THE MAGEE WOMENS RESEARCH INSTITUTE CLINICAL TRAINEE RESEARCH AWARD

FACE PAGE (must be typewritten)

APPLICANT INFORMATION	
NAME (Last, first, middle) [redacted]	DEGREE(S): [redacted]
	ARE YOU A RESIDENT OR FELLOW: Fellow
POSITION TITLE: [redacted]	OFFICE MAILING ADDRESS (building, room, street, city, state, zip code)
YEAR(S) IN TRAINING: [redacted]	[redacted]
YEAR(S) IN CURRENT PROGRAM: [redacted]	
DEPARTMENT: [redacted]	
TEL: [redacted] FAX: [redacted]	E-MAIL ADDRESS: [redacted]

APPLICATION TITLE: Characterization of the Macrophage Transcriptional Response to the Mid-Gestation Placenta

HUMAN SUBJECTS RESEARCH	☐ No ☒ Yes	IRB APPROVAL DATE: IRB:PRO16100021
VERTEBRATE ANIMALS	☐ No ☐ Yes	IACUC APPROVAL DATE:
TOTAL FUNDS REQUESTED	$4000	

FACULTY SPONSOR	DEPARTMENT CHAIR OR DIRECTOR OF FELLOWSHIP/RESIDENCY PROGRAM
Name [redacted]	Name [redacted]
Title [redacted]	Title [redacted]
SIGNATURE [redacted]	SIGNATURE [redacted]

APPLICANT SIGNATURE	DATE
[redacted]	[redacted]

THE MAGEE WOMENS RESEARCH INSTITUTE CLINICAL TRAINEE RESEARCH AWARD

FACE PAGE (must be typewritten)

APPLICANT INFORMATION		
NAME (Last, first, middle) [redacted]		DEGREE(S): MD/PhD ARE YOU A RESIDENT OR FELLOW: Fellow
POSITION TITLE: [redacted] YEAR(S) IN TRAINING: [redacted] YEAR(S) IN CURRENT [redacted]		OFFICE MAILING ADDRESS (building, room, street, city, state, zip code) [redacted]
TEL (412) 641-1018	FAX (412) 641-2252	E-MAIL ADDRESS: [redacted]

APPLICATION TITLE:	Characterization of the Macrophage Transcriptional Response to the Mid-Gestation Placenta

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VERTEBRATE ANIMALS ☐ No ☐ Yes		IACUC APPROVAL DATE:
TOTAL FUNDS REQUESTED	$4000	

FACULTY SPONSOR	DEPARTMENT CHAIR OR DIRECTOR OF FELLOWSHIP/RESIDENCY PROGRAM
Name Carolyn Coyne, Ph[redacted]	Name Kata Himes, MD MS
Title Professor	Title Assistant Professor
SIGNATURE	SIGNATURE

APPLICANT SIGNATURE	DATE

PROPOSED BUDGET

Personnel – List **effort** for **all** personnel to be involved in carrying out the proposed research, whether or not salary is requested, **beginning with P.I.**

Name	Role	% Effort	Salary Requested	Fringe Benefits*	Total
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]			
		[redacted]		[redacted]	[redacted]
Equipment				Subtotal	
Supplies (list)				Subtotal	
Other Expenses	RNA sequencing			Subtotal	4x$1350
				Total	$5,400

*MWRIF Staff fringe benefit rate is 25.0%

BUDGET JUSTIFICATION
(JUSTIFY PERSONNEL COSTS ONLY)

Tissue culture and placental supplies will be provided by laboratory funding. This proposal is requesting supplemental funds to assist the cost of RNA sequencing.

ABSTRACT: The placenta – the primary secretory organ of pregnancy – has an important exocrine function to regulate maternal physiology. Clinical evidence suggests that pregnancy has a profound effect on the maternal immune system: pregnant women experience more severe infections than non-pregnant individuals and the sequalae of autoimmunity changes during pregnancy. We have previously developed an *in vitro* model to study placental exocrine function and described antiviral activity of secreted products of the placenta. We hypothesize that placental secretory products modulate distinct pathways of circulating leukocytes to alter maternal immune responsiveness. Our preliminary data show that placental trophoblast conditioned media alters macrophage differentiation and activation. This study extends to characterize the effects of trophoblast conditioned media and further investigate the mechanism by which the placenta regulates macrophages through transcriptional profiling.

TRAINING GOALS:

This proposed project will help Dr. [redacted] acquire technical skills, develop an understanding of immune function and placental biology, and augment communication skills. These will all help her to achieve her goal of a career as a physician scientist. In her PhD thesis work, Dr. [redacted] previously worked on bacterial genetics and host pathogen interactions but has not done work in immunology. She will learn the technique of activating and differentiating macrophages from the promyeloid THP-1 cell line. She will perform placental villi dissection, isolate placental trophoblast conditioned media and complete RNA isolation. These techniques are new to her and thus allow her development new technical skills that she can use as she transitions to her own independent laboratory. This project will help her to develop skills based in immunology and eukaryotic gene profiling. Through this work, she will develop an understanding of intracellular signaling pathways that regulate macrophage cell function by interpreting and analyzing data from transcriptomic profiles. These technical and data processing skills will facilitate Dr. [redacted]s development as a physician scientist. She will both learn new skills at the bench but also develop a solid background in macrophage and placental biology to better appreciate how pregnancy can regulate maternal immune function. As her goal is to have a career in both clinical medicine and bench research, developing a diverse set of scientific tools will directly benefit her. Dr. [redacted]'s project is also clinically relevant to her training in reproductive infectious disease and maternal fetal medicine. Development of an understanding of immune and inflammatory pathways will enhance her knowledge of the pathophysiology of infections in pregnancy.

Furthermore, Dr. [redacted] will continue to develop skills in manuscript writing and communicating her research. These will be critical for development of a successful career in academic medicine. Dr. [redacted] is expected to submit her results to a national meeting and present her work as an oral presentation in lab meetings and at Maternal Fetal Medicine and Reproductive Infectious Disease departmental meetings at Magee-Women's Hospital.

HYPOTHESIS: Secreted products from mid-gestation placental trophoblasts alter the transcriptional profile of activated macrophages

BACKGROUND AND SIGNIFICANCE: Studies on the influence of pregnancy on immunity typically focus on the maternal-fetal interface and the barriers to fetal infection[1,2]. Clinical observations that women are more susceptible to a variety of infections during pregnancy and that autoimmune conditions improve during pregnancy suggest that profound immunomodulation occurs, particularly in the second and third trimesters[3,4]. To date, studies on systemic immune regulation in pregnancy are limited to comparing circulating populations of immune cells[2]. The mechanisms through which these cells are regulated and change during pregnancy remain unclear. As such, the pathogenesis of increased morbidity and mortality with systemic infections in pregnancy is unknown.

The placenta is the predominant organ of pregnancy. In addition to functioning as the site of gas and nutrient exchange with fetal circulation, the placenta is a dynamic endocrine and exocrine organ that modulates physiologic adaptations to pregnancy. Secreted hormones, extracellular vesicles and growth factors have been identified in maternal system circulation and are thought to play an important role in maintaining pregnancy. Our lab has developed techniques to study how secreted placental products modulate immune mechanisms at the maternal-fetal interface and regulate vertical infections[5–7]. Our lab and others have defined a subset of secreted products from human placentas in mid-gestation that have substantial exocrine activity[7–10]. These techniques in placental dissection and isolation of secreted products from second- trimester placentas allow us the unique ability to directly evaluate the effect that secreted placental products have on immune cell populations.

Changes in macrophages/monocytes have been demonstrated in pregnancy in circulating immune cell populations. Monocytes are a macrophage precursor and compose approximately 5-10% of white blood cells (WBCs) present in whole blood. In pregnancy, this proportion increases to 20-25% starting as early as 20 weeks[11,12]. Both monocytes and tissue macrophages have been shown to have differential regulation during pregnancy as compared to nonpregnant controls[13–16]. The monocyte/macrophage can mediate a wide variety of immune functions including initiating a proinflammatory cascade, immunoinhibitory response, and instigation of adaptive immunity. As such, they are an ideal target through which the placenta could modulate systemic immunity. Macrophages have extensive heterogeneity and plasticity that make them the ideal target for modulating an immune response[17]. The spectrum of macrophage function can be detected with morphologic changes, secretion of cytokines and is characterized by transcriptional profile alterations[18–20].

Our goal in this study is to identify the macrophage transcriptional response to secreted placental products from the second-trimester placenta.

SPECIFIC AIMS:

Aim 1- Develop an *in vitro* model of macrophage activation and differentiation and characterize macrophage responses to secreted products from the human placenta

Our goal is to develop an *in vitro* model of macrophage activation and differentiation using the THP-1 cell line to characterize responses to placental trophoblast secreted products. We will use established protocols to generate placental conditioned media after incubation with human second trimester placentas and develop a protocol to activate and differentiate macrophages from the THP-1 promyeloid cell line. Previous studies have demonstrated that THP-1 cells display similar morphologic and phenotypic characteristics to *in vivo* macrophage populations in humans when activated with phorbol 12-myristate 13-acetate (PMA) and exposed to cytokines[17,21]. Briefly, THP-1 cells are activated with PMA to become uncommitted macrophages (termed M0). These are then incubated with different cytokines (IFN-ɤ/LPS or IL-4) to differentiate into M1 or M2 macrophages, each displaying different immune functions and expression signatures. These are distinguished phenotypically with different morphology on light microscopy and have different cytokine profiles. Preliminary data demonstrate that THP-1 cells need to be activated into M0 macrophages to develop a phenotypic response to placenta conditioned media (data not shown). Our goal is to activate and differentiate THP-1 cells into these macrophage cell populations and compare them to undifferentiated M0 cells exposed to placental

conditioned media. These will be characterized by cytokine production and by cell morphology on light microscopy. After characterization from the THP-1 cell line, we plan to extend these studies to primary cells collected from pregnant and non-pregnant healthy donors.

Aim 2- Characterize macrophage transcriptional response to placental conditioned media

Aim 2 is designed to develop an understanding of the transcriptional regulation mediated by placental conditioned media. Macrophage polarization and phenotypes are diverse and overlapping. Transcriptional profiling of mRNA transcript levels will be important in understanding the phenotype after placental conditioned media exposure. Briefly, whole transcriptome shotgun sequencing using next generation sequencing technology will be performed in collaboration with the Health Sciences Sequencing Core. mRNA transcript levels from placental conditioned media-exposed macrophages will be compared to transcripts from both unexposed and M1/M2 polarized macrophages.

Experimental design- Secreted products from second trimester placentas will be collected through the generation of placental conditioned media as previously described. Briefly, placental tissue from elective second-trimester pregnancy terminations are dissected and decidua, villi and the fetal membrane are isolated. Tissue fragments are incubated at 37 degrees C in media overnight, and supernatants are collected for conditioned media. Conditioned media from at least four different placentas will be used, and all experiments will be performed in triplicate and from multiple (at least three) independent donors.

Figure 1

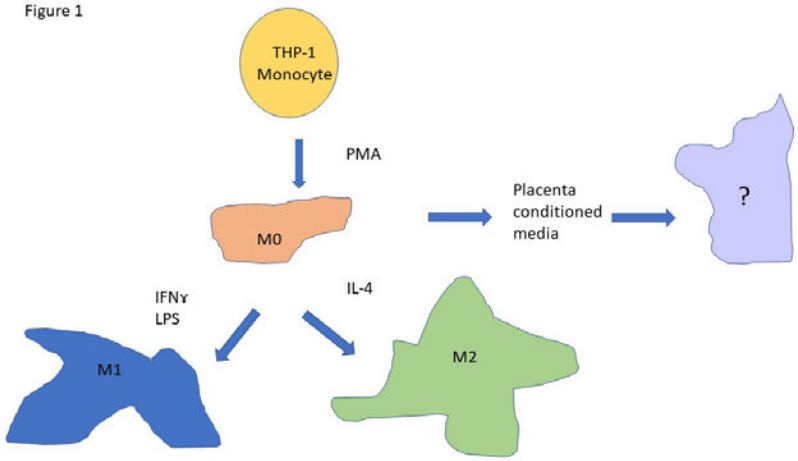

Figure 1 demonstrates a schematic of THP-1 activation and polarization. Briefly, as previously described, THP-1 cells are activated into M0 cells by incubation with PMA[21,22]. After activation and a 24-hour wash out period, the M0 cells are then placed in placenta conditioned media, control media, or incubated with LPS/IFN-ɤ for M1 cells or IL-4 for M2 cells[17,23,24]. We will identify adhesion and morphology phenotypes in response to differentiation or exposure to placental conditioned media with light microscopy. After 24 hours, supernatants are collected for cytokine production and cells are collected, lysed and RNA is isolated (GenElute Mamalian Total RNA Miniprep kit, Sigma). The RNA is then sent to the Health Sciences Sequencing Core at Children's Hospital of Pittsburgh for cDNA isolation and RNA sequencing. Resulting mRNA transcripts will be compared using GraphPad Prism statistical analysis software.

TIMELINE: The macrophage activation and differentiation experiments each take a week and will be done in triplicate using conditioned media from four different placentas. The time to develop the technical skills and gather high quality RNA will take approximately three months. After submission to the core facility, the sequencing takes approximately one month. The following three months will be for data analysis. Following data analysis, we anticipate an additional month for writing and presentation at meetings. Dr. [redacted] has approximately one day in clinic a week, leaving her with 80% protected time during her research blocks. She is a currently a second-year fellow, completing a combined reproductive infectious disease fellowship and maternal fetal medicine fellowship with two years remaining after this academic year.

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