambach P, Fulton TR, et al. *A systematic review of ethical issues in vaccine studies involving pregnant women.* Hum Vaccin Immunother 2016;1-8.

6 Moraes TJ, Lefebvre DL, Chooniedass R, et al. *The Canadian healthy infant longitudinal development birth cohort study: biological samples and biobanking.* Paediatr Perinat Epidemiol 2015;29:84-92.

7 HayGlass KT, Graham C, Chooniedass R, et al. *In vivo immune signatures of healthy human pregnancy: inherently inflammatory or anti-inflammatory?* PLoS One 2016 (submitted).

8 Chung KF. *Targeting the interleukin pathway in the treatment of asthma*. Lancet 2015;386:1086-96.

9 Huson MA, Grobusch MP, van der Poll T. *The effect of HIV infection on the host response to bacterial sepsis.* Lancet Infect Dis 2015;15:95-108.

10 Coccaro EF, Lee R, Coussons-Read M. *Elevated plasma inflammatory markers in individuals with intermittent explosive disorder and correlation with aggression in humans.* JAMA Psychiatry 2014;71:158-65.

11 Lindmark E, Diderholm E, Wallentin L, et al. *Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy*. JAMA 2001;286:2107-13.

12 Hasnain SZ, Borg DJ, Harcourt BE,et al. *Glycemic control in diabetes is restored by therapeutic manipulation of cytokines that regulate beta cell stress.* Nat Med 2014;20:1417-26.

13 Hanson MA, Gluckman PD. *Early developmental conditioning of later health and disease: physiology or pathophysiology?* Physiol Rev 2014;94:1027-76.

14 Dotterud CK, Storro O, Simpson MR, et al. *The impact of pre- and post-natal exposures on allergy related diseases in childhood: a controlled multicentre intervention study in primary health care.* BMC Public Health 2013;13:123.

15 Dick S, Friend A, Dynes K, et al. *A systematic review of associations between environmental exposures and development of asthma in children aged up to 9 years.* BMJ Open 2014;4:e006554.

16 Castro-Rodriguez JA, Forno E, Rodriguez-Martinez CE, et al. *Risk and protective factors for childhood asthma: what is the evidence?* J Allergy Clin Immunol Pract 2016;4:1111-22.