

Department of Health and Human Services

9 5 0 0 1 4

1 R01

Dual: ES

IRG: ECD

Received: \_\_\_\_\_

1. TITLE OF PROJECT (Do not exceed 56 characters, including spaces and punctuation.)

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION  NO  YES  
(If "Yes," state number and title) The Role of Gene-Environmental Interactions Underlying the  
Number: PA-02-102 Title: Health Disparity of Premature Birth

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR New Investigator  No  Yes

3a. NAME (Last, first, middle) 3b. DEGREE(S)  
MD, MSCR

3c. POSITION TITLE Assistant Professor 3d. MAILING ADDRESS (Street, city, state, zip code)

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Ob, Gyn and Reproductive Sciences

3f. MAJOR SUBDIVISION School of Medicine

3g. TELEPHONE AND FAX (Area code, num. TEL: FAX: E-MAIL ADDRESS:

4. HUMAN SUBJECTS RESEARCH 4a. Research Exempt  No  Yes If "Yes," Exemption No. 5. VERTEBRATE ANIMALS  No  Yes

No  Yes 4b. Human Subjects Assurance No. FWA00003567 4c. NIH-defined Phase III Clinical Trial  No  Yes 5a. If "Yes," IACUC approval Date 5b. Animal welfare assurance no.

6. DATES OF PROPOSED PERIOD OF SUPPORT (month, day, year-MM/DD/YY) 7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD 8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT  
From Through 7a. Direct Costs (\$) 7b. Total Costs (\$) 8a. Direct Costs (\$) 8b. Total Costs (\$)  
4/1/05 3/31/10 \$318,885 \$484,705 \$1,852,832 \$2,788,603

9. APPLICANT ORGANIZATION Name Address 10. TYPE OF ORGANIZATION Public:  Federal  State  Local Private:  Private Nonprofit For-profit:  General  Small Business  Woman-owned  Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER DUNS NO. Congressional District Institutional Profile File Number (if known)

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE Name Title Research Administrator & Compliance Officer Address Office of Research Administration Tel: FAX: E-Mail: 13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION Name Title Director, Address Tel: FAX: E-Mail:

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. SIGNATURE OF PI/PD NAMED IN 3a. (In ink. Not acceptable.) DATE

15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. SIGNATURE OF OFFICIAL NAMED IN 13. (In ink. "Per" signature not acceptable.) DATE

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

This proposal from a new investigator is in response to a PA to address gene-environment interactions that contribute to the racial disparity in preterm birth. Our group has found that there are specific alterations in the lower genital tract inflammatory milieu early in pregnancy that predispose pregnant women to upper genital tract infection and early preterm birth. Furthermore, we have found that these alterations in the lower genital tract inflammatory environment are more common among African-American women, and that African-American women are more likely than Caucasian women to possess cytokine promoter gene polymorphisms that are related to infection-related preterm birth. Appreciation of the influence of gene-environment interactions on lower genital tract inflammation is critical to understanding the racial disparity underlying the predisposition to early spontaneous preterm birth. Stress is an exposure that has plausibility for exertion of influence on immunity. The various facets of perceived stress converge to exert influence on human health and disease via a physiologic stress response. Corticotropin Releasing Hormone (CRH) is one such stress-related biological effector that is related to preterm birth. Another important physiologic manifestation of stress is via catecholamines. We posit that these biologic responses to stress may exert influence on preterm birth via changes in lower genital tract immunity that predispose to upper genital tract infection/inflammation. We also hypothesize that cytokine promoter polymorphism genotype influences the nature of the lower genital tract inflammatory changes that occur in women who experience stress. We aim to determine if maternal prenatal psychological stress and the biological stress response in pregnancy promote a lower genital tract inflammatory milieu, as represented by concentrations of important pro- and anti-inflammatory cytokines, vaginal pH, and vaginal neutrophils, that increases the risk of preterm birth. To this end, we will enroll 800 women over 3 1/2 years in a prospective cohort study. Recent stressful life-events, perception of stress and of racism will be ascertained by questionnaire. Degree of residential segregation and exposure to violence will be assessed on the community level. Plasma CRH and platelet catecholamines will be used to assess the physiological stress response and genital tract specimens for important pro- and anti-inflammatory cytokines, vaginal pH, and vaginal neutrophils will be obtained at different gestational age intervals throughout pregnancy. We aim to determine if known functional cytokine promoter polymorphism status alters the impact of stress on lower genital tract inflammation, thus establishing a gene-environment interaction. To that end, we will extract DNA from blood specimens to genotype all 800 subjects for several cytokine promoter polymorphisms in order to determine if there are genotype patterns that modify the impact of stress on lower genital tract immunity. We aim to identify how racial differences in psychological stress, its physiological response, and the interactive contribution of cytokine promoter polymorphism status impact lower tract immunity, which may in turn increase the risk of preterm birth.

PERFORMANCE SITE(S) (organization, city, state)

\_\_\_\_\_

KEY PERSONNEL. See instructions on Page 11. Use continuation pages as needed to provide the required information in the format shown below.

Name	Organization	Role on Project
_____	_____	Principal Investigator
_____	_____	Co-Investigator

Type the name of the principal investigator/program director at the top of each printed page and each continuation page. (For type specifications, see instructions on page 6.)

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## Research Plan

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\*Type density and type size of the entire application must conform to limits provided in instructions on page 6.

Appendix (Five collated sets. No page numbering necessary for Appendix.)

Check if Appendix is included

Number of publications and manuscripts accepted or submitted for publication (Not to exceed 10) \_\_\_\_\_

Other items (list): \_\_\_\_\_

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY					FROM 04/01/05	THROUGH 03/31/06		
PERSONNEL (Applicant organization only)		TYPE APP (months)	% EFFO RT O PRO	INS T BAS SALA	DOLLAR AMOUNT REQUESTED (omit cent)			
NAME	ROLE ON PROJECT				REQUESTE D	FRINGE BENEFITS	TOTALS	
	Principal Investigator	12	30%	175,700	52,710	12,123	64,833	
	Co-Investigator	12	10%	160,347	16,035	3,688	19,723	
	Co-Investigator	12	5%	175,700	8,785	2,021	10,806	
	Co-Investigator	12	2.5%	175,700	4,393	1,010	5,403	
	Co-Investigator	12	5%	94,044	4,702	1,081	5,783	
	Co-Investigator	12	7.5%	University of Pittsburgh Subcontract Budget)				
	Co-Investigator	12	10%	Penn State University Subcontract Budget)				
	Research Coordinator	12	100%	56,154	56,154	13,084	69,238	
	Laboratory Technician	12	50%	40,538	20,269	4,723	24,992	
	Laboratory Technician	12	50%	44,412	22,206	5,174	27,380	
	Data Manager	12	20%	38,514	7,703	1,795	9,498	
SUBTOTALS →					192,957	44,699	237,656	
CONSULTANT COSTS								
EQUIPMENT (Itemize)								
SUPPLIES (Itemize by category)								
Clerical and computer sup		750						
Assay supplies:		37,718						
TRAVEL								
PI's attendance at annual scientific meeting for presentation of study findings							1,500	
PATIENT CARE		INPATIENT						
		OUTPATIENT						
ALTERATIONS AND RENOVATIONS (Itemize by category)								
OTHER EXPENSES (Itemize by category)								
Participant payments:		5,000		Food Frequency Questionna		8,000		
Malpractice insurance:		8,895						
Publication costs (beginning in the 02 year)							21,895	
<b>SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD</b>							<b>299,519</b>	
CONSORTIUM/CONTRACTUAL		DIRECT COSTS					13,222	
COSTS		FACILITIES AND ADMINISTRATION COSTS					6,144	
<b>TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (Item 7a, Face Page) →</b>							<b>318,885</b>	
<b>SBIR/STTR Only: FIXED FEE REQUESTED</b>								

**BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD  
DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD <i>(from Form Page 4)</i>	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: <i>Salary and fringe benefits. Applicant organization only.</i>		237,656	244,786	252,129	259,693	267,484
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		38,468	78,472	80,826	83,251	43,296
TRAVEL		1,500	1,545	1,591	1,639	1,688
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES		21,895	36,192	36,498	36,813	24,138
SUBTOTAL DIRECT COSTS		299,519	360,995	371,044	381,396	336,606
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	13,222	13,648	14,089	14,543	15,012
	F&A	6,144	6,341	6,545	6,755	6,973
<b>TOTAL DIRECT COSTS</b>		<b>318,885</b>	<b>380,984</b>	<b>391,678</b>	<b>402,694</b>	<b>358,591</b>
<b>TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD</b> <i>(Item 8a, Face Page)</i> _____					<b>\$1,852,832</b>	
<b>SBIR/STTR Only Fee Requested</b>						
<b>SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period</b> <small>(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&amp;A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.)</small>					\$	

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

**BUDGET JUSTIFICATION****Personnel:**

\_\_\_\_\_, Principal Investigator (30%) is an Assistant Professor in the Department of Obstetrics, Gynecology, and Reproductive Sciences and Director of the Prematurity Center of \_\_\_\_\_. Dr. \_\_\_\_\_ is fellowship trained in Maternal-fetal Medicine and Reproductive Infectious Diseases and Immunology, and has completed a Masters of Science in Clinical Research. Dr. \_\_\_\_\_ will be responsible for the overall administration and management of activities on this project. \_\_\_\_\_ will be primarily responsible for the supervision of recruitment by the research nurse coordinator, and will assist with acquisition of data and biological samples. Dr. \_\_\_\_\_ will supervise the laboratory component involving assay of cytokine promoter polymorphisms, CRH, and platelet catecholamines. \_\_\_\_\_ will direct the analysis and interpretation of data in collaboration with the Co-Investigators. Dr. \_\_\_\_\_ will also be responsible for the preparation and reporting of all presentations and manuscripts.

*Co-Investigators:*

\_\_\_\_\_, Ph.D. (10%) is the Director of Research for the Reproductive Infectious Diseases and Immunology at \_\_\_\_\_. \_\_\_\_\_ has extensive research experience in the arena of infection and preterm birth. Dr. \_\_\_\_\_ will supervise the microbiologic and immunologic laboratory components of this project. Additionally, \_\_\_\_\_ will provide input regarding analysis and interpretation of these data.

\_\_\_\_\_, M.D. (5%) has served for the past 19 years as the Principal Investigator of the \_\_\_\_\_ site of the \_\_\_\_\_ funded by the NICHD (\_\_\_\_\_) . As Director of the Maternal-Fetal Medicine private practice, Dr. \_\_\_\_\_ will be primarily responsible for overseeing recruitment in that setting.

\_\_\_\_\_, M.D., M.S. (2.5%) Professor of Psychiatry and Obstetrics and Gynecology and Reproductive Sciences and Epidemiology. Dr. \_\_\_\_\_ is an experienced researcher in women's mental health, particularly postpartum disorders. \_\_\_\_\_ has had consistent NIMH funding since \_\_\_\_\_ and has served on numerous NIH study sections, as well as the NIMH Data Safety and Monitoring Board, and the FDA Advisory Group to Revise Labelling for Drugs during Pregnancy and Lactation, and the National Children's Study Psychosocial Stress and Infancy Workshop. Dr. \_\_\_\_\_ will provide input on the stress measures to be included in this investigation. \_\_\_\_\_ will continue to provide consultation about the interpretation of stress measures and integration into the biopsychosocial model. \_\_\_\_\_ will assist in analysis and interpretation of the study results for publication. \_\_\_\_\_ will devote 2.5% effort to each year of the study and salary support is requested.

\_\_\_\_\_, Ph.D. (5%), is the Director of the Data Management Center for the Maternal-Fetal Medicine and Reproductive Infectious Diseases Division at \_\_\_\_\_. \_\_\_\_\_ will direct the data management and biostatistical services for this project.

Dr. \_\_\_\_\_ also has extensive experience and publications in the epidemiology of reproductive infectious disease and will provide content expertise in this capacity.

\_\_\_\_\_, Ph.D., (7.5%) will assist in geocoding the study subjects to census tracts and linking the census tract data with 2000 US census socioeconomic indicators. In addition, \_\_\_\_\_ will assist in analysis of the ways the neighborhood mechanisms, such as economic deprivation and social stress (e.g. violent crime) interact with individual level characteristics to influence proximate biomedical pathways hypothesized to influence preterm delivery. Please see the \_\_\_\_\_ Subcontract Budget which follows.

\_\_\_\_\_, PhD (10%), Assistant Professor of Psychology, \_\_\_\_\_, will be responsible for overseeing completion of the platelet catecholamine assays, preliminary statistical analyses, and analysis of psychological and physiological indicators of stress. Please see the \_\_\_\_\_ Subcontract Budget which follows.

\_\_\_\_\_, BSN (100%) is the Research Coordinator for the Prematurity Center at \_\_\_\_\_. \_\_\_\_\_ will be primarily responsible for subject recruitment and retention, administration of survey instruments, and acquisition and transport of biological samples.

\_\_\_\_\_ (50%) is a laboratory technician with experience and skill in DNA extraction, gene polymorphism assays, CRH assays, and measurement of platelet catecholamines.

\_\_\_\_\_ (50%) is a laboratory technician with experience and skill in quantitative vaginal culture and cytokine assay using the Luminex LabMAP® system.

\_\_\_\_\_ (20%) is a data manager with extensive experience in the management of data files for large human clinical studies. \_\_\_\_\_ will coordinate the data entry, database management, and pre-analysis data preparation.

Fringe benefits are requested at 23% for Drs. \_\_\_\_\_, which is the \_\_\_\_\_ research rate for medical faculty, effective July 1, 2004; and, at 23.3% for all remaining personnel, which is the current DHHS-approved rate for the \_\_\_\_\_

Personnel costs are projected at a 3% annual increase in the 02 through 05 years.

**Supplies:**

*Clerical and computer supplies:* \$750/year for the purchase of stationery, envelopes, file folders, labels, diskettes, and printer cartridges.

*Assay costs:* The following is a breakdown of assays to be performed over the five-year period.

Supply costs are projected at a 3% increase in the outlying years.

The Table below outlines the proposed number of tests to be performed over the course of this study.

	Year 1	Year 2	Year 3	Year 4	Year 5
GC/Ct SDA	200	400	400	400	200
TV culture	200	400	400	400	200
Quantitative vaginal culture and Gram stain	200	400	400	400	200
CRH	200	400	400	400	200
Platelet catecholamine	200	400	400	400	200
Food frequency Questionnaire	100	200	200	200	100
Cytokines	200	400	400	400	200
Gene Polymorphisms	100	200	200	200	100
cotinine	100	200	200	200	100

Funds are requested for the performance of the above noted laboratory and clinical tests as follows:

Test	Cost per test
GC/Ct SDA+ TV culture	\$16.95
Quantitative vaginal culture and Gram stain	\$27.98
CRH	\$10.00
Platelet catecholamine	\$10.00
Cytokines	\$116.16
Gene Polymorphisms	\$10.00
cotinine	\$5.00

**Travel:**

Funds are requested in the amount of \$1,500 per year to help defray national travel expenses for the Principal Investigator's attendance at one annual scientific meeting each year to consult with colleagues and present study findings.

Travel costs are projected at a 3% increase in the outlying years.

**Other Expenses:**

Funds are requested for the following:

*Participant payments:* Payments of \$50 per participant are requested and the amounts each year are \$5,000 in the 01 year; \$10,000/year in the 02-04 years; and, \$5,000 in the 05 year. These payments are intended to provide participants with nominal compensation for their time and effort, as well as to help offset child care and/or travel expenses.

*Malpractice insurance:* Funds are included for medical liability insurance for Drs. \_\_\_\_\_, which is proportionate to the level of clinical effort for each on the proposed study. This cost is included as a Direct Costs in this budget, as per the OMB Circular A-122, Attachment B, Paragraph 22g.

*Food Frequency Questionnaire:* The FFQ will assess nutrient intake of participants and has approximately 120 food items and a component to assess dietary supplement use. The cost of \$80 includes analysis and is calculated at \$8,000 in the 01 year; \$16,000 in the 02 through 04 years; and, \$8,000 in the 05 year.

*Publication costs:* \$1,000/year is requested, beginning in the 02 year, to help offset page charges, graphics and reprints.

**Consortium/Contractual Costs:**

Funds are requested to provide payment to the \_\_\_\_\_ for the participation of Drs. \_\_\_\_\_ and \_\_\_\_\_, respectively, as Co-Investigators on the proposed study (see Subcontract Budgets which follow).

**STATEMENT OF INTENT TO ENTER INTO A CONSORTIUM AGREEMENT**

For a Research Proposal Entitled: \_\_\_\_\_  
\_\_\_\_\_

Investigators: \_\_\_\_\_, Ph.D.  
\_\_\_\_\_

\_\_\_\_\_ Consortium Institution

The appropriate programmatic and administrative personnel of each institution involved in this grant application are aware of the *National Institutes of Health Consortium Grant Policy* and are prepared to establish the necessary inter-institutional agreement(s) consistent with that policy.

\_\_\_\_\_  
Institution

\_\_\_\_\_  
Institution

\_\_\_\_\_  
Signature

\_\_\_\_\_  
\_\_\_\_\_, MD

\_\_\_\_\_  
Typed Name

\_\_\_\_\_  
Typed Name

Director, Office of Research  
Title

Director,  
Title

\_\_\_\_\_  
Date

\_\_\_\_\_  
Date

SUBCONTRACT BUDGET

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

**DETAILED BUDGET FOR INITIAL BUDGET PERIOD  
DIRECT COSTS ONLY**

FROM  
4/1/05

THROUGH  
3/31/06

PERSONNEL <i>(Applicant organization only)</i>		TYPE APPT. <i>(months)</i>	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED <i>(omit cents)</i>		
NAME	ROLE ON PROJECT				SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator	12	7.5%	76,220	5,848	1,404	7,252
<b>SUBTOTALS</b> →					<b>5,848</b>	<b>1,404</b>	<b>7,252</b>
CONSULTANT COSTS							
EQUIPMENT <i>(Itemize)</i>							
SUPPLIES <i>(Itemize by category)</i>							
TRAVEL							
PATIENT CARE COSTS		INPATIENT					
		OUTPATIENT					
ALTERATIONS AND RENOVATIONS <i>(Itemize by category)</i>							
OTHER EXPENSES <i>(Itemize by category)</i>							
<b>SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD</b>							<b>\$ 7,252</b>
CONSORTIUM/CONTRACTUAL COSTS		DIRECT COSTS					
		FACILITIES AND ADMINISTRATIVE COSTS					<b>3,517</b>
<b>TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD</b> <i>(Item 7a, Face Page)</i> →							<b>\$ 10,769</b>

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

**BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD  
DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD <i>(from Form Page 4)</i>	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
PERSONNEL: <i>Salary and fringe benefits. Applicant organization only.</i>		7,252	7,469	7,693	7,924	8,162
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		7,252	7,469	7,693	7,924	8,162
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A	3,517	3,622	3,731	3,843	3,959
<b>TOTAL DIRECT COSTS</b>		10,769	11,091	11,424	11,767	12,121
<b>TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD</b> <i>(Item 8a, Face Page)</i> _____					<b>\$57,173</b>	
<b>SBIR/STTR Only Fee Requested</b>						

**SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period**

(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&amp;A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.)

\$

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Principal Investigator/Program Director (last, First, Middle):

CHECKLIST

TYPE OF APPLICATION (Check all that apply.)

- NEW application. (This application is being submitted to the PHS for the first time.)
SBIR Phase I, SBIR Phase II, STTR Phase I, STTR Phase II, SBIR Fast Track, STTR Fast Track
REVISION of application number:
COMPETING CONTINUATION of grant number:
SUPPLEMENT to grant number:
CHANGE of principal investigator/program director.
FOREIGN application or significant foreign component.

1. PROGRAM INCOME (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Table with 3 columns: Budget Period, Anticipated Amount, Source(s)

2. ASSURANCES/CERTIFICATIONS (See instructions.)

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/certifications are provided in Section III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

- Debarment and Suspension; •Drug- Free Workplace (applicable to new [Type 1] or revised [Type 1] applications only); •Lobbying; •Non-Delinquency on Federal Debt; •Research Misconduct; •Civil Rights (Form HHS 441 or HHS 690); •Handicapped Individuals (Form HHS 641 or HHS 690); •Sex Discrimination (Form HHS 639-A or HHS 690); •Age Discrimination (Form HHS 680 or HHS 690); •Recombinant DNA and Human Gene Transfer Research; •Financial Conflict of Interest (except Phase I SBIR/STTR) •STTR ONLY: Certification of Research Institution Participation.

- Human Subjects; •Research Using Human Embryonic Stem Cells• •Research on Transplantation of Human Fetal Tissue •Women and Minority Inclusion Policy •Inclusion of Children Policy• Vertebrate Animals•

3. FACILITIES AND ADMINISTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

- DHHS Agreement dated:
DHHS Agreement being negotiated with
No DHHS Agreement, but rate established with
No Facilities And Administrative Costs Requested.
Regional Office.
Date

CALCULATION\* (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)

Table showing calculation of F&A costs for years 02 through 05, with a total of 18,673.

\*Check appropriate box(es):

- Salary and wages base
Modified total direct cost base
Other base (Explain)
Off-site, other special rate, or more than one rate involved (Explain)
Explanation (Attach separate sheet, if necessary.):

4. SMOKE-FREE WORKPLACE Yes No (The response to this question has no impact on the review or funding of this application.)

Collaborative/Consortium Institution

STATEMENT OF INTENT TO ENTER INTO A COLLABORATIVE/CONSORTIUM AGREEMENT

The appropriate programmatic and administrative personnel of each organization involved in this grant application are aware of the NIH consortium agreement policy and are prepared to establish the necessary inter-institutional agreement(s) consistent with that policy.

Grantee Institution

Consortium Institution

\_\_\_\_\_  
(Name of Grantee Institution)

\_\_\_\_\_  
(Name of Consortium Institution)

\_\_\_\_\_  
(Title of Grantee Official)

\_\_\_\_\_  
Signature of Consortium PI (Optional)

\_\_\_\_\_  
Official of the Grantee Institution

\_\_\_\_\_  
Signature of Authorized Institutional  
Official of the Consortium Institution

\_\_\_\_\_, MD  
Director, \_\_\_\_\_

\_\_\_\_\_, Associate VP for Research  
Date: \_\_\_\_\_

SUBCONTRACT BUDGET

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY					FROM 4/1/05	THROUGH 3/31/06	
PERSONNEL <i>(Applicant organization only)</i>		TYPE APPT. <i>(months)</i>	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED <i>(omit cents)</i>		
NAME	ROLE ON PROJECT				SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator	12	10%	47,007	4,701	1,269	5,970
<b>SUBTOTALS</b> →					<b>4,701</b>	<b>1,269</b>	<b>5,970</b>
CONSULTANT COSTS							
EQUIPMENT <i>(Itemize)</i>							
SUPPLIES <i>(Itemize by category)</i>							
TRAVEL							
PATIENT CARE COSTS		INPATIENT					
		OUTPATIENT					
ALTERATIONS AND RENOVATIONS <i>(Itemize by category)</i>							
OTHER EXPENSES <i>(Itemize by category)</i>							
<b>SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD</b>							<b>\$ 5,970</b>
CONSORTIUM/CONTRACTUAL COSTS		DIRECT COSTS					
		FACILITIES AND ADMINISTRATIVE COSTS					2,627
<b>TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD</b> <i>(Item 7a, Face Page)</i> →							<b>\$ 8,597</b>

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

**BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD  
DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD <i>(from Form Page 4)</i>	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
PERSONNEL: <i>Salary and fringe benefits. Applicant organization only.</i>		5,970	6,179	6,396	6,619	6,850
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		5,970	6,179	6,396	6,619	6,850
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A	2,627	2,719	2,814	2,912	3,014
<b>TOTAL DIRECT COSTS</b>						

**TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD** *(Item 8a, Face Page)* \_\_\_\_\_ **\$46,100**

**SBIR/STTR Only Fee Requested**

**SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period**  
 (Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) \$

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

**CHECKLIST**

**TYPE OF APPLICATION** (Check all that apply.)

- NEW application.** (This application is being submitted to the PHS for the first time.)
  - SBIR Phase I     SBIR Phase II: SBIR Phase I Grant No. \_\_\_\_\_
  - STTR Phase I     STTR Phase II: STTR Phase I Grant No. \_\_\_\_\_
  - SBIR Fast Track
  - STTR Fast Track
- REVISION** of application number: \_\_\_\_\_  
(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)
- COMPETING CONTINUATION** of grant number: \_\_\_\_\_  
(This application is to extend a funded grant beyond its current project period.)
  - No
  - Yes. If "Yes,"  Previously reported
  - Not previously reported
- SUPPLEMENT** to grant number: \_\_\_\_\_  
(This application is for additional funds to supplement a currently funded grant.)
- CHANGE** of principal investigator/program director.  
Name of former principal investigator/program director: \_\_\_\_\_
- FOREIGN** application or significant foreign component.

**1. PROGRAM INCOME** (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

**2. ASSURANCES/CERTIFICATIONS** (See instructions.)

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/certifications are provided in Section III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

- Human Subjects; •Research Using Human Embryonic Stem Cells
- Research on Transplantation of Human Fetal Tissue •Women and Minority Inclusion Policy •Inclusion of Children Policy •Vertebrate Animals

- Debarment and Suspension; •Drug- Free Workplace (applicable to new [Type 1] or revised [Type 1] applications only); •Lobbying; •Non-Delinquency on Federal Debt; •Research Misconduct; •Civil Rights (Form HHS 441 or HHS 690); •Handicapped Individuals (Form HHS 641 or HHS 690); •Sex Discrimination (Form HHS 639-A or HHS 690); •Age Discrimination (Form HHS 680 or HHS 690); •Recombinant DNA and Human Gene Transfer Research; •Financial Conflict of Interest (except Phase I SBIR/STTR) •STTR ONLY: Certification of Research Institution Participation.

**3. FACILITIES AND ADMINISTRATIVE COSTS (F&A)/ INDIRECT COSTS.** See specific instructions.

- DHHS Agreement dated: \_\_\_\_\_  No Facilities And Administrative Costs Requested.
- DHHS Agreement being negotiated with \_\_\_\_\_ Regional Office.
- No DHHS Agreement, but rate established with \_\_\_\_\_ Date \_\_\_\_\_

**CALCULATION\*** (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)

a. Initial budget period:	Amount of base \$	5,970	x Rate applied	44	% = F&A costs	\$	2,627
b. 02 year	Amount of base \$	6,179	x Rate applied	44	% = F&A costs	\$	2,719
c. 03 year	Amount of base \$	6,396	x Rate applied	44	% = F&A costs	\$	2,814
d. 04 year	Amount of base \$	6,619	x Rate applied	44	% = F&A costs	\$	2,912
e. 05 year	Amount of base \$	6,850	x Rate applied	44	% = F&A costs	\$	3,014
<b>TOTAL F&amp;A Costs \$</b>							<b>14,086</b>

\*Check appropriate box(es):

- Salary and wages base     Modified total direct cost base     Other base (Explain)
- Off-site, other special rate, or more than one rate involved (Explain)

Explanation (Attach separate sheet, if necessary.):

**4. SMOKE-FREE WORKPLACE**  Yes     No (The response to this question has no impact on the review or funding of this application.)

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel in the order listed for Form Page 2.  
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME  _____, MD	POSITION TITLE  <b>Assistant Professor, Department of Obstetrics, Gynecology &amp; Reproductive Sciences</b>
-----------------------	--

EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
_____	BA	_____	Medical Science Medicine OB/GYN
_____	MD	_____	
_____	Residency	_____	
_____	Fellowship	_____	Maternal-Fetal Medicine Reproductive Infectious Diseases & Immunology Clinical Research
_____	Fellowship	_____	
_____	MS	_____	

**A. Positions and Honors**

**Positions and Employment**

- \_\_\_\_\_ Instructor, OB/GYN, \_\_\_\_\_
- \_\_\_\_\_ Clinical Instructor, Department of Obstetrics, Gynecology & Reproductive Sciences, \_\_\_\_\_
- \_\_\_\_\_ Assistant Professor, Department of Obstetrics, Gynecology & Reproductive Sciences, \_\_\_\_\_

**Other Experience and Professional Memberships**

- \_\_\_\_\_ Junior Fellow, \_\_\_\_\_
- \_\_\_\_\_ Fellow, \_\_\_\_\_
- Associate Member, Society for Maternal-Fetal Medicine
- Member, Infectious Diseases Society for Obstetrics and Gynecology
- Associate Member, Society for Gynecologic Investigation

**Honors**

_____ _____ _____	
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Principal Investigator/Program Director (Last, first,

**B. Selected peer-reviewed publications (in chronological order).**

**Completed Research Support**

Principal Investigator/Program Director (Last, first, middle):

[Redacted]

[Redacted]

[Redacted]

[Redacted]



Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

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**OTHER SUPPORT**

\_\_\_\_\_

**ACTIVE:**

\_\_\_\_\_  
\_\_\_\_\_ 10%

The purpose of this Network is to conduct studies generated by the Steering Committee of the Maternal Fetal Medicine Network, and to propose those research protocols that represent areas of interest/controversy in current clinical settings.

\_\_\_\_\_  
\_\_\_\_\_ \$858,491 DC \_\_\_\_\_ 20%

This comprehensive investigation will answer major women's health problems regarding the dynamic interplay among fetal and maternal genetics, sex-specific genomic imprints and consequences of our first environmental exposures. Specifically, Project 2, on which Dr. \_\_\_\_\_ is Co-P.I., addresses sex-specific genomic imprints in genetically controlled and experimentally-manipulated pregnancies.

\_\_\_\_\_  
\_\_\_\_\_ \$225,980 DC \_\_\_\_\_ 5%

This study will test the hypothesis that decreased concentrations of pro-inflammatory cytokines in the lower genital tract early in pregnancy indicate a greater susceptibility to ascending microbial invasion. Increased cytokines in the cervix early in pregnancy indicate pre-existing microbial invasion.

**PENDING:**

NIH \_\_\_\_\_ \$545,043 DC \_\_\_\_\_ 15%

The purpose of this proposal is to establish an \_\_\_\_\_ which will be part of a \_\_\_\_\_ that will identify and study common problems related to pharmacologic agents used in pregnancy.

NIH \_\_\_\_\_ \$99,677 DC\* \_\_\_\_\_ 10%

The purpose of this RFA is to stimulate research on immune function in early life in order to define the cellular and molecular mechanisms underlying the development of asthma and to use this information to devise prevention strategies.

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_____		
_____	\$416,666 DC*	10%

The proposed biomedical research is motivated by the need for precise fetal anatomical measurements in prenatal diagnosis. The overall goals are 1) to create an advanced prenatal screening technique and 2) make it available to a broad segment of the population. The screening technique is high-resolution three-dimensional reconstruction of fetal anatomy from 2D ultrasound images.

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_____		
_____	6/1/04 – 5/31/08 \$1,188,933 DC	10%

This project proposes to address the problem of preeclampsia, preterm birth and the failure of the fetus to exercise its full growth potential, and intrauterine growth restriction by forming the \_\_\_\_\_ in order to provide infrastructure to recruit and retain a diverse group of women.

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel in the order listed for Form Page 2.  
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME _____, Ph.D.		POSITION TITLE Associate Professor	
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
_____	B.A.	_____	English
_____	M.P.H.	_____	Epidemiology
_____	Ph.D.	_____	Epidemiology
_____	Post-doc	_____	Biostatistics

**A. Positions and Honors**

**Positions and Employment**

- Research Associate in Epidemiology and in Obstetrics & Gynecology, \_\_\_\_\_
- Assistant Professor of Epidemiology and Obstetrics & Gynecology, \_\_\_\_\_
- Visiting Associate Professor of Obstetrics, Gynecology and Reproductive Sciences, \_\_\_\_\_
- Associate Professor of Obstetrics, Gynecology and Reproductive Sciences, \_\_\_\_\_
- Associate Professor of Epidemiology, \_\_\_\_\_

**Other Experience and Professional Memberships**

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Honors**

None.

**B. Selected peer-reviewed publications**

1. \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Principal Investigator/Program Director (Last, first, middle):

5.

[Redacted content]

**C. Research Support**

**On-going Research Support**

[Redacted content]

Principal Investigator/Program Director (Last, first, middle):

[Redacted text block containing multiple lines of obscured information]

**Completed Research Support**

[Redacted text block containing multiple lines of obscured information]

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

### BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.  
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
_____, Ph.D.	Professor – Department of Obstetrics, Gynecology and Reproductive Sciences

**EDUCATION/TRAINING** (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
_____	B.A.	_____	Bacteriology/Public Health
_____	Ph.D.	_____	Bacteriology/Public Health

#### A. Positions and Honors

##### Positions and Employment

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##### Other Experience and Professional Memberships

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##### Honors

\_\_\_\_\_

Principal Investigator/Program Director (Last, first, middle):

**B. Selected peer-reviewed publications during the past 3 years (from a total of 164)**

[Redacted content]

Principal Investigator/Program Director (Last, first, middle):

17.

Principal Investigator/Program Director (Last, first, middle):

[Redacted content]

**C. Research Support**  
**On-going Research Support**

[Redacted content]

Principal Investigator/Program Director (Last, first, middle):

**Completed Research Support**

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel in the order listed for Form Page 2.  
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME  _____, M.D., M.S.	POSITION TITLE Professor of Psychiatry, Obstetrics and Gynecology and Reproductive Sciences, and Epidemiology (Tenured)
-------------------------------	--

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
_____	B.S.	_____	Chemistry & Biology
_____	M.S.	_____	Nutrition
_____	M.D.	_____	Medicine

**A. Positions and Honors:**

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Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

[Redacted content]

**B. Selected Peer-Reviewed Publications (from over 70):**

6. \_\_\_\_\_

[Redacted content]

Principal Investigator/Program Director (Last, first, middle):

[Redacted content]

**C. Research Support:**

[Redacted content]



Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

### BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.  
Photocopy this page or follow this format for each person.

NAME		POSITION TITLE	
_____		Assistant Professor of Psychology	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
_____	B.S. B.A. M.S., Ph.D.	_____	Molecular Biology Psychology Psychology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honor. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

#### Current Position

\_\_\_\_\_ Assistant Professor of Psychology, \_\_\_\_\_

#### Awards

_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

#### Selected Publications

_____	_____
_____	_____
_____	_____
_____	_____

#### Professional Presentations

Principal Investigator/Program Director (*Last, first, middle*):

[Redacted content]

Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.  
Follow sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME		POSITION TITLE	
		Assistant Professor	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
	BA		Sociology
	MA		Demography
	PhD		Demography

### A. Positions and Honors.

#### Positions and Employment

#### Other Experience and Professional Memberships

#### Honors

### B. Selected peer-reviewed publications (in chronological order).

Principal Investigator/Program Director (Last, first, middle):


### C. Research Support

#### Ongoing Research Support


Principal Investigator/Program Director (Last, first, middle): \_\_\_\_\_

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## RESOURCES

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**FACILITIES:** Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

**Laboratory:**

Dr. \_\_\_\_\_'s laboratory is located on the 5<sup>th</sup> floor of the \_\_\_\_\_ located across the street from \_\_\_\_\_ laboratory is entirely equipped for culture of facultative and anaerobic bacteria and for cytokine assays. Dr. \_\_\_\_\_'s laboratory is on the 2<sup>nd</sup> floor of \_\_\_\_\_ and is fully equipped for cytokine promoter gene polymorphism assay Corticotropin Releasing Hormone assays, and platelet catecholamine assays.

**Clinical:**

The Antepartum Clinic and the MFM Private Practice office are located in \_\_\_\_\_ The Clinics are staffed by Obstetrics and Gynecology faculty, fellows, and residents, who will cooperate in referring women to the study staff.

**Animal:**

N/A

**Computer:**

Dr. \_\_\_\_\_ and the data management staff are well equipped with computers in their offices. The other investigators also have computers in their offices. Dr. \_\_\_\_\_ and \_\_\_\_\_ staff have extensive computer resources for demographic and vital statistics work. All of the investigators at the \_\_\_\_\_ are on a single University-wide secure email system that supports sending data files, slides, and draft manuscripts among all of the offices.

**Office:**

All of the investigators, clinical study staff, and laboratory technicians have their own offices and work space.

**MAJOR EQUIPMENT:** List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

An anaerobic Chamber Hood , BD ProbeTec System are both located in Dr. \_\_\_\_\_'s laboratory, as is a Luminex LabMAP® system for cytokine assay. Superspeed centrifuges, ELISA plate readers, water baths, walk-in cold rooms for storage of culture media, spectrophotometers, gas chromatograph, microscopes, incubators (ambient air, CO<sub>2</sub> and anaerobic), -20°C and -80°C freezers, ultrapure water purification system, biologic safety cabinets and anaerobic gloveboxes are all available in Dr. \_\_\_\_\_'s lab as well. HPLC and flow cytometry are available in Dr. \_\_\_\_\_'s laboratory.

+

The disparity in preterm delivery rates between African-American and white women is well established and is not explained by demographic factors alone. Infection is the etiology of many spontaneous preterm births, especially the very early births that have the greatest morbidity and mortality. Our group and others have found that there are specific alterations in the lower genital tract inflammatory milieu in early pregnancy that predispose pregnant women to upper genital tract infection and early preterm birth. Furthermore, we have found that these alterations in the lower genital tract inflammatory environment are more common among African-American women, and that African-American women are more likely than their Caucasian counterparts to possess cytokine promoter gene polymorphisms that are related to infection-related preterm birth. An understanding of the influence of environmental factors and gene-environment interactions on lower genital tract inflammation is critical to understanding the racial disparity underlying the predisposition to infectious/inflammatory adverse pregnancy outcomes such as early spontaneous preterm birth.

Stress is an exposure that has both epidemiologic and biologic plausibility for exertion of influence on immunity. The physiologic response to perception of life stress exerts a protean influence that includes immune response. The literature supports the notion that the various facets of perceived stress converge to exert influence on human health and disease via a biologic and endocrinologic stress response. Corticotropin Releasing Hormone (CRH) is one such stress-related biological effector that is related to preterm birth. Another important physiological manifestation of psychological stress is catecholamines. We posit that these biologic responses to stress may exert influence on preterm birth via changes in lower genital tract immunity that predispose to upper genital tract infection/inflammation. We also hypothesize that cytokine promoter polymorphism genotype influences the nature of the lower genital tract inflammatory changes that occur in women who experience stress.

#### **A. Hypotheses and Specific Aims**

**Specific Aim 1:** We aim to determine if maternal prenatal psychological stress and the biological stress response in pregnancy promote a lower genital tract inflammatory milieu, as represented by concentrations of important pro- and anti-inflammatory cytokines, vaginal pH, and vaginal neutrophils, that increases the risk of preterm birth. To this end, we will enroll 800 women over 3 ½ years in a prospective cohort study. Recent stressful life-events, perception of stress and of racism will be ascertained by questionnaire. Degree of residential segregation and level of community violence will be assessed on the community level. Plasma CRH and platelet catecholamines will be used to assess the physiological stress response and genital tract specimens for important pro- and anti-inflammatory cytokines, vaginal pH, and vaginal neutrophils will be obtained at different gestational age intervals throughout pregnancy.

**Hypothesis 1:** We hypothesize that women who experience physiologic stress and thereby have elevated CRH and platelet catecholamine concentrations during pregnancy have alterations in the inflammatory milieu of the lower genital tract that are associated with preterm birth.

**Specific Aim 2:** We aim to determine if known functional cytokine promoter polymorphism status alters the impact of stress on lower genital tract inflammation, thus establishing a gene-environment interaction. To that end, we will extract DNA from blood specimens to genotype all subjects for cytokine promoter polymorphisms in order to determine if there are individual genotypes or genotype patterns that modify the impact of stress on lower genital tract immunity.

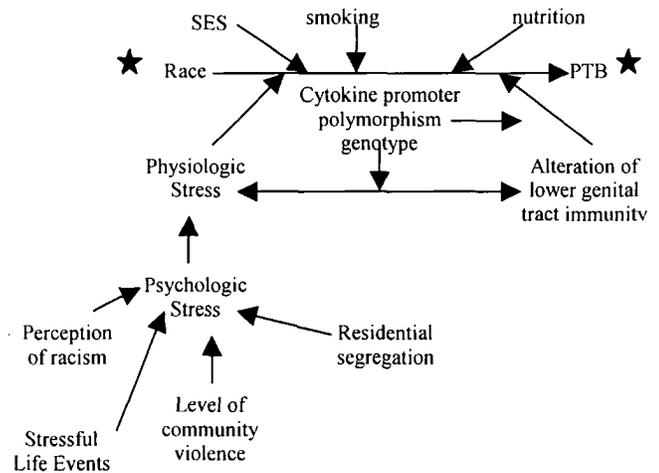
**Hypothesis 2:** We hypothesize that cytokine promoter polymorphism genotype influences the nature of the lower genital tract inflammatory changes that occur in women who experience stress. That is, the degree of lower genital tract inflammatory change that occurs in women who experience stress will depend, in part, on their genetic predisposition to cytokine response.

**Specific Aim 3:** We aim to identify how racial differences in psychological stress, its physiological response, and the interactive contribution of cytokine promoter polymorphism status impact lower tract immunity, which may in turn increase the risk of preterm birth.

**Hypothesis 3:** We hypothesize that African-American women will report more psychological stress and will exhibit a greater level of subsequent physiological stress response during pregnancy compared with their Caucasian counterparts. We further hypothesize that the interaction between cytokine promoter polymorphism status and stress among African-American women will contribute to an unfavorable innate immune profile than among their Caucasian counterparts.

## B. Background and Significance

In the Background and Significance section, we will develop the model for our approach to the exploration of the relationship of stress and lower genital tract immunity on preterm birth, and how that relationship might contribute to racial disparity in preterm birth. We will describe relevant literature and its limitations for each of these important links in our model. The first section is concerned with the link between race and preterm birth, shown in the figure to the right. We will denote the topic of each section with a star.



### Race and Preterm Birth

Prevention of preterm birth is among the most important unmet needs in modern obstetrics. It is responsible for nearly 75% of perinatal mortality and approximately half of cases of long-term neurologic morbidity. The disparity in preterm delivery rates between white and African-American women is well established. Being African-American confers an additional risk for being born either early, at a lower weight, or both.<sup>1</sup> Studies have failed to account for this disparity when adjusting for low maternal education, poor economic status, poor prior pregnancy outcome, poor nutrition, alcohol and tobacco use, illegal drug use, and sexually transmitted infections.<sup>2-5</sup>

Several epidemiologic factors have been associated with preterm birth. Among the most consistent and significant sociodemographic risk factors are African-American race, extremes of maternal age, unmarried status, and low socioeconomic status.<sup>2, 4-12</sup> While numerous investigators have supported significant univariate associations of these factors with preterm birth, their complex interrelationship is less clear. African-American race, in particular, stands out among the most important risk factors for preterm birth and low birthweight. However, categorization by race is a social construct which has little biological basis; African-Americans and Caucasians are genetically indistinguishable.<sup>1, 9</sup> David et al found that the rate of low birthweight infants to immigrant Sub-Saharan African-born women was half that of African-American women.<sup>13</sup> Additionally, Pallotto et al determined that Caribbean-born immigrants had a lower rate of low birthweight than African-American women.<sup>14</sup> Race may be a surrogate marker for unknown and unmeasured risk factors.<sup>9</sup> Several investigators have attempted to explain the increased risk of preterm birth in African-American women by adjusting for other risk factors that are associated with both race and preterm birth (Table 1).<sup>2-5</sup>

**Table 1. Unadjusted and Adjusted Association of PTB and African-American Race**

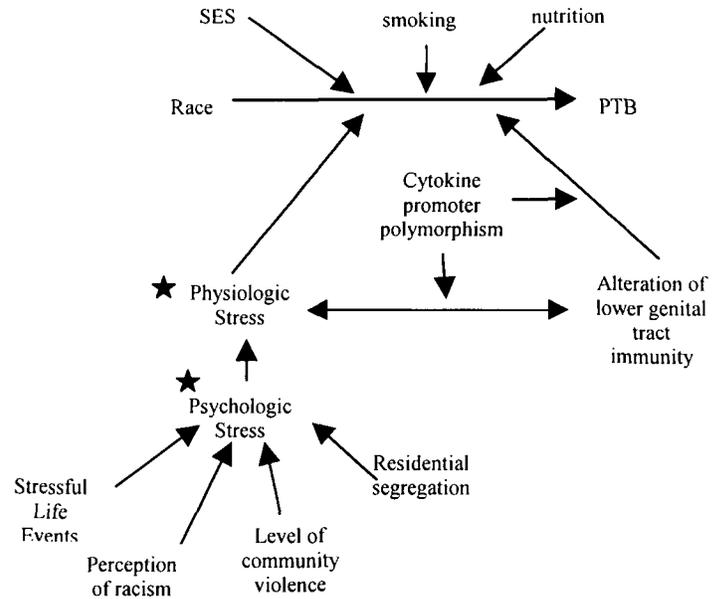
Study	Outcome	Univariate OR	Adjusted OR	Adjustment
McGrady, 1992	Preterm birth (PTB)	1.67	1.95	Medical Complications
Starfield, 1991	Low birthweight	2.21	1.61	Poverty, education, age, parity, marital status, smoking
Virji, 1991	PTB	2.01	1.56	Smoking, alcohol, use of prenatal care, weight gain, education, age
Lieberman, 1987	PTB	1.94	1.03	Hematocrit, Economic, Demographic/ Behavioral Variable Index (age < 20, single marital status, education < high school, welfare use)

Indeed, most investigators have found that some, but not all, of the racial disparity in preterm birth and/or low birthweight is explained by associated confounding risk factors. The previously described studies are limited by failure to account for all potential confounding or effect-modifying environmental factors, poorly quantified assessment of those factors (such as the use self-report for smoking status), and lack of an integrated model that

includes interaction with important biologic predictors. In order to evaluate the racial disparity in preterm birth, important sociodemographic and environmental factors (such as nutritional, smoking, or socioeconomic status) should be assessed in a reliable and valid fashion together with other risk factors, such as stress.

### Psychological & Physiologic Stress and Preterm Birth

Many large epidemiologic studies support the independent association of maternal prenatal psychological stress and preterm birth.<sup>11, 15-20</sup> More stressful life events or greater perceptions of stress during pregnancy are associated with higher rates of premature birth, obstetric complications, lower birth weight, and poor neonatal health.<sup>21-23 24-26</sup> These findings are inconsistent, as several other studies have not supported this relationship.<sup>27-29</sup>



Many of the inconsistencies in the data using psychosocial measures of stress and anxiety during pregnancy could be due to differences in demographic characteristics in populations of pregnant mothers (i.e., socioeconomic status, racial proportions, age), in the timing of the psychological assessment of stress/anxiety (i.e., retrospective or prospective during particular stages of pregnancy), or an inattention to factors that may mimic the stress response (e.g., caffeine and nicotine). A prospective assessment of psychological stress and its physiological response with attention to environmental exposures that might confound the physiological stress response might best provide an understanding of the relationship between stress and preterm birth. Additionally, assessment of community-specific stressors and chronic stressors may better predict adverse health outcomes in racial/ethnic minority groups that increase with length of time in the United States.<sup>30-32</sup> We will discuss the community-level stressors of residential segregation and community violence later in this section.

Stress might influence preterm birth indirectly or directly. Stress may have an indirect effect because individuals who report higher levels of stress engage in poorer health behaviors such as increased smoking, alcohol consumption, caffeine consumption, less sleep, and less healthy eating.<sup>33, 34 35</sup> These unhealthy behaviors are related to preterm birth and low birth weight.<sup>36, 37</sup> Psychological stress may exert a direct effect of preterm birth via physiological stress. Perception of stress and stressful life events are associated with higher levels of corticosteroids and catecholamines circulating in the body.<sup>38-40</sup> The changes in stress-related hormones circulating in the body have three pathways through which they may increase the risk of preterm birth; via direct changes in the **growth and development of the fetus, parturitional trigger of labor, and changes in immunologic response to genital infection and inflammation.** The immunologic response pathway is the focus of this proposal.

Direct changes in the growth and development of the fetus are found on both axes of the stress-response system. First, changes in corticosteroid levels (via the hypothalamic-pituitary axis) have been shown to decrease glucose utilization and decrease growth-promoting factors to result in smaller offspring in animal models.<sup>41</sup> Elevations in maternal cortisol have been found to inhibit fetal production of ACTH, and in turn, inhibit fetal production of cortisol.<sup>42, 43</sup> Secondly, elevations in maternal catecholamines via the sympathetic axis decrease blood flow to maternal organs, including the uterus of pregnant animals.<sup>41</sup> Reduced blood flow to the uterus results in a decreased delivery of oxygen and nutrients to the developing fetus, slowing growth by limiting the fetal supply of glucose and growth factors.<sup>41, 44</sup> As described in greater detail in Preliminary Data, higher maternal levels of platelet catecholamines at the beginning of the third trimester of human pregnancy are related to lower birthweight and shorter birth length of infants. Platelet catecholamines are a reliable estimate of an individual's circulating

catecholamines during the 7-10 days prior to venipuncture. This estimation of catecholamine secretion is based on the fact that platelets accumulate epinephrine (E) and norepinephrine (NE) over their life cycle and that platelet catecholamine content reflects the amounts of E and NE in the peripheral circulation during the past 7-10 days.<sup>45-51</sup> The platelet catecholamine assay has been shown to be consistent with 18-hour urinary catecholamines collected and combined across collections for each day during a 7-day period ( $r = 0.6 - 0.77$ ; Baum, unpublished data). The collection and analysis of catecholamines using the platelet catecholamine assay is less time consuming, less invasive, and has a higher adherence rate than collection via urinary catecholamine techniques.

The proposed biologic link between stress and the timing of parturition is CRH. CRH is a critical molecule in the hypothalamic-pituitary-adrenal (HPA) response to stress. In pregnancy, the placenta produces a large quantity of CRH that is released into both maternal and fetal circulation. CRH of placental origin has been hypothesized to be the parturitional trigger in human labor. Through stimulation of the fetal adrenal and the resultant hormonal cascade, CRH may have an important role in determining the timing of the "gestational clock".<sup>42</sup> In fact, CRH rises precociously in women who deliver preterm compared to women who deliver at term. Hobel and colleagues confirmed the association of early rise of CRH with prematurity and with self-reported prenatal stress.<sup>52</sup> Using a case-control design, these investigators compared maternal plasma CRH levels and perceived stress level among 18 women who delivered at term to those among women who delivered preterm. Hobel and coworkers noted that plasma CRH concentrations were higher at three gestational age intervals (18-20 weeks', 28-30 weeks', and 35-36 weeks') among women who delivered preterm compared with women who delivered at term. Additionally, they noted that maternal stress level, as measured by the Perceived Stress Scale, at 18 to 20 weeks' was significantly related to CRH concentration at 28 to 30 weeks'.<sup>52</sup> These data support the relationship between stress, CRH, and preterm birth. This work is limited, however, by a small sample size that prevents any meaningful assessment of other important sociodemographic, ethnic/racial, or biologic risk factors for prematurity. In order to achieve our specific aims for this project, in addition to collecting data on perceived stress, we will collect information on other important covariates including community violence, residential segregation, and smoking.

The physiologic stress response may also promote preterm birth through an effect on the immune system. These effects may be mediated through CRH and the glucocorticoid pathway or through a catecholamine pathway. In a validated murine model, macrophage function is impaired under stress.<sup>53, 54</sup> Endogenous glucocorticoids, produced during the physiologic response to stress, have a profound impact on the production of IL-1, IL-2, TNF- $\alpha$ , and IFN- $\gamma$  in gestational tissues.<sup>55-57</sup> Catecholamines are known to exert a powerful impact on the immune system by downregulation of proliferation and differentiation, and induction of apoptosis of immune cells. Neutrophil chemotaxis and bactericidal potential are diminished by catecholamine stimulation.<sup>58</sup> Catecholamines increase the production of the anti-inflammatory cytokine IL-10 from monocytes in patients after myocardial infarction.<sup>59</sup> Increased emotional distress in daughters of breast cancer patients is associated with decreased natural cytotoxic activity, elevated catecholamines and decreased secretion of Th1 cytokines.<sup>60</sup>

There are little data regarding the impact of stress on reproductive tract immunity, but those data that are available are intriguing. Petraglia and coworkers evaluated the relationship between CRH and preterm and term birth with and without microbial invasion of the amniotic cavity. They noted that women in preterm labor with bacteria in the amniotic fluid had significantly higher CRH concentrations in maternal circulation and in placental extracts than those in preterm labor without infection.<sup>61</sup> This suggests that infection in the amniotic fluid is linked to the fetal endocrine response, but the mechanism of this connection is unclear. Arck and colleagues noted that the decidua of women with first trimester spontaneous abortion who experience "high stress" contain more mast cells, CD8(+) T cells and TNF-alpha(+) cells, as noted immunohistochemically.<sup>62, 63</sup> One particularly fascinating recent study by Culhane et al. examines the relationship between chronic stress and bacterial vaginosis. Bacterial vaginosis (BV) is of relevance to our proposal because of its mechanistic and epidemiologic link to preterm birth, particularly those preterm births that occur as a result of infection and inflammation.<sup>64, 65</sup> Another relevant aspect of this study is the evaluation of the influence of race on the relationship of stress to the frequency of BV. These investigators sought to assess the contribution of chronic social stressors to race/ethnic differences in the rate of BV among pregnant women in inner-city Philadelphia. They conducted a cross-sectional study in a 2304 women at the first prenatal visit. Bacterial vaginosis was diagnosed by Gram stain microbiologic criteria. Psychological stress was measured at the individual and community levels with the use of interviews and chart review. As supported by the literature, these investigators found that black women had significantly higher rates of BV (64%) compared with white women (35%). Exposure to chronic stressors at the individual level differed by race (eg, 32% of the black women reported threats to personal safety compared with 13% of white women). Culhane et al noted that there were

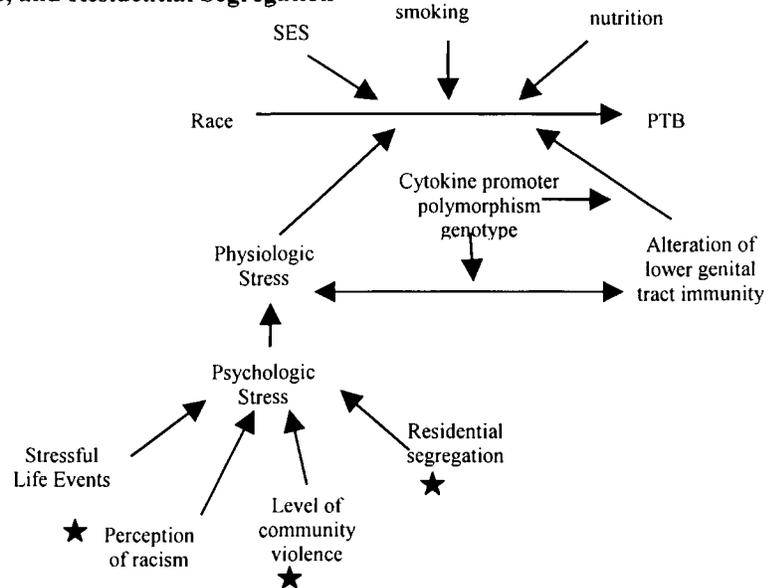
significant racial differences in exposure to stress at the community level (eg, 63% of the black women lived in neighborhoods with aggravated assault rates that were above the citywide mean compared with 25% of the white women). After the adjustment for sociodemographic, behavioral risk, and perceived stress, stressful exposures were associated positively with BV in pregnancy in a sample of women of low income in the inner city. The measurement of stressors at multiple levels explained a significant proportion of the racial disparity in the rates of occurrence of bacterial vaginosis. These findings support our proposed line of investigation. It is distinctly plausible that the association of stress (and the racial disparity in the experience of stress) with BV is mediated by a stress-related alteration in the lower genital tract immunologic environment. That altered inflammatory milieu may favor the disruption of normal vaginal ecology that is BV. Our project offers the opportunity to explore this connection.

### Contributors to Stress: Racism, Community Violence, and Residential Segregation

Three contributors to stress that are likely to be more influential among African-American women include perception of racism, level of violence in the community, and residential segregation.

### Perception of Racism and Preterm Birth

There is a large body of literature that supports adverse social and medical consequences of the experience of racism. The experience of racism may translate into psychological stress that can manifest as physiologic stress. That physiologic stress in response to the perception of racism contributes to health-related consequences such as a greater frequency of hypertension.<sup>66</sup>



The perception of racism has been demonstrated to be related to self-esteem and the ability to cope with stressful events.<sup>67, 68</sup> In a study of African-American women by Kwate et al, perception of racism was related to psychological stress. Among smokers and drinkers, the perception of racism within the past year was positively correlated with number of cigarettes and drinks consumed. Lifetime racism was negatively related to perceived health, and positively related to lifetime history of physical disease and frequency of recent common colds. In this cohort, these relationships were largely unaccounted for by other variables. In addition, demographic variables such as income and education were not related to experiences of racism.<sup>69</sup> Of particular relevance to this proposal, Collins et al found that among low-income African American mothers in Chicago, the odds of giving birth to a very low-birth-weight infant (<1,500 gm) were 3 times greater (95% CI = 0.9–11.3) among women reporting having experienced racial discrimination, adjusting for maternal age, parity, prenatal care, social support, smoking, alcohol, and drug use. Although this association did not reach statistical significance, these data suggest that, among low-income African women, perceived racial discrimination independently elevated their already high risk of having a very low-birth-weight infant.<sup>70</sup> Our proposal is uniquely suited to evaluate prospectively the perception of racism as a psychological and physiological stressor during pregnancy, in the context of other socioeconomic factors and a biologic immunological mechanism that could tie these processes to preterm birth.

### Community violence and preterm birth

Living in a violent community adversely influences the maternal environment. Two studies have previously assessed the relationship between community violence and adverse birth outcomes. In a cross-sectional study, Collins et al investigated the effect of the violent crime rate on low birthweight, small for gestational age, and preterm delivery.<sup>71</sup> Using Illinois vital records and Chicago police department records, the researchers were able to assign each singleton infant born in a census tract with less than \$10,000 median income/year to one of four categories of median violent crime rate and to a specific police district.<sup>71</sup> The violent crime rate was determined by the number of murders, rapes, robberies, and aggravated assault per 1000 residents in each police district. Collins et al found that following adjustment for maternal age, education, marital status, parity, and prenatal care, there was a significant relationship between community violence and small for gestational age (AOR: 1.5; 95% CI 1.1-2.1).<sup>71</sup> Zapata et al investigated the influence of social and political violence on a variety of pregnancy complications in Santiago, Chile under the dictatorship of Gen. Pinochet.<sup>72</sup> One-hundred sixty one healthy pregnant women were recruited from six health care clinics in Santiago for this longitudinal study. Each clinic was in a separate district that was assessed for bomb threats, military presence, undercover surveillance, and political demonstrations. The pregnancy complications assessed were gestational hypertension, preeclampsia, eclampsia, bleeding during the first, second, and/or third trimester, threat of miscarriage, rupture, and/or premature labor, painful preterm contractions that require bed rest, sexual abstinence, or hospitalization, stillbirth, and fetal growth retardation. The researchers found that after controlling for age, low social support, high level of alienation, and perception of neighborhood milieu, the risk of pregnancy complications was five times higher for women living in a high violence district than those living in low violence district (AOR: 5.0; 95% CI 1.93-12.77;  $p < 0.01$ ). Our proposed study would be the first to investigate community level of violence longitudinally, in relation to perception of stress, biological stress response, and consequent immunological manifestations.

### Residential Segregation and preterm birth

Residential segregation, using the index of dissimilarity, measures the unevenness of residential distribution of African-Americans to Caucasians in a census tract<sup>14</sup>. Residential segregation based on race occurs in a number of cities.<sup>71</sup> There have been several studies that have found an association between residential segregation and mortality and with tuberculosis.<sup>73-78</sup> However, there has only been one study investigating birth outcomes with residential segregation. Polednak collected infant mortality data from 38 metropolitan statistical areas with a population of more than one million people from 1982-1986.<sup>79</sup> Residential segregation was a significant predictor of African-American infant mortality. Polednak hypothesized that the relationship between segregation and adverse reproductive outcome is mediated by the high concentration of extreme poverty, poorer quality of the neighborhood, and a higher prevalence of individual risk factors. There are currently no prospective studies in the medical literature that have addressed the issue of residential segregation and preterm birth. In the context of this proposal, we hypothesize that residential segregation will contribute to preterm birth in part by its contribution to psychological and physiological stress.

### Cytokine Promoter Gene Polymorphisms

Polymorphisms have been described for many human cytokine genes.<sup>80-82</sup> These polymorphisms represent normal allelic variation, frequently within the regulatory region of cytokine genes. Enormous interest has developed in the study of the role of cytokine gene polymorphisms in human disease. In an exhaustive review, Bidwell and colleagues have catalogued approximately 70 known human cytokine gene polymorphisms.<sup>83</sup> More than one polymorphism may be present for any given cytokine, though it is clear that many polymorphisms do not have an impact on cytokine production or function. However, a number of these polymorphisms are known to impact on cytokine expression. Based on *in vitro* and *in vivo* evaluation of cytokine messenger RNA and protein production, it has been possible to characterize individuals as "high", "low", or "intermediate" producers for a given cytokine. Such polymorphisms have been described for a variety of important cytokines including TNF- $\alpha$ , TGF- $\beta$ 1, IL-1, IL-2, IL-4, IL-6 and IL-10.<sup>84-90</sup>

The relevance of cytokine gene polymorphisms and clinical outcomes has been investigated in transplantation medicine. In heart and kidney transplant recipients, cytokine genotype may confer susceptibility to early rejection. In heart transplant recipients, a certain combination of polymorphisms at -308 in the TNF- $\alpha$  gene and at -1082 in the IL-10 gene (high TNF- $\alpha$ /low IL-10) was associated with a higher level of graft rejection. In kidney transplant recipients, the high TNF- $\alpha$  and high IL-10 genotype polymorphisms are associated with a greater number of rejection episodes.<sup>84, 91</sup>

Specific polymorphisms are associated with increased susceptibility to certain infectious diseases and increased severity of autoimmune disease.<sup>92</sup> A polymorphism in promoter region of TNF- $\alpha$  (at position -308) that is associated with high production of TNF- $\alpha$  (allele TNFA2) is a significant risk factor for severe and fatal disease in patients with meningococcal infection.<sup>92</sup> Similarly, Gambian children with malaria that were homozygous for the TNFA2 allele were at highest risk for severe neurological sequelae. The frequency of TNF- $\alpha$  high allele is also associated with RA and SLE.<sup>93</sup> There are two recent reports of the relationship of a cytokine promoter polymorphism to preterm birth. Both investigations examined TNF- $\alpha$  polymorphism with the promoter region -308. Dizon-Townson and colleagues performed a case-control study in Utah to evaluate the allelic frequency of the aforementioned TNF- $\alpha$  promoter polymorphism among women who delivered at less than 37 weeks (after preterm labor or preterm rupture of membranes) compared with women who delivered at term. This study did not exclude women based upon race; the investigators do not report the distribution of race in the overall sample or by allelic variant. They did not find any statistically significant difference in the frequency of the polymorphism by preterm birth status.<sup>94</sup> Roberts and colleagues performed a case-control study of African-American women with delivery prior to 37 weeks after idiopathic preterm labor or preterm premature rupture of membranes compared with African-American women who delivered after 37 weeks and had no history of preterm delivery. These investigators evaluated the carrier frequency TNFA2 allelic variant. In this study, there was not a significant association between preterm birth after idiopathic preterm labor and TNFA2. There was, however, an odds ratio of 3.2 for the association of TNFA2 to preterm birth after preterm premature rupture of membranes. As will be outlined in the Preliminary Data section, we have demonstrated an association of cytokine promoter polymorphism status with both preterm birth and chorioamnionitis.<sup>95,96</sup> We will only assess known functional cytokine promoter polymorphisms in this project.

### C. Preliminary Data

The Preliminary Data section is divided into **nine** sections, each having a different goal:

- (1) To describe the qualifications of this multi-disciplinary research team to accomplish to proposed research
- (2) To describe the clinic population at \_\_\_\_\_ from whom the Preliminary Data were derived and to show that African-American women have an elevated risk of prematurity as measured by low birth weight compared with white women
- (3) To describe our pilot data regarding psychological and physiological stress among women seeking routine prenatal care at \_\_\_\_\_
- (4) To describe our pilot data regarding community level of crime exposure and residential segregation among women who had a singleton delivery at \_\_\_\_\_
- (5) To describe our pilot data that demonstrate a racial disparity in cervical pro-inflammatory cytokines early in pregnancy
- (6) To show that women with decreased cervical pro-inflammatory cytokines early in pregnancy are at an increased risk of intraamniotic infection during labor and may have an increased risk of preterm delivery.
- (7) To demonstrate that elevated vaginal pH and elevated number of vaginal neutrophils are associated with an increased risk of preterm delivery
- (8) To describe our findings regarding the genetic regulation of inflammation and its association with preterm birth and chorioamnionitis
- (9) To justify our use of multiplex technology to assay cervical cytokine concentrations

#### 1. Characteristics of the Investigative Team

The combination of investigators who will work together to accomplish the proposed studies bring expertise in health implications of biopsychosocial processes (Drs. \_\_\_\_\_) and infection, inflammation and preterm birth. (Drs. \_\_\_\_\_). Dr. \_\_\_\_\_ is a new investigator in the field and has published a total of five publications with an additional five publications currently under consideration at peer review journals. Despite \_\_\_\_\_ brief list of publications, Dr. \_\_\_\_\_ is considered a rising star in the field reproductive infectious disease as evidenced by the fact that \_\_\_\_\_ was awarded the \_\_\_\_\_

\_\_\_\_\_. In \_\_\_\_\_ was also awarded the \_\_\_\_\_  
 Thus, Dr. \_\_\_\_\_ brings a ferocious energy and unique skill set to the proposed research. Dr. \_\_\_\_\_ will be supported by a number of established senior research investigators including Dr. \_\_\_\_\_, who is a professor in the Department of Psychiatry and who has established a

behavioral medicine clinic at [redacted]. Other members of the research team are funded senior investigators each of whom has a commitment to insure that Dr. [redacted] is successful in conducting the proposed research. Dr. [redacted]'s lab is internationally recognized for performance of the microbiologic techniques to be used in this proposal and has collaborated with Dr. [redacted] on several of [redacted] manuscripts either published or in press. Not only are [redacted] and the [redacted] uniquely suited to carry out the proposed multidisciplinary project, women of [redacted] also serve as an excellent resource for this study. Racial disparities and health outcomes, high-risk behaviors and circumstances of life for minority women in [redacted] provide an optional population in which to perform the proposed studies. The [redacted], directed by Dr. [redacted], and has a satellite office at [redacted] and will work with the investigative team to target appropriate populations. In addition, the [redacted] has an established community advisory board, which encourages participation of minorities in research. Dr. [redacted] will utilize this community advisory board to ensure appropriate enrollment of the highest risk women in the study.

## 2. Patient Population at [redacted]

From 1998 to 2000, among all of the deliveries performed at [redacted] (n = 22,178), the [redacted] antepartum clinics provided prenatal care for 4275 of the women. To evaluate the number of women who had a spontaneous preterm delivery, we removed those who had twins or higher multiples (n = 63), cesarean delivery (n = 675), and induced labor (n = 537). Of the remaining 3000 women receiving care in our clinic, 390 delivered a spontaneous preterm neonate at less than 37 completed weeks gestational age. The women who seek care at [redacted] clinics are 55% African-American, 44% white, and 1% other groups. The overall frequency of delivery less than 37 weeks gestational age among [redacted] patients is 13% with approximately 60% of the spontaneous preterm deliveries being infection associated. Only white and African-American women are considered in the assessment of factors associated with low birth weight delivery.

**Table 2. Risk of Delivery less than 2500 gm in [redacted] Patients;**

Factor	N	LBW		P	OR	95% CI
		N	%			
Mother's age				NS		
Teen	671	65	10			
20+	2187	239	10			
Race				<0.001		
African-American	1658	213	13		1.8	(1.4, 2.3)
White	1312	103	8		Reference	
Smoke during pregnancy				<0.001		
Yes	1083	141	13		1.6	(1.2,2.0)
No	1798	160	9		Reference	
Cocaine during pregnancy				<0.001		
Yes	135	32	24		2.3	(1.5, 3.6)
No	2835	284	10		Reference	

## 3. Psychological and physiological stress and impact on pregnancy outcomes among women seeking routine prenatal care at [redacted]

We investigated the role of maternal stress/anxiety during pregnancy on early infant development. The purpose of the study was to investigate the effects of psychological stress and the resulting changes in the sympathetic branch of the nervous system (i.e., blood pressure, heart rate, and platelet catecholamines epinephrine and norepinephrine) on birth weight and length, Apgar scores, and obstetric complications. Participants were enrolled prior to the 20th week of pregnancy from [redacted]. Sixty-five healthy women were enrolled. The women were typically low-stress and low-risk as most were married, living with partner (81.5%), highly educated (75.4% with 4+ years college), high socioeconomic status (Mean Hollingshead Index = 46/66; Mean Family Income = \$51K), Caucasian (84.5%), and younger than 35 years of age (mean = 29.5 years; range: 18 – 38). Women were excluded from participation if they smoked, used alcohol or drugs, or had pre-pregnancy diabetes or hypertension.

Upon recruitment from local practices prior to 20th week gestation, blood was drawn for platelet catecholamines and questionnaires (Perceived Stress Scale, Recent Life Events, State-Trait Anxiety Index,

SCL-90R, Pregnancy Outcome Questionnaire) were administered at 25-28 weeks gestation. Heart rate and blood pressure were measured at each prenatal visit after consent. Outcome data were collected from medical records following delivery. Discriminant Function Analyses were used to assess group differences between women with and without complications during gestation and labor, differences in gestational age at delivery, and high and low Apgar scores. Individual stepwise regression analyses were used to assess the relation between psychological or physiological stress with birth length and birth weight.

Mean psychological stress indices were greater among women with complicated pregnancies in general and among those with preterm and postterm deliveries (Table 3). With respect to physiological measures of stress, when adjusted for diastolic and systolic blood pressure, platelet epinephrine was negatively associated with birth length ( $p < 0.10$ ). Higher norepinephrine levels were associated with higher birth weight ( $p < 0.05$ ). No differences were found for physiological measures of stress between normal and pre- or post-term births. We cannot estimate the relationship with preterm birth alone because of limited sample size. Higher anxiety/stress associated with pregnancy (Pregnancy Outcome Questionnaire) was more prevalent in women who delivered shorter and lower birthweight infants. No other psychological measures of stress contributed significantly to the regression equations.

**Table 3. Psychological Stress Measures, mean scores stratified by complication status and by gestational age at birth**

	Complications Absent (N = 42)	Complications present (N = 22)	Term birth (37-41 wk)	Preterm or Postterm Birth (<37 or ≥41.5 wk)
Pregnancy Anxiety	27.61*	34.50*	27.04*	34.71*
Perceived Stress	22.72 <sup>+</sup>	26.50 <sup>+</sup>	22.50 <sup>+</sup>	28.27 <sup>+</sup>
State-Trait Anxiety	51.92	67.50	51.62	66.78
Generalized	0.62 <sup>+</sup>	0.90 <sup>+</sup>	0.61 <sup>+</sup>	0.82 <sup>+</sup>
Somatization				
Total Life Events	2.37 <sup>+</sup>	3.02 <sup>+</sup>	2.36	2.79
Adjustment to Life Events	14.95*	22.40*	15.19	20.06

\*  $p < 0.05$ , <sup>+</sup>  $p < 0.10$

These data demonstrate a fairly consistent relationship between psychological measures of stress with pregnancy complications. Trends in the physiological data support the hypothesized relation between sympathetic activation via stress and higher rates of preterm/low birthweight infants, although low sample size and limited blood collected for this pilot data reflects a need for further investigation. Detection of platelet catecholamines has been improved since the collection of these data and will likely better detect differences in sympathetic activation in future use. These pilot data are relevant to the current proposal for several reasons. First, they demonstrate our ability to ascertain psychological stress. Second, they demonstrate our ability to collect, process, and analyze platelet catecholamines as a representative of the sympathetic activation component of physiological stress.

#### 4. Community Level of Crime Exposure and Residential Segregation among Women who had a Singleton Delivery at \_\_\_\_\_

We performed a cross-sectional study of all women who were residents of the \_\_\_\_\_ and underwent a singleton delivery at \_\_\_\_\_ from \_\_\_\_\_. Residential segregation data were collected from public databases. The components required to calculate residential segregation were collected from the US Census Bureau and the \_\_\_\_\_ City Planning Office. Total population for the year \_\_\_\_\_, as well as the population of self-identifying Caucasian and African-American inhabitants of Pittsburgh were also ascertained from these sources. These populations were categorized by neighborhood composed of one or more census tracts. Community violence data were collected by the \_\_\_\_\_ Police Department. The crimes were categorized as violent (e.g. homicides, rapes, assaults, robberies) or non-violent (e.g. arson, theft). Only community exposure to violent crimes was considered. The data from the Police Department was categorized into neighborhood for crimes occurring \_\_\_\_\_. An event was considered a crime if it was reported, investigated, or considered a case-closed.

**Table 4. Crime and segregation indices in \_\_\_\_\_, overall and stratified by race**

Variable	Urban unstratified by race n= 3735	Urban Caucasian n = 2219	Urban African- American n = 1516	Odds Ratio or p value
<b>Crime rate per 1000 persons (median)</b>	53.5	47.8	76.9	<b>&lt;0.0001</b>
<b>High Crime Rate (%)</b> High: > 53 crime/1000 persons Low: ≤53 crimes/1000 persons	51.9% 48.1%	37.7% 62.3%	72.8% 27.9%	<b>4.4 (3.9-5.1)</b>
<b>% of community members that are African-American</b>	20%	10%	70%	
<b>Residential segregation(%)</b> High: >63% African-American population Low: ≤63% African-American population	27.0% 73.0%	4.6% 95.6%	40.3% 59.7%	<b>30.4 (24.3-38.0)</b>

African-American \_\_\_\_\_ lived in communities with a higher median crime rate than their Caucasian counterparts, and had more than four times the odds of living in a high crime community. Residential segregation is an unfortunate but very real aspect of life in \_\_\_\_\_ as evidenced by our data. African-American women had thirty times the odds of living in a highly segregated community as their white counterparts.

**Table 5. Pregnancy outcomes, overall and stratified by crime exposure**

Variable	Urban Pittsburghers N=3735	Low Crime N=1795	High Crime N=1940	Odds ratio or p value
Birth Weight (g)(mean)	3269	3312	3229	<0.0001
Spontaneous preterm birth < 34 weeks (%)	4.6%	3.8%	5.5%	1.5 (1.1-2.0)
Small for gestational age (%)	10.7%	9.5%	11.7%	1.3 (1.02-1.6)

**Table 6. Pregnancy outcome, overall and stratified by degree of segregation**

Variable	Urban Pittsburghers N=3735	Low Segregation N=1795	High Segregation N=1940	Odds ratio or p value
Birth Weight (g)(mean)	3269	3319	3134	<0.0001
Spontaneous preterm birth < 34 weeks (%)	4.6%	4.1%	6.3%	1.6 (1.1-2.2)
Small for gestational age (%)	10.7%	9.7%	16.9%	1.9 (1.5-2.4)

Spontaneous preterm birth and small for gestational age were more frequent among women who lived in high crime and highly segregated communities. In these pilot data, race demonstrated significant statistical interaction in multivariable modeling with both of our community level variables ( $p < 0.01$ ) and that each demonstrated an influence on adverse pregnancy outcome independent of race. These data demonstrate several important points relevant to our proposed project. First, we are able to successfully ascertain community level variables of crime and residential segregation with an established infrastructure. Please see Appendix A for manuscripts based on Dr. \_\_\_\_\_ work in this arena. Secondly, the demographics of \_\_\_\_\_ are such that we anticipate an adequate distribution of crime exposure and segregation to permit feasible evaluation of these variables in prospective study. Thirdly, we demonstrated

in our pilot data that level of crime in the community and degree of residential segregation are related to preterm birth.

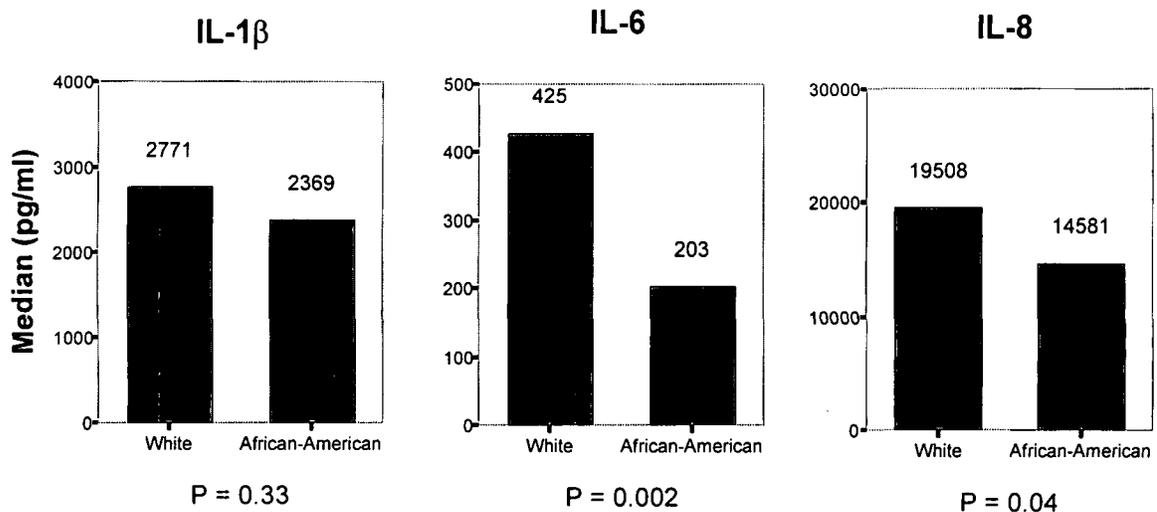
### 5. Median Values of Pro-inflammatory Cytokines (IL-1 $\beta$ , IL-6, and IL-8) Measured Early in Pregnancy in African-American Compared with White Women

cervical pro-inflammatory cytokines were measured in pregnant women enrolled early in gestation for prenatal care.<sup>97</sup> The purpose of the study was to evaluate the relationship of sexually transmitted infections and the production of pro-inflammatory cytokines measured at an early gestational age. Please refer to Appendix A for the manuscript based upon these data. Women were enrolled from February 1996 through February 1997 in the antepartum clinics at Magee-Womens Hospital. Women had cervical swabs obtained for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and had vaginal swabs obtained for the diagnosis of bacterial vaginosis using bacterial morphotypes evaluated by the Nugent 10 point scoring system.<sup>98</sup> These samples were collected between 8 and 20 weeks gestational age. We enrolled 517(31%) of the 1672 women that attended the antepartum clinics in this time period.

Women signed a written consent form approved by the Magee-Womens Hospital institutional review board. Exclusions were antibiotic therapy within the past two weeks, multiple gestation, indication for cervical cerclage, vaginal bleeding, and significant medical problems. Among the 517 women who agreed to the study, 506 had interpretable cytokine assays, and 424 women had complete delivery information available from their medical record. Demographic and obstetric information was obtained from the medical record. African-American and white race were self-reported. The diagnosis of intraamniotic infection was based upon clinical signs and symptoms during labor: temperature elevation above 38°C combined with two of the following signs; maternal or fetal tachycardia, uterine tenderness greater than expected, foul smelling vaginal discharge, and white blood count more than 18,000. Women had two vaginal swabs taken for the diagnosis of bacterial vaginosis and *T. vaginalis*. Bacterial vaginosis was diagnosed by vaginal pH  $\geq$  4.7 and a score of 7 through 10 from a Gram stained vaginal smear using the Nugent method. *T. vaginalis* and *N. gonorrhoeae* were identified by culture and *C. trachomatis* was assayed by nucleic acid amplification methods.

Additionally, two cervical swabs were taken for the assay of interleukin-1 $\beta$ , interleukin-6, and interleukin-8. Concentrations of IL-1 $\beta$ , IL-6, and IL-8 were measured by a commercially available ELISA assay kit (R & D Systems, Minneapolis, MN). Each assay was run with an intra- and inter-assay variation of < 10%. The following graphs display the median cytokine values for African-American women compared with white women. African-American women had lower cytokine levels for IL-1 $\beta$ , IL-6, and IL-8; however, the decreased values were statistically significant for IL-6 and IL-8.

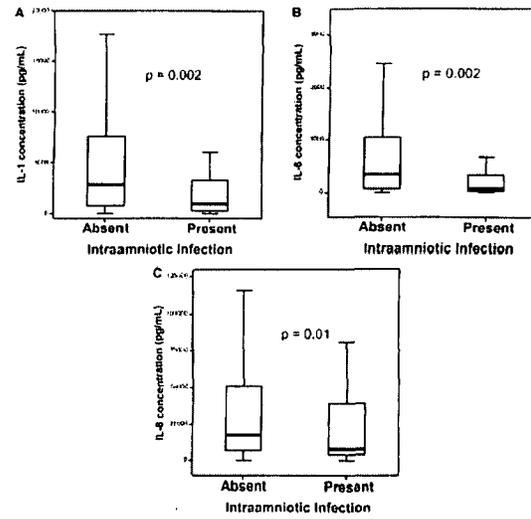
#### Median Cervical Cytokine Values Compared Between African-American (N=257) and White Women (N=249); Specimens at 8 to 20 Weeks



These data support our notion of a racial disparity in the cervical inflammatory environment.

## 6. Risk of chorioamnionitis and preterm birth associated with cervical immune hyporesponsiveness at 8-20 Weeks Gestational Age

Because of the wide range and lack of normality of the distribution of cytokine concentrations, we dichotomized the concentrations into less than the 25<sup>th</sup> percentile and greater than or equal to the 25<sup>th</sup> percentile. When cervical cytokine values were categorized as women producing less than the 25<sup>th</sup> percentile and producing more than the 25<sup>th</sup> percentile, women who had low levels of cytokines measured early in pregnancy had an elevated risk of intraamniotic infection (IAI). Women with decreased IL-1 $\beta$  had a 3.4 fold increased risk of IAI (95% CI 1.5, 7.9); for decreased IL-6 the risk of IAI was 2.5 (95% CI 1.1, 5.6); and for IL-8 the risk of IAI was 2.6 (95% CI 1.2, 5.7).



Decreased cytokine values were also associated with preterm delivery (< 37 weeks). IL-1 $\beta$  less than the 25<sup>th</sup> percentile was associated with a 1.6 fold increased risk of preterm delivery (95% CI 0.8, 3.4) and IL-8 was associated with a 1.5 fold increased risk of preterm delivery (95% CI 0.8, 3.0). Decreased IL-6 was not associated with an elevated risk of preterm delivery; however > the 90<sup>th</sup> percentile of IL-6 was associated with a 60% increase in preterm delivery. These findings suggest that decreased cervical cytokines or extremely elevated cytokines measured very early in pregnancy may fail to protect the upper genital tract from ascending *bacteria* and increase the risk of subsequent preterm birth or intraamniotic infection. We next evaluated the influence of low concentration of multiple cytokines. Because the proinflammatory cytokines have complementary functions, we hypothesize that low concentrations of multiple cytokines indicates a more global immune hyporesponsiveness. We hypothesize that as the number of cytokines with low concentration increases, so does the risk of subsequent intraamniotic infection. The table below displays the risk for intraamniotic infection for women by each combination of cytokine concentrations less than the 25<sup>th</sup> percentile. As shown, the incidence of intraamniotic infection increased from 4.4% among those with no low cytokine concentrations to 8.0% of those with one low cervical cytokine concentration and 17.2% among those with two or more low cytokine concentrations ( $p=0.001$ ,  $\chi^2$  test for trend). After adjustment, women with two or three low cervical cytokines had a five-fold increased risk of infection (Table 7). The strength of the association of clinical risk factors (stressors such as intrauterine pressure catheter use and prolonged rupture of membranes) dramatically increases in women who have low cytokine concentrations

**Table 7: Unadjusted and adjusted odds ratios for intraamniotic infection based on zero, one, and two or more cervical cytokine concentrations < 25<sup>th</sup> percentile**

Number of low cytokine concentrations	N	Number of cases of IAI	%	Unadjusted OR(95% CI)	Adjusted OR†(95% CI)	
Zero	228	10	4.4	Ref	Ref	-
One	88	7	8.0	1.9 (0.7-5.1)	2.0 (0.7-5.8)	0.22
Two or three	87	15	17.2	4.5 (2.0-10.6)	5.1 (2.0-13.0)	0.02*

\* $p < 0.05$

†adjusted for prelabor rupture of membranes, intrauterine pressure catheter use, prolonged rupture of membranes, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and race

These data in sections 4&5 above make several important points relevant to the current proposal. First, we have demonstrated that we can recruit women and collect and analyze genital specimens for cytokines, and that Dr. [redacted] research laboratory is experienced with not only these assays, but other advanced microbiologic techniques. Secondly, we demonstrated that women with a hyporesponsive lower genital tract inflammatory milieu in early pregnancy have a trend towards an increased frequency of preterm birth and an increase in subsequent clinical chorioamnionitis. Thirdly, we demonstrated that there is a racial disparity in the inflammatory milieu of the lower genital tract in early pregnancy and that African-American women are more likely to have an inflammatory profile that places them at risk for later adverse pregnancy outcome.

#### 7. Vaginal Markers of Inflammation and Preterm Birth

Our group has also evaluated aspects of lower genital tract inflammation other than cytokines.<sup>99</sup> In order to determine the association of other vaginal markers of inflammation with infection-mediated preterm birth, we evaluated data from the [redacted]. Please refer to Appendix A for a copy of this manuscript.

[redacted]. In this study, history and vaginal and cervical Gram stain and cultures were obtained from 13,914 women from 23 to 26 weeks' gestation. In this group of women, we sought to determine the association of vaginal markers of inflammation with preterm birth. Elevated vaginal pH, an important clinical criterion for the diagnosis of bacterial vaginosis, is a marker of upper genital tract infection and inflammation both in pregnancy and in the non-pregnant state. [redacted] Neutrophils in the vagina are associated with a positive amniotic fluid culture and elevated amniotic fluid IL-6 among women with preterm labor and intact membranes.<sup>101</sup>

**Table 8. Relationship of Vaginal PMNs and Elevated Vaginal pH to preterm birth**

Delivery (wk)	N (%)	Polymorphonuclear leukocytes >5 odds ratio (95% CI)*	pH $\geq$ 5.0 odds ratio (95% CI)*	Polymorphonuclear leukocytes >5 and pH odds ratio (95% CI)
<37	1527 (11.5)	1.2 (1.1-1.4)	1.3 (1.1-1.5)	1.4 (1.2-1.6)
<34	424 (3.1)	1.2 (1.1-1.5)	1.7 (1.4-2.1)	1.6 (1.2-2.2)
<32	228 (1.7)	1.6 (1.2-2.1)	2.0 (1.5-2.6)	2.7 (1.8-4.0)
<30	130 (0.9)	1.9 (1.3-2.7)	2.0 (1.4-2.9)	2.9 (1.7-4.9)
<28	66 (0.5)	2.2 (1.3-3.6)	3.0 (1.8-4.9)	7.4 (2.8-19.6)

\*Adjusted for previous problem, race, age, smoking, N gonorrhoeae, C trachomatis, T vaginalis, and antibiotic use.

The earlier in gestation a preterm birth occurs, the more likely infection or inflammation is present in the fetal compartment.<sup>103</sup> The concomitant presence of vaginal neutrophils and elevated vaginal pH is consistently associated with preterm birth. Furthermore, the strength of that association increases as the gestational age at delivery decreases, as shown in Table 8. Thus, these markers of vaginal inflammation may be important environmental factors in infection-mediated preterm birth. Vaginal pH and neutrophils interact to increase the risk of preterm birth. Table 9 demonstrates this interaction. Importantly, there is racial disparity in the frequency of this marker of vaginal inflammation. In our analysis, the presence of vaginal neutrophils and elevated vaginal pH occurred in 10.4% of African-American women and 8.6% of white women ( $p < 0.001$ ). In this proposal, we will also utilize vaginal pH and neutrophils to describe the inflammatory milieu of the lower genital tract as it related to risk of preterm birth.

**Table 9. PMNs, pH, and PTB < 32 weeks**

Polymorphonuclear leukocytes >5	pH $\geq$ 5.0	Proportion of deliveries <32 weeks of gestation (%)	Odds ratio (95% CI)*
-	-	1.2	Reference
+	-	1.8	1.6 (1.1-2.1)
-	+	2.2	1.7 (1.1-2.6)
+	+	3.6	2.9 (2.0-4.3)

\*Unadjusted.

### 8. Genetic control of inflammation and risk of chorioamnionitis and preterm birth

Central to this proposal is the notion of a gene-environment interaction that influences stress and the lower genital tract inflammatory derangements that promote preterm birth. Our group has published on the relationship of cytokine promoter polymorphism genotype to chorioamnionitis and to preterm birth.<sup>95,96</sup> Please refer to Appendix A for copies of these manuscripts. In approaching the genetic predisposition to chorioamnionitis, we chose to consider the pro-inflammatory cytokine, TNF- $\alpha$ . A polymorphism in the promoter region of TNF- $\alpha$  (at position -308) is associated with high production of TNF- $\alpha$ . The substitution of adenosine (A) for guanine (G) at this position is responsible for the increase in promoter activity. The individual with guanine at both positions (homozygous G/G) displays normal production of TNF- $\alpha$ . This is known as the TNFA1 allelic variant. The high production allelic variant (TNFA2) may be homozygous (A/A) or heterozygous (G/A). Since the genetic predisposition towards increased TNF- $\alpha$  production is related to preterm premature rupture of the membranes<sup>104</sup>, we sought to ascertain its relationship to another infectious complication of pregnancy: intraamniotic infection in labor. In this retrospective cohort study, previously banked DNA from 149 women who had spontaneous labor from 37 to 42 weeks' gestation was used. Polymerase chain reaction was utilized for polymorphism assay. Demographic and clinical information were obtained from the medical record. Clinical chorioamnionitis was defined as at least one temperature elevation above 38°C combined with at least two of the following signs; maternal or fetal tachycardia, uterine tenderness greater than expected, foul smelling vaginal discharge, and white blood count more than 18,000. Chorioamnionitis was present in 18 women (12.1%). Among women who did not carry TNFA2, the chorioamnionitis rate was 7.4%. Among women who carried TNFA2, the chorioamnionitis rate was 24.4%. The relative risk for chorioamnionitis with carriage of TNFA2 was 3.3 (95% CI 1.3-7.1). This increased risk was not altered after adjustment for race, type of rupture of membranes, intrauterine pressure catheter use, smoking, and prolonged rupture of membranes. Carriage of the TNFA2 allele is associated with a more than three-times increased risk of clinical chorioamnionitis, even when accounting for important clinical and microbiologic risk factors.

When considering the genetic predisposition to preterm birth, we chose to evaluate interleukin-6 (IL-6). IL-6 is a critical cytokine in the cascade of host response to infection. IL-6 activates the acute phase response, stimulates T lymphocytes, induces the terminal differentiation of B lymphocytes, and induces C-reactive protein production.<sup>105</sup> In the setting of intrauterine infection and preterm labor, the amniotic fluid concentration of IL-6 is increased in excess of that of other proinflammatory products.<sup>106</sup> Increases in IL-6 concentration are seen in maternal serum, cervix, and amniotic fluid in preterm labor.<sup>107-109</sup> In large part, the production of IL-6 is under genetic regulation. A polymorphism in the promoter region of IL-6 (at position -174) on chromosome 7 is associated with production of IL-6. The substitution of cytosine (C) for guanine (G) at this position is responsible for a decrease in promoter activity. The individual with guanine at both positions (homozygous G/G) or one position (heterozygous G/C) displays normal production of IL-6. Those individuals who are homozygous C/C display lower production of IL-6. The C/C variant of this polymorphism is related to IL-6 production and to the incidence and severity of inflammatory conditions such as juvenile chronic arthritis and end-stage renal disease.<sup>110, 111</sup>

The distribution of the G and C alleles in the IL-6 -174 promoter polymorphism is known to vary by ethnicity.<sup>111</sup> Among 145 subjects in Washington, D.C., the frequency of the C allele was 35% among whites and 9% among African-Americans. In that population, there were no African-Americans with the C/C allele combination, nor were there any Afro-Caribbeans with the C/C allele in a similar study in the United Kingdom.<sup>110</sup>

Because of the important role that IL-6 may play in preterm birth and because of the known racial disparity in preterm birth, we sought to assess the relationship between IL-6 promoter polymorphism allelic variant and spontaneous preterm birth < 34 weeks' and the relationship of polymorphism status with race. Polymerase chain reaction was used to evaluate DNA from 156 controls who delivered after spontaneous labor at term and 51 cases who had spontaneous preterm birth < 34 weeks'.

**Table 10. IL-6 -174 polymorphism genotype among term and preterm births, overall and stratified by race**

	<i>Term overall</i> (n = 156)	<i>Preterm overall</i> (n = 51)	<i>Term white</i> (n = 110)	<i>Term African American</i> (n = 46)	<i>Preterm white</i> (n = 39)	<i>Preterm African American</i> (n = 12)
Genotype						
G/G	68 (43.6%)	32 (62.8%)	33 (30.0%)	35 (76.1%)	21 (53.9%)	11 (91.7%)
G/C	58 (37.2%)	17 (33.3%)	47 (42.7%)	11 (23.9%)	16 (41.0%)	1 (8.3%)
C/C	30 (19.2%)	2 (3.9%)	30 (27.3%)	0 (0%)	2 (5.1%)	0 (0%)
Allele						
G	0.62	0.79	0.51	0.88	0.74	0.96
C	0.38	0.21	0.49	0.12	0.26	0.04

Among the 156 controls, the C/C variant was present in 30 (19.2%). Among the 51 cases, the C/C variant was present in 2 (6.3%) [OR 0.17 95%CI 0.04-0.74]. Among Caucasians, the C/C variant was present in 30 (27.2%) controls but only 2 (5.2%) cases. No African-Americans carried the C/C variant. The racial disparity was statistically significant. (p<0.001). The IL-6 promoter -174 allelic variant C/C is significantly less frequent among women with sPTB < 34 weeks'. There is a dramatic racial disparity in the distribution of allelic variants of this polymorphism. These preliminary data are relevant to the current proposal for several reasons. First, we have a demonstrated ability to recruit subjects, collect samples and data, process and analyze DNA data, and interpret results of studies of the genetic epidemiology of preterm birth and other adverse pregnancy outcomes. Second, our available subject population provides appropriate genetic and ethnic diversity to evaluate adequately the contribution of cytokine promoter polymorphism genotype in the context of the other environmental exposures outlined in this proposal.

## 9. Use of multiplex technology to assay cervical cytokine concentrations

In general, ELISA is the most commonly used technique to determine the concentration of cytokines in human body fluids or cellular supernatant.<sup>112-114</sup> One important disadvantage to ELISA technology relevant to our proposal is the available volume of cervical fluid. The average quantity of undiluted cervical fluid that may be obtained with a Dacron tipped swab is ~250  $\mu\text{L}$ . A single ELISA cytokine assay requires 50-100 $\mu\text{L}$  of sample per cytokine; when samples are run in duplicate or triplicate, available sample becomes an ever-pressing issue. Multiplexed particle-based flow cytometric technology permits the simultaneous assay of numerous analytes with a single 50 $\mu\text{L}$  aliquot of sample.<sup>114, 115</sup> Multiplex-based platforms quantify cytokine and antibody concentrations in plasma with a high degree of agreement and correlation to ELISA, while providing improved sensitivity.<sup>116-119</sup> We sought to validate this technology in cervical fluid so as to support its use for measurement of cytokines in cervical fluid. We used the Luminex LabMAP™ system as the platform and evaluated a Beadlyte® analyte kit from Upstate USA (Lake Placid, NY) designed to assay 22 human cytokines from single aliquot of 50 $\mu\text{L}$  of specimen. A monoclonal antibody specific for a cytokine is covalently linked to a fluorescent bead set, which captures the cytokine. A complementary biotinylated monoclonal cytokine antibody then completes the immunological sandwich and the reaction is detected with streptavidin-phycoerythrin. We utilized 49 banked cervical fluid specimens from a study done at our institution of the cervical inflammatory response before and after treatment of bacterial vaginosis in pregnancy.<sup>120</sup> These samples were previously analyzed in duplicate using ELISA for IL-1 $\beta$ , IL-6, and IL-8 as part of the original study. Each sample was frozen at -80°C after initial assay and was never thawed until this correlation study. After thawing, multiplex assay was performed. We found a very high degree of correlation between concentration determined by ELISA and concentration determined by multiplex assay ( $r^2 = 0.95$  to 0.97) for each of the cytokines with previous ELISA values. This high degree of correlation, the efficiency in terms of time for assay and volume required, and the number of analytes per assay makes multiplex technology the optimal choice for evaluation of cervical cytokine concentration in this proposal.

In summation our Preliminary Data supports several critical points regarding our proposal. First, \_\_\_\_\_ are a rich resource population to study racial disparity in preterm birth, particularly with respect to our unique approach to include assessment of community level contributors to stress. Secondly, our group has a documented track record of research recruitment from the antenatal clinics of \_\_\_\_\_, with an ability to collect, process, transport, store, and analyze all biological specimens for all the assays required to complete this study. Our plan to use multiplex technology to evaluate a multitude of cytokines will provide the opportunity to simultaneously evaluate many aspects of the immunologic environment of the lower genital tract, and affords us the possibility of revealing complex interrelationships among these critical inflammatory molecules that were not possible in previous work. Likewise, through our pilot data, we have demonstrated our ability to collect, analyze, and interpret to individual and community level variables related to stress and contributors to stress vital to this proposal. Our preliminary data supports our hypothesis of a lower genital tract innate immune profile that places a woman at risk for preterm birth and/or intrauterine infection during pregnancy (hyporesponsive proinflammatory cytokine pattern early in pregnancy and elevated vaginal pH and neutrophils at the end of the second trimester). Of particular relevance to our proposal in response to this Program Announcement is our data regarding the genetic control of inflammation and its influence on preterm birth and intrauterine infection. Our plan to evaluate the interactive contribution of stress and cytokine promoter polymorphisms is biologically plausible and data-driven.

### D. Research Design and Methods

#### 1. Availability and Characteristics of Study Population

Disparities in maternal and child health outcomes are particularly stark in \_\_\_\_\_. According to a \_\_\_\_\_ report by \_\_\_\_\_ (see Appendix B) that examines health disparities by race in \_\_\_\_\_, the rate of gonorrhea is 48 times higher in African-American females than white females while the rate of chlamydia is 20 times greater in African-American women. The infant mortality rate among African-Americans living in \_\_\_\_\_ is the second highest nationally and four times the Healthy People 2010 goal. The rate of unintentional injury death rates in the county is 1.5 times greater in African-American women than in Caucasian women. The social and economic status of African-Americans in \_\_\_\_\_ is among the worst in the United States and a high percentage of African-American children are living in poverty. Analysis of data from \_\_\_\_\_ demonstrates preterm birth rates among African-Americans that are double those of Caucasians. At \_\_\_\_\_,

\_\_\_\_\_ racial disparity among a number of fetal outcomes and maternal risk behavior are astonishing (see Appendix C). Recruitment will occur in our ambulatory clinics and the private offices of the Maternal-Fetal Medicine faculty. Approximately 1700 pregnant women are seen annually at these sites. Our clinic provides care to approximately 1300 women annually and about half of these are Caucasian while the other half is African-American. Patients seeking perinatal care at our clinics have access to social workers, dietitians, family planning and genetic counselors. A full range of prenatal care is available including appropriate laboratory testing, ultrasound, genetic counseling and Maternal-Fetal Medicine consultative services. Programs to address problems such as domestic violence, smoking, drug abuse, and depression are available and clearly described to all new obstetrical patients.

The private offices of the Maternal-Fetal Medicine faculty are adjacent to the central clinic location. Approximately 500 patients are delivered annually by faculty and fellows. Four nurse clinicians assist in providing care to these patients who are mostly high risk. The racial mix is about 10% African American and 90% Caucasian. The majority of patients have commercial insurance, are highly educated, and represent a middle to upper-middle socioeconomic class.

The patients in the clinic and the MFM office are available for participation in this proposal as their care is totally supervised by MFM faculty. Dr. \_\_\_\_\_, an MFM faculty member, supervises the prematurity high-risk clinic, and Dr. \_\_\_\_\_ the MFM Division director, supervises the private MFM office. The necessary infrastructure for clinical research has existed at our institution for many years. As active participants in the NICHD-sponsored \_\_\_\_\_ for more than 15 years, we

have participated in many research trials that have required active patient recruitment. The proposed study will not interfere with any network trial. We have established a Human Investigation Committee to coordinate patient recruitment in our resident clinics so that recruitment is optimized.

**Table 11. Patient population in our clinic and in the private MFM offices.**

Deliveries	Clinic 1998-1999		MFM Office 1998-1999	
	n	%	n	%
Cesarean	456	16.7%	348	32.6%
Vaginal	2267	83.3%	720	67.4%
<b>Race</b>				
White	1251	45.9%	915	85.7%
Black	1378	50.6%	109	10.2%
Asian	1		1	
Hispanic	3		1	
<b>Insurance</b>				
Commercial	327	12.0%	770	72.4%
Medicaid	2326	85.4%	287	26.9%
Self	33	1.2%	6	0.6%
Missing	38	1.4%	5	0.5%

This is a prospective, observational cohort study. Eligible patients will fulfill the following **inclusion criteria**:

(1) pregnancy prior to 20 weeks' gestation and (2) singleton gestation.

Eligible subjects will not exhibit any of the following **exclusion criteria**:

1. vaginal bleeding
2. fetal anomalies
3. known thrombophilias
4. diabetes-pregestational
5. chronic hypertension requiring medication
6. documented cervical funneling
7. current or planned cervical cerclage
8. Immunocompromise (HIV +, use of systemic steroids within 6 months, use of post-transplant immunosuppressive medications)
9. Autoimmune disease (Inflammatory bowel disease, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Scleroderma)
10. Use of illegal drugs or controlled substances

All eligible subjects will be approached at the first prenatal visit and the study will be explained. A pamphlet describing the study will be given to the eligible subjects with a phone number to call for further information. The recruiter will seek out the eligible subjects at subsequent visits to determine their level of interest. Consenting subjects will be followed longitudinally throughout pregnancy. Planned enrollment is 800 subjects (see sample size calculation, below). Determination of gestational age is made based on the following procedure:

1. The first day of the last menstrual period (LMP) is determined, and a judgment made as to whether or not the patient has a "sure" LMP date
2. If the LMP date is unsure, ultrasound measurements obtained at the patient's first ultrasound examination are used to determine the gestational age, by the standard method of ultrasound gestational age determination.
3. If the date of the LMP is certain, and ultrasound confirms this gestational age within the number of days in the table below, the LMP-derived gestational age is used as the gestational age estimate.
4. If ultrasound-determined gestational age does not confirm the LMP-generated gestational age within the number of days specified in the table below, the ultrasound is used to determine estimated gestational age.

**Table 12. Criteria for establishing gestational age by LMP and ultrasound**

Gestational Age at first ultrasound by LMP	Ultrasound agreement with LMP
Up to 19 6/7 weeks	± 7 days
20 0/7 weeks to 29 6/7 weeks	± 14 days
30 0/7 weeks or more	± 21 days

### Participation of Children

We will not exclude children from this investigation as long as the inclusion and exclusion criteria are met. The perinatal and obstetrical services at all sites should routinely provide a full complement of care to pregnant minors. These women will not be excluded from study participation based on their age.

### 3. Study Procedures: Interviews, Examinations, and Specimen and Data Collection

The research nurses and recruiters will keep logs of all eligible subjects. The reasons for ineligibility will be tallied. Among eligible subjects, we will tally those who are approached, those who are not approached, and reasons why eligible subjects decline to participate.

Consenting subjects will have the following assessments performed during pregnancy or at delivery:

- a. First Visit – 8 to 16 weeks' gestational age  
The first study visit may be the woman's first or second prenatal visit. 83% of \_\_\_\_\_ clinic patients have their first prenatal visit by the 8<sup>th</sup> week.
  1. Obtain informed consent
  2. Obtain vaginal and cervical specimens for sexually transmitted infections, Gram stain, vaginal microbiology, and cervical cytokine assays.
  3. Interview for medical history, drug/alcohol use, general demographic information, and reduced Block Food Frequency questionnaire, Schedule of Recent Life Events (SRE), Edinburgh Postnatal Depression Survey (EPDS), State-Trait Anxiety Index (STAI), and Social Function-12 (SF-12)
  4. Urine specimen for cotinine measurement
- b. Second study visit – 18 to 20 weeks' gestational age
  1. Obtain blood specimen for assay of maternal plasma CRH and platelet catecholamines and cytokine promoter gene polymorphism
  2. Obtain vaginal and cervical specimens for sexually transmitted infections, Gram stain, vaginal microbiology and cervical cytokine assays.
  3. Interview using Perceived Stress Scale (PSS), Hassles Scale, and Subjective SES survey
- c. Third study visit – 28 to 30 weeks' gestational age
  1. Obtain blood specimen for assay of maternal plasma CRH and platelet catecholamines
  2. Interview using PSS, Hassles Scale, SRE, and Subjective SES survey
- d. Admission/Delivery
  1. Collection of pregnancy outcome data on all subjects

The research nurse will track all appointments of all subjects. One week prior to a subject's second study visit, the research nurse will send a postcard to the subject reminding her of the upcoming appointment. The subjects will be given the research nurse's pager and office phone to call with questions or schedule changes. The chart of all recruited subjects will be identified by a brightly colored face sheet that will identify the subject as a study participant and provide the contact information for the investigators and research nurse. All hospital visits and the delivery will be tracked by the research nurse so that pregnancy outcome data can be retrieved promptly.

#### **Rationale and Methods of Measurements**

##### **a. Interview – Determination of Race, Reproductive History, and Sexual History**

The interview will be a face-to-face interview with a trained study interviewer using a standardized, closed question format questionnaire. The interview takes twenty to thirty minutes. The clinical assistant conducting the interview will be trained to ask questions in an empathetic and nonjudgmental manner. The definitions used for race/ethnicity will be based on those used by the US Census, the OMB, and established epidemiologic methodology. Lin and Kelsey describe the purpose of identifying race in epidemiologic and medical studies as an effort to identify "biological inheritance." (Lin, 2000) Ethnicity refers to a person's social group characterized by a distinctive social and cultural tradition. Members of an ethnic group often have shared experiences, common genetic heritage, and common features in their way of life. (Lin 2000) The US census and the US Office of Management and Budget use four broad categories (White, Black, American Indian, and Asian/Pacific Islander) to define race. We appreciate that measures of race are imperfect and do not completely reflect one's genetic and environmental predisposition to disease. In addition to collecting information on the race and ethnicity of the enrolled woman, the father of their fetus, and the fetus' grandparents, we will also collect family history of infectious/immunologic disease, pregnancy complications, and maternal birthweight.

Reproductive history is being obtained on the subject because the risk of preterm delivery and being small-for-gestational age repeats from pregnancy to pregnancy.<sup>121</sup> The following variables will be collected for each of the women's previous pregnancies: month, year, gestational weeks, delivery method, birth weight, race of father, vital status at birth, and vital status now.

Sexual history is being obtained because it is associated with other risk factors for preterm delivery. The information about alcohol use and illegal drug use just prior to and during the early part of pregnancy is being collected by directed interview. This method is being used for obtaining data on alcohol use during pregnancy rather than using biologic measurements because the biologic measurements for alcohol lack accuracy and because they would not distinguish well between the time period before pregnancy and just after pregnancy began. The information about illegal drug use during pregnancy is not being collected by biologic measures because it would be excessively expensive, put the study personnel in an adversarial relationship with the study enrollees, and may be offensive to the primary care providers of the women enrolled in the study.

##### **b. Food Frequency Questionnaire for Nutritional Information**

In order to capture as much of the multi-faceted nature of "nutritional status" as possible, we will assess pre-pregnancy weight by subject self-report, weight gain over pregnancy, and Body Mass Index. Additionally, each subject will complete the reduced Block Food Frequency Questionnaire (rBFFQ). The rBFFQ reliably and validly assesses total energy as well as micro- and macronutrient intake. The rBFFQ, after analysis with the DIETANAL software package, provides estimates of intake of energy (kcal), protein, fat (saturated, polyunsaturated, and monounsaturated), carbohydrate, alcohol, cholesterol, dietary fiber, calcium, iron, vitamin A, and vitamin C. (Block 1998, Willett, 1998, Wirfalt 1998) The rBFFQ takes between 20 and 30 minutes to complete.

**c. Self-report Plus Biologic Measures for Tobacco Use**

Objective assessment of tobacco exposure will be accomplished by measuring the major stable metabolite of nicotine, cotinine. Cotinine measurements not only assess the direct intake of nicotine from active smoking but also serve as a marker of passive exposure. Cotinine levels have previously been used in smoking cessation programs and as a marker of exposure in relation to other disease states. In many previous studies, tobacco exposure has been determined retrospectively by medical record information or prospectively by patient self-report. The smoking habits reported by women during pregnancy do not necessarily provide an accurate measure of tobacco exposure, and cotinine measurements provide a more accurate assessment of maternal and fetal tobacco exposure. (Bardy 1993) Haddow measured serum cotinine levels in relationship to birthweight and found that cotinine measurements were more strongly related to birth weight than smoking history. (Haddow 1987) The unreliability of patient reported smoking was confirmed in a study at Magee-Womens Hospital in which nearly a third of women with cotinine concentrations greater than 50 ng/ml denied smoking. (Lain 1999)

**d. Assessment of stress and its psychological effects**

We have selected these measures to tap multiple domains of stress and its sequelae. Recent life events and daily hassles will be quantified as well as the resultant stress that each patient perceives. Physiologic dysregulation increases when the woman's ability to cope with the stress that she experiences is compromised. We will evaluate levels of symptoms (depression and anxiety as continuous measures) as well as the woman's overall functional ability. See Appendix E for the questionnaires.

Perceived Stress Scale (PSS) is the most widely used psychological instrument for measuring the perception of stress. The PSS is a 14-item instrument developed to assess perceptions of stress levels as well as perceptions of one's ability to deal with stressful events. The PSS has shown adequate internal ( $r = .84 - .86$ ) and test-retest reliability ( $r = .85$ ), as well as good predictive validity in the comparison of life events and outcomes to perceptions of stress.<sup>122</sup> An interesting finding using the PSS to measure perceived stress prospectively predicted important aspects of the common cold and did so differently from other indices of stress (negative life events were associated with greater rates of clinical illness).<sup>123</sup> The PSS takes between 7 and 15 minutes to complete.

Schedule of Recent Events (SRE) is a 54-item questionnaire that provides an estimate of the total stress burden over the past six months by accounting for the frequency of life changes and events and their perceived impact.<sup>124</sup> The SRE has demonstrated excellent test-retest reliability ( $r = .90$ ) in short-term studies and convergent validity in samples of individuals who have and have not undergone significant life changes in the past year (e.g., severe illness) and in retrospective and prospective studies of the effects on life changes on the development of coronary artery disease.<sup>125</sup> Further, divergent validity has been shown in comparison of daily hassles with major life changes in the SRE ( $r = .25-.33$ ).<sup>125</sup> The SRE takes between 20 and 30 minutes to complete.

The Hassles Scale is a 117-item questionnaire indicating the number of events in the past month that are considered to be irritants, annoyances, or pressures and the intensity of those "hassles".<sup>125</sup> Hassles include areas of work, family, social activities, the environment, daily annoyances (e.g., misplacing keys), finances, and health. Two scores are created using this questionnaire; a frequency of hassles reported in the past month, and an intensity of hassles reported. The scale has been used widely and has an average test-retest reliability of  $r = .79$ .<sup>125-127</sup> The Hassles Scale takes between 25 and 35 minutes to complete.

The Edinburgh Postnatal Depression Screening Scale<sup>128</sup> is a measure that has been validated for use in screening for depression during pregnancy as well as its original use for postpartum screening.<sup>129, 130</sup> This measure performs well (when validated against a structured diagnostic research interview for the diagnosis of Depression) compared to other screens during pregnancy. This has also been demonstrated by Dr. \_\_\_\_\_'s group.

The State-Trait Anxiety Inventory (STAI) was initially conceptualized as a research instrument for the study of anxiety in adults. It is a self-report assessment device which includes separate measures of state and trait anxiety. Scores on the STAI have a direct interpretation: high scores on their respective scales mean more trait or state anxiety and low scores mean less. The stability of the STAI scales was assessed on male and female samples of high school and college students. For the Trait-anxiety scale the coefficients ranged from .65 to .86, whereas the coefficient for the State-anxiety scale was .62. Correlations are presented in the manual between this scale and other measures of trait-anxiety: the Taylor Manifest Anxiety Scale, the IPAT Anxiety Scale, and the Multiple Affect Adjective Check List. These correlations are .80, .75, and .52, respectively.

The Social Function 12 (SF-12) is the most widely used measure of health-related functioning and distinguishes change over time.<sup>131</sup> It allows comparisons of functioning with national norm data.

**e. Measurement of Neighborhood Factors :Community level of crime exposure & racial segregation**

This study uses locally acceptable neighborhood definition as published by the \_\_\_\_\_ and the \_\_\_\_\_ Department of Planning. This neighborhood structure has been spatially captured into a spatial data using Geographic Information System (GIS). Essentially, the neighborhoods within the city of \_\_\_\_\_ are composed of census tracts which are merged using GIS to define a given neighborhood. Outside the city, the municipalities conveniently serve as communities. Thus we are able to link other geospatial databases, such as the census. The GIS methodology is flexible and enables us to reconfigure neighborhoods based on analytical needs. Defining communities with very small populations leads to considerable sampling variability in outcomes of interest. Therefore, smaller communities might have to be aggregated to attain sufficient population size. Such merging of communities can only be done by taking into account characteristics of the communities and spatial proximity. These geospatially designed neighborhoods have been linked to census data at the appropriate level of aggregation. The participants will be assigned a census tract number following geo-coding of their home addresses. The census tracts will be combined according to local Planning Offices to create neighborhoods. Participants will have a residential segregation index number calculated for them by their neighborhood. Likewise, the overall rate of community violence will be computed for the nine months of gestation, as well as a community violence rate by month for each participant's neighborhood. Data required for calculating the residential segregation index has been collected

by the Planning Office whose source was the 2000 U.S. Census data. Community violence data was collected by the Police Department. The data is categorized by violent crimes (homicides, rapes, assaults, robberies) and non-violent crimes (arson, theft, etc.). Dr. \_\_\_\_\_ is experienced (see Appendix A) in these techniques and will serve in this capacity for this project.

#### f. Perception of racism

The racism survey by Krieger is a widely used, short 3-item instrument designed to assess racial discrimination in six settings-- school, getting a job, work, obtaining work, receiving medical care or dealing with the legal system.<sup>132, 133</sup> The tool also assesses how individuals respond to discrimination-- do they accept it as a fact of life or do something about it. The Krieger perception of racism survey takes between 5 and 10 minutes to complete.

#### 5. Laboratory Specimens and Measurements for Gene Polymorphisms

**Blood Collection:** Having given informed consent, 800 women will be entered to the study for one-time blood collection.

Approximately 5-10 cc peripheral blood will be collected in yellow top (ACD) tube.

**Evaluation of polymorphisms:** The DNA extraction and SSP-PCR will be carried out according to standard techniques for HLA polymorphism (34). Commercially available kits are purchased from One-Lambda Inc. (CA). Each well includes an internal positive control of a pair of primers unrelated to the polymorphism of interest called a 'housekeeping gene' to detect possible PCR inhibition. A negative control well (without the target DNA) is also included in each run to rule out non-specific amplification. Each primer set is designed to give an amplified fragment of a specific size, which can be detected by gel electrophoresis and ethidium bromide staining. Patterns of positive and negative amplification yield the relevant phenotype. For other cytokine gene polymorphism like IL-1 $\alpha$ , IL-1 $\beta$ , IL-1Ra, and IL-4, we will have access to the specific reagents and we will set the PCR-SSP for each cytokine gene polymorphism.

**Table 13. Cytokine polymorphisms to be investigated.**

Cytokine	Position	Allelic Variation	Cytokine Production
IL-4	-590	T	Higher activity
		C	Lower activity
IL-1 $\beta$	-511	C	High
		T	Low
TNF- $\alpha$	-308	A	High
		G	Low
TNF- $\alpha$	-863	C	High
		A	Low
IL-10	-1082,-819,-592	G,C,C*	High
		A,C,C*	Low
		A,T,A*	Low
TGF- $\beta$ 1	Codon 10	Leucine	High
	Codon 25	Proline	Low
IL-6	-174	Arginine	High
		Proline	Low
IL-8	Microsatellite locus A2 on 4q12-13	G	High
		C	Low
IL-8	Microsatellite locus A2 on 4q12-13		High

\*Heterozygote G,C,C/A,C,C or A,T,A is intermediate

#### 6. Laboratory Specimens and Measurements for Cervical Cytokines

**Cervical Specimen Collection:** At the time of speculum examination a dacron swab will be placed in the cervix and left there for 10 seconds to achieve saturation. We will then place the swab in a plastic tube containing 400  $\mu$ l of Purified Bovine Serum (final dilution of 1:5), and store the sample at -20°C. When analyzing the sample, we will thaw it at room temperature, place the swab and the diluent in a spin-X centrifuge filter unit and centrifuge at 12,000 rpm for 20 minutes. Aliquots of the filtered solution will be stored at -80°C until performing the assays.

**Measurement of Cytokines:** We will use the Luminex LabMAP™ system as the platform and the Beadlyte® analyte kit from Upstate USA (Lake Placid, NY) designed to assay 22 human cytokines from a single aliquot of 50  $\mu$ L of specimen. The cytokines we will assay are IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, GM-CSF, IFN- $\gamma$ , TNF- $\alpha$ , Eotaxin, MCP-1, RANTES, MIP-1 $\alpha$ , and IP-10. Monoclonal antibody specific for each cytokine is covalently linked to a fluorescent bead set, which captures the cytokine. A complementary biotinylated monoclonal cytokine antibody then completes the immunological sandwich and the reaction is detected with streptavidin-phycoerythrin. The standards are used to generate a standard curve from which the unknowns are quantified. Each assay is run with an intra- and inter-assay variation of <7%.

#### 7. Laboratory Specimens and Measurements for Sexually Transmitted Infections, Other Genital Pathogens, and Normal Vaginal Flora

##### a. STI's, Bacterial Vaginosis, Facultative Bacteria, and Anaerobic Bacteria: Rationale for Inclusion in the Study

STI's and bacterial vaginosis are being diagnosed for a number of reasons. First, they have been associated with preterm delivery, race, and with cervical cytokine levels, so they are expected to be important confounding variables which will need to be controlled in the analysis. Second, other investigators have found an interaction in risk between cytokine promoter gene polymorphism for TNF- $\alpha$  and bacterial vaginosis in the risk for preterm delivery (Macones et al, SMFM abstract 2002). We expect to find a similar interaction in this study and may find an interaction with BV and other cytokine polymorphisms. Additionally, STI's and BV have been associated with cervical cytokine production. It will be important to know information about STI's and BV to appropriately interpret the relationship of cytokine production to race or pro- and anti-inflammatory cytokine production with preterm delivery. Other facultative bacteria such as group B Streptococcus, E. coli, absence of Lactobacillus spp., and Lactobacillus spp. that do not produce hydrogen peroxide have all been associated with African-American race and with preterm delivery. These commensal vaginal flora may be important confounding variables to be adjusted for in the analysis of cytokine production and preterm delivery. Anaerobic bacteria are BV-associated bacteria. Their assessment in this study is important because different components of the BV-associated bacteria may be more important in the risk of preterm delivery associated with BV and with the interaction of BV with gene polymorphisms.

**b. Vaginal Specimens for STD's and Bacterial Vaginosis: Collection and Treatment**

The specimens for N. gonorrhoeae and C. trachomatis are obtained with a dry swab and will be placed into a Culturette Direct for use with the Strand Displacement Amplification System made by \_\_\_\_\_. The specimens for T. vaginalis are obtained with a swab and will be placed into PCR transport medium. The specimen for the diagnosis of bacterial vaginosis is rolled from the swab onto a glass slide and air-dried. pH is measured by placing pH paper against the vaginal wall for 10 seconds, then comparing it to a colorimetric standard on the pH paper box. These vaginal and cervical specimens are transported to Dr. \_\_\_\_\_'s Research Microbiology Laboratory in the \_\_\_\_\_.

Women who are identified as having BV will be offered treatment with metronidazole in accordance with CDC guidelines, 2 gm in a single oral dose. Laboratory personnel will contact the care provider with the BV smear result and the care provider will discuss treatment options with the patient. Study personnel will record which women choose to have treatment and which women choose not to be treated. We are recommending offering women treatment because it is unethical to offer them different care than other women seen at \_\_\_\_\_ clinics who are offered treatment if they are tested.

The laboratory personnel will notify the care provider of women who are identified with N. gonorrhoeae, C. trachomatis, and T. vaginalis. The care provider will recommend to the patient that she be treated in accordance with CDC guidelines. The administration of these or other antibiotics between the first study visit and delivery will be recorded and adjusted for in the analysis, but there are no other existing data to suggest how they may affect upper genital tract bacterial carriage. Therefore, we have no estimates of what effect to expect in this group of patients.

**c. Microbiology Laboratory Procedures**

**i. Bacterial Vaginosis and Vaginal Neutrophils**

A swab obtained for the diagnosis of bacterial vaginosis will be transported to the Research Microbiology Laboratory under the direction of Dr. \_\_\_\_\_. The laboratory is across the street from \_\_\_\_\_, so specimens will not require any special handling for transportation. The methods for diagnosis of bacterial vaginosis have been published by Nugent and colleagues.<sup>98</sup> Bacterial vaginosis is diagnosed by a Gram stained slide prepared from a vaginal swab. The vaginal swab will be rolled onto a glass slide, heat fixed, and Gram stained. The Gram stained slide is examined under an oil immersed microscope head (x 1,000 magnification) for bacterial morphotypes. Additionally, the Gram stained slide will be examined under x 1,000 magnification for the presence of neutrophils. Vaginal neutrophils will be counted in 5 non-consecutive fields without cervical mucus and averaged. Only areas that have a single layer of epithelial cells will be evaluated. In our laboratory, vaginal smears (n=50) were evaluated by five different readers in a blinded fashion. The degree of agreement with respect to those smears having  $\leq 5$  or  $> 5$  neutrophils per oil-field was 95%, suggesting that the inter-observer reproducibility of neutrophil detection performed in this manner.<sup>99</sup>

**ii. Culture for Facultative and Anaerobic Vaginal Flora**

Vaginal swabs are inserted into a Port-A-Cul anaerobic transport tube (\_\_\_\_\_). The sample is transported to the laboratory within 24 hours. Upon arrival, the specimen is logged into the computer database. Inside an anaerobic chamber, the specimen is removed from the tube and plated onto Columbia sheep blood agar (BA), two human bi-layer tween agar plates (HBT), Brucella sheep blood agar (BR), A8 agar, and Mycoplasma broth (M broth). A smear on a slide is made for a Gram stain. The BA, one HBT, A8, and M broth are incubated in 6% CO<sub>2</sub>, 36° C for 48 hours and the BR and one HBT are incubated in an anaerobic chamber (5% hydrogen, 5% CO<sub>2</sub>, and 90% nitrogen, at 36° C for 4 – 7 days. All bacteria are quantified and identified according to standard methods.<sup>134</sup>

**iii. N. gonorrhoeae, C. trachomatis, and T. vaginalis**

The BDProbeTec ET C. trachomatis and N. gonorrhoeae amplified DNA assays, when used with the BDProbeTec ET system, uses homogeneous Strand Displacement Amplification (SDA) technology as the amplification method and fluorescent energy transfer (ET) as the detection method to test for the presence of C. trachomatis and N. gonorrhoeae DNA in clinical specimens. The DNA assays are based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescent-labeled detector probe. The SDA reagents are dried in two separate disposable microwell strips. The processed sample is added to the priming microwell that contains the amplification primers, fluorescent-labeled detector probe, and other reagents necessary for amplification. DNA amplification techniques will also be used for the identification of T. vaginalis. Amplicor PCR (\_\_\_\_\_) will be used on clinical specimens brought to the laboratory in PCR transport medium.

**g. Laboratory Specimens and Measurement for Urinary Cotinine**

**Urine Collection:** Urine will be obtained from subjects at the initial study visit. Urine will be obtained as a clean-catch specimen.

**Cotinine Measurement:** Cotinine levels will be determined by ELISA using a commercially-available kit from BioQuant (San Diego, CA) as follows. A 15 $\mu$ L aliquot of urine is incubated with a 100  $\mu$ L dilution of horseradish peroxidase labeled cotinine derivative in micro-well plates, coated with fixed amounts of oriented high affinity purified polyclonal antibody. The wells are washed thoroughly and a chromogenic substrate added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of cotinine in the sample. The technique is sensitive to 20 ng/mL cotinine.

**h. Collection of blood, extraction of plasma, and measurement of Plasma CRH** Blood will be collected into chilled glass tubes containing EDTA (1mg/mL blood) and aprotinin (500 kIU/mL blood) and will be centrifuged at 4°C. Plasma will be stored at -70°C until extraction. Plasma will be extracted with Sep-Pak C-18 cartridges (Waters Assoc, Milford, MA) for assay of immunoreactive CRH. Acidified plasma will be loaded onto columns previously activated with 60% acetonitrile in 1% trifluoroacetic acid. The absorbed peptide will be eluted with 3 mL acetonitrile, trifluoroacetic acid buffer and eluent will be dried in a speed vacuum concentrator. The dried extract will be stored at -80°C and will be resuspended in radioimmunoassay buffer at the time of assay, Plasma CRH will be measured by specific double-antibody radioimmunoassay utilizing antisera and iodinated peptides. ( Peninsula Laboratories; Belmont, CA)

**i. Collection of blood, extraction of plasma, and measurement of platelet catecholamines:** Blood will be extracted into 2-5ml glass tubes containing EDTA and will be centrifuged at 0°C at 1000rpms for 10 minutes to obtain the plasma. The platelets are separated from the plasma (centrifuged at 3400rpms for 10 minutes at 0°C) and are washed (lysis buffer; 155 mM NH<sub>4</sub>Cl, 10mM KHCO<sub>3</sub>, 0.1 mM EDTA) to remove red blood cells. The platelets are resuspended in lysis buffer, and the wash and centrifuge process is repeated. The pellet of platelets is stored at -80°C until assay for catecholamines. Frozen pellets are thawed in ice with 750  $\mu$ L PCA and resuspended by vortexing. The platelet suspension is centrifuged at 3000 rpm for 25 minutes at 5°C. The supernatant is added to 30mg of alumina and let sit for 18 minutes. The mixture is centrifuged for 1 minute at 14,000 rpm and the resulting supernatant is removed. The alumina is washed twice with 1ml ddH<sub>2</sub>O: 1M Tris/0.2M EDTA, pH 8.6 (1:40 dilution) and recentrifuged after each wash for 1 minute at 14,000 rpm. 150 $\mu$ L cold 0.1N PCA is added to the alumina. The mixture is mixed for 1 minute and recentrifuged for 1 minute at 14,000 rpm. The supernatant is transferred to microsedimentation tubes and centrifuged for 2 minutes at 3,000 rpm to sediment the alumina. The supernatant is transferred to a glass autosampler and inserted (85 $\mu$ L) onto a HPLC (Beckman Ultrashpere C<sub>15</sub> ODS 5 $\mu$ m column, 4.6mm x 15 cm with Beckman 125 pump and 507e refrigerated autosampler, @ 5°C. Catecholamine concentrations are determined from plasma by HPLC with electrochemical detection and an alumina extraction procedure.<sup>135</sup> The interassay coefficient of variation for epinephrine (E) is 6.8%, and the coefficient of variation for norepinephrine (NE) is 7.1%.

**Statistical analysis****a. Preliminary Analysis**

Ongoing preliminary analysis monitored twice yearly during the study will include: (1) the proportion of white and African-American women enrolled and with follow-up, (2) the proportion of women producing the 10<sup>th</sup> percentile, 25<sup>th</sup> percentile, 75<sup>th</sup> percentile, and 90<sup>th</sup> percentile of each of the pro- and anti-inflammatory cervical cytokines at each study visit separately, (3) the distribution of gene polymorphisms for each of the pro- and anti-inflammatory cytokines, (4) and the number of weeks between visit one and two and the difference in cytokine levels between visit one and two. (5) completeness and distribution of psychological stress measures (6) inter- and intra-assay reliability of biochemical assays.

We will additionally monitor the frequency of women smoking during pregnancy, quitting between the first and second visit, and the agreement between self-report measure of smoking and cotinine determination of smoking exposure. We will determine the proportion of women who are positive for BV, *N. gonorrhoeae*, *C. trachomatis*, *T.vaginalis*, group B *Streptococcus*, *E.coli*, and hydrogen peroxide positive and negative *Lactobacillus* spp. Monitoring these frequencies during the study will be done to evaluate the function of our collaborations with the laboratory arms of the project team. Monitoring of these laboratory measures will give us the opportunity to identify problems with obtaining specimens, transport to the laboratory, and laboratory supplies and procedures.

Preliminary analyses will also include determining the parametric shape of the concentrations of pro- and anti-inflammatory cytokines. Our experience with the pilot data presented in section C of this proposal suggests that cytokine values are not normally distributed; there is no transformation that brings them close to a normal distribution, and they do not fit any other commonly known distribution. The parametric shape of the cytokine variables will influence the type of analysis to be used at the end of the study to determine the study findings. At the end of the study, comparison of cytokine concentrations with respect to categorical variables such as race, preterm delivery, intraamniotic infection, gene polymorphisms, and environmental exposures will be performed using non-parametric tests and percentiles: 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup> and 90<sup>th</sup>.<sup>136</sup> We have chosen these percentiles to evaluate because we have no *a priori* information on how cytokine values should be discretized. Knapp and Miller recommend evaluating percentiles of variables for use in analysis when the variable does not conform to a distribution. We have chosen percentiles rather than Receiver Operating Characteristics Curves (ROC) because ROC curves presume that the variable being tested will have excellent sensitivity and specificity. We do not expect high or low concentrations of cytokines to have high sensitivity, we are evaluating them for a statistical association and a point estimate of risk in combination with other factors associated with preterm delivery not as a stand-alone screening test.

**Analyses in Support of Specific Aim 1:**

Specific Aim 1: To determine if maternal prenatal psychological stress and the biological stress response alter the lower genital tract inflammatory milieu in pregnancy, as represented by concentrations of important pro- and anti-inflammatory cytokines, vaginal pH, and vaginal neutrophils.

The exposures of interest are the various measures of psychological stress and physiological stress. The outcomes of interest are concentrations of important pro- and anti-inflammatory cytokines, vaginal pH, and vaginal neutrophils. The first step in the analysis will be to evaluate the overall distribution of the continuous variables in order to inform the decision to utilize parametric or non-parametric tests.

Preliminary analyses will also include determining the parametric shape of the PSS, Hassles, and SRE scores. We will determine if there is a transformation that brings these to a normal distribution, or if they fit any other commonly known distribution. The parametric shape of the scores will influence the type of analysis to be used at the end of the study to determine the study findings. In our experience, these scores typically are transformed best with logarithmic or square transformation. Cytokine concentrations are typically not Gaussian, and, in our experience are not readily transformed to a normal distribution. We will categorize cytokine concentrations into percentiles: 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup> and 90<sup>th</sup>. We have chosen these percentiles to evaluate because we have no *a priori* information on how these concentrations should be discretized. Vaginal pH and vaginal neutrophil number will be evaluated as categorical variables. The categories of vaginal pH are defined customarily by the categories noted on pH paper. Vaginal neutrophils are counted in 5 non-consecutive fields without cervical mucus and averaged. This average number of neutrophils will be categorized as 0, <1, 1, 1-3, 3-5, and >5 per oil-field. We will also evaluate pH and neutrophils, categorized as in our previous publications (Appendix A) and Preliminary Data. We will use discriminate function analyses and logistic regression to determine if the scale variables of stress discriminate between women with each category of neutrophils, pH, and categorized cytokine concentration, univariately and in a multivariable model. Other variables such as smoking, illegal drug use during pregnancy, nutritional status, BV or sexually transmitted infections, and reproductive history will be evaluated as potential effect modifiers or confounders. If they are not effect modifying variables and they alter the risk of preterm delivery associated with altered lower tract immunity or stress, they will be included in logistic models as controlling variables. (91, 92)

Since we will ascertain psychological and physiological stress at several time points over pregnancy, we plan time-dependent and matched analyses to see if changes over pregnancy provide any additional information regarding the relationship of stress with lower genital tract immunity.

**Analyses in Support of Specific Aim 2**

Specific Aim 2: To determine if known functional cytokine promoter polymorphism status alters the impact of stress on lower genital tract inflammation, thus establishing a gene-environment interaction.

All polymorphism genotype data will be evaluated for Hardy-Weinberg equilibrium prior to use in statistical modeling. Based on the published functional polymorphism literature, all polymorphism genotypes will be categorized into functional categories. Each functional cytokine promoter polymorphism gene phenotype will be included in the logistic regression models generated through the analyses in support of Specific Aim 1. These analyses will also be stratified by polymorphism status to determine if the magnitude of the influence of stress variables on lower tract immunity variables differs by genotype.

**Analyses in Support of Specific Aim 3**

Specific Aim 3: To evaluate the magnitude of psychological stress and its physiological response by race. We further aim to assess the interactive contribution of cytokine promoter polymorphism status and stress to the lower genital tract immunological milieu, stratified by race.

We will describe the psychological and physiological stress variables with respect to central tendency and distribution, overall and stratified by race. Since each subject will have each measure at several time points, we cannot assume independence of these data. We will perform time-dependent and paired analyses to see if the change in stress over gestation differs by race.

**Estimates of Statistical Power for Primary Aim**

The primary purpose of this proposal is to ascertain the relationship between stress and altered lower genital tract immunity. For estimate of cohort size, we estimated the frequency of exposure to high psychological stress (by PSS) at 25% based on our preliminary data in our population and based on the literature. We assumed that 10% of our population would be exposed to high physiologic stress as represented platelet catecholamine assay and CRH based on our preliminary data and a review of the literature. With respect to altered lower genital tract immunity, based on our preliminary data we estimate that 21.5% of women will have hyporesponsive cytokine concentrations (2 or 3 cytokines < 25<sup>th</sup> percentile)<sup>97</sup> and that 8.3% of women will have vaginal pH  $\geq$  5.0 and vaginal neutrophils > 5 per oil-field.<sup>99</sup> We assume  $\alpha=0.05$  for sample size estimates. The table, below, describes the power of cohort of varying sizes would have to detect a relationship between stress (either physiologic or psychological) and altered lower genital tract immunity (represented either by cytokine concentrations or by vaginal pH & PMNs) over a range of odds ratios.

**Table 14. Power table for cohort, based on cohort size**

		Physiologic stress to cytokines	Psychological stress to cytokines		Physiologic stress to vaginal pH & PMNs	Psychological stress to vaginal pH & PMNs
Cohort size	Odds ratio detectable			Odds ratio detectable		
1000	1.3	68%	61%	2.0	75%	67%
	1.5	96%	93%	2.3	88%	83%
	1.7	99%	99%	2.5	93%	90%
900	1.3	65%	57%	2.0	72%	64%
	1.5	94%	90%	2.3	86%	80%
	1.7	99%	99%	2.5	92%	88%
800	1.3	61%	54%	2.0	68%	59%
	1.5	92%	87%	2.3	83%	76%
	1.7	99%	98%	2.5	89%	84%
700	1.3	56%	49%	2.0	64%	55%
	1.5	89%	83%	2.3	78%	71%
	1.7	98%	97%	2.5	86%	80%
600	1.3	51%	45%	2.0	59%	51%
	1.5	84%	78%	2.3	74%	66%
	1.7	97%	95%	2.5	82%	75%

Based on these estimates, a cohort of 800 women provides reasonable power to address our main hypothesis. This sample size is entirely feasible to enroll over the course of the study.

#### **h. Time schedule for study**

**Months 1-3:** Identify new employees. Hire and train new employees. Finalize copies of data collection instruments. **Months 4-54:** Enroll and follow subjects. **Months 55-60:** Identify and collect all outstanding laboratory data. Perform final data analysis.

**i. Strengths and Limitations Strengths:** This study is significant for several reasons. First, the problem addressed in this study, the racial disparity in preterm birth, is a problem of tremendous medical, economic, and national public health importance. Second, we are exploring the connection between stress, a critical biobehavioral exposure with protean health effects, with the immune predisposition to preterm birth. Identifying this connection will provide terrific insight into mechanisms of prematurity and create new avenues of investigation for future descriptive and interventional studies. Importantly, our study will evaluate the stress-immune connection not in isolation, but in the context of the complex world of biology, individual behavior, and community factors. The notion that the relationship between stress and reproductive immunity is modified by the genetic regulation of inflammation is wholly plausible and yet novel. Racial disparities in health outcomes, high-risk behaviors, and circumstances of life in \_\_\_\_\_ provide a solid population in which to perform such a study, and in whom future interventional studies are desperately needed.

**Limitations:** Our ascertainment of psychological and physiological stress is limited by our measures. We have attempted to comprehensively assess psychological stress with a number of validated measures, but there may be aspects of psychological stress not addressed with our techniques. The same is true with our "biomarkers" of physiologic stress. CRH and platelet catecholamines are valid, established methods of assessing physiologic stress. By evaluating both, we hope to gain a broader view of the biologic response to psychological stress. Still, the breadth of that view may be incomplete, as limited by these particular markers. Another limitation of our proposal is an inability to gather data on racial or ethnic groups other than Caucasian-Americans or African-Americans. The population of \_\_\_\_\_ does not afford us the opportunity to evaluate other ethnic groups or evaluate groups based on time since immigration as a measure of acculturation, as might be possible in other cities in the US.

**E. HUMAN SUBJECTS****1. Study Population****a. Eligible subjects**

We will recruit 800 healthy women from our outpatient clinics at \_\_\_\_\_ and the private offices of the Maternal-Fetal Medicine group. Criteria for eligibility include:

1. pregnancy prior to 20 weeks gestation
2. singleton gestation

Exclusion criteria include:

1. vaginal bleeding
2. fetal anomalies
3. known thrombophilias
4. diabetes-pre-gestation
5. chronic hypertension requiring medication
6. documented cervical funneling
7. current or planned cervical cerclage
8. Immuno-compromise (HIV positive, use of systemic steroids within six months, use of post-transplant immunosuppressive medication)
9. Autoimmune disease (inflammatory bowel disease, Systemic Lupus Erythematosus, rheumatoid arthritis, scleroderma)

**b. Characteristics of patient population who deliver at \_\_\_\_\_**

The characteristics of women delivering at \_\_\_\_\_ are summarized in the table below by site of care.

**Table 18. Characteristics of women who deliver at \_\_\_\_\_**

Deliveries	Clinic 1998-1999		MFM Office 1998-1999	
	n	%	n	%
Cesarean	456	16.7%	348	32.6%
Vaginal	2267	83.3%	720	67.4%
<b>Race</b>				
White	1251	45.9%	915	85.7%
Black	1378	50.6%	109	10.2%
Asian	1		1	
Hispanic	3		1	
Mixed/Other	87	3.2%	25	2.4%
<b>Maternal Education</b>				
Less than high school	625	23.0%	75	7.0%
High school	1171	43.0%	391	36.6%
Some college	586	21.5%	239	22.4%
College graduate	146	5.4%	279	26.1%
Missing	195	7.2%	84	7.9%
<b>Social Status</b>				
Married	314	11.5%	500	46.8%
Unmarried	2409	88.5%	568	53.2%
<b>Insurance</b>				
Commercial	327	12.0%	770	72.4%
Medicaid	2326	85.4%	287	26.9%
Self	33	1.2%	6	0.6%
Missing	38	1.4%	5	0.5%

**c. Gender and racial exclusion**

Men will not be recruited, as the study requires pregnant women. We will not exclude subjects on the basis of race. It is important in the design of our study to include equal and sufficient numbers of African-American and white women.

**d. Participation of Children**

We will not exclude children from this investigation as long as the inclusion and exclusion criteria are met. The perinatal and obstetrical services at \_\_\_\_\_ routinely provide a full complement of care to

pregnant minors. These women will not be excluded from study participation based on their age. Parental assent will be obtained for those children who are not emancipated minors based on a prior delivery.

## 2. Sources of Research Material

Data will consist of biological specimens, pregnancy outcome data, and a questionnaire. Pregnancy outcome data will be collected from the medical record at the time of delivery. Biological fluids will include placentas for pathologic examination, maternal blood for determination of cytokine polymorphisms, maternal vaginal secretions for measurement of cytokines (both pro and anti-inflammatory), and vaginal secretions for microorganisms and determination of bacterial vaginosis. These data will be obtained specifically for research purposes related to this proposal only. Patient confidentiality will be high priority and subjects will only be identified by case number and not by name whenever possible. Some material or data will be obtained as part of patient care and will not be specific for this project. For example, material relating to pregnancy outcome will be recorded in the patient's medical record. That information extracted from the medical record will only be used for this study. Biological samples such as urine are obtained as part of routine prenatal care, particularly during the first prenatal visit. Likewise, biological specimens may be obtained in women who are symptomatic of other infections.

In addition to the biological fluids and data from the chart, subjects will be interviewed regarding food intake. A reduced Block Food Frequency questionnaire will be administered once during the pregnancy. The interview will assess reproductive and sexual history, alcohol and illegal drug use, tobacco use, and race.

## 3. Recruitment and Consent Procedures

We will recruit eligible patients from our resident clinics, our neighborhood outreach clinics and from the Maternal-Fetal Medicine offices housed at \_\_\_\_\_ The recruiting research nurse will screen the charts of all new obstetrical patients in order to identify eligible subjects. Eligible subjects will be approached at the first prenatal visit and the study will be explained. A pamphlet describing the study will be given to the subjects and a phone number provided in order for the subjects to call for further information. The recruiter will seek out the eligible subject at subsequent visits to determine her level of interest. We will offer a small incentive (\$50) to those who consent to participate. Consenting subjects will be followed longitudinally throughout pregnancy. Consent will be obtained only during the course of routine prenatal care. Patients will be told that the purpose of this study is to evaluate the relationship of the immune system to preterm birth. Once consent is obtained, consent forms will be filed with the Institutional Review Board. The Institutional Review Board will have approved the consent forms.

## 4. Potential Risks

### a. Questionnaires & Interviews

The Food Frequency questionnaire will be filled out by the study subject under the supervision of the research nurse. There is very little risk from this aspect of the study. The other questionnaire topics are stress, reproductive history, alcohol and illegal drug use, and sexual activity before and early in pregnancy. This sensitive information will be kept confidential by keeping the forms with study number only, keeping computer data files with study number only, and keeping the linked list of names to study number in a locked file cabinet. The file cabinets where forms are filed are in a locked cabinet within a locked room. Computers that contain patient data without identifiers have logon passwords.

### b. Biological fluids

There is little risk in pregnancy from a pelvic examination. Obtaining specimens from the cervical vaginal secretions is very commonly done in pregnancy and poses no risk to the pregnancy. Sterile swabs are used for obtaining samples for microbiological evaluation and similar swabs are used to obtain material for cytokine determination. Likewise, there is very little risk from obtaining a blood sample in order to determine the genetic polymorphisms for the cytokines. The venipuncture will produce some discomfort and may produce some discoloration at the site of puncture.

## 4. Minimizing Risks

The issue of confidentiality of cytokine polymorphisms for maternal blood is important and will receive special attention. The risk of confidentiality will be kept to a minimum by utilizing case numbers where feasible rather than the patient's name. The genetic polymorphism data will not be utilized for anything other than this study and the consent form will make clear how this material is to be obtained and used. Likewise, the microbiological information will be kept confidential, but will be provided to the healthcare provider when evidence of vaginal infection exists. All positive lab results will be reported to the principal investigator who will notify the patient's attending physician. Collected data will be reviewed anonymously to assure the quality of those data. The cytokine polymorphisms are not yet known to be predictive of disease, therefore, these data do not require report to the patient.

## 5. Risk/Benefit Ratio

The women participating in this study will have little to gain personally during the index pregnancy. However, the risk of participation in this study is very small and the benefit to other women and the study subject in a subsequent pregnancy is substantial. By characterizing the relationship between stress, the immune system and preterm birth, a major public health issue will be addressed. If it can be demonstrated that certain environmental or genetic polymorphisms predispose women to infection complications during pregnancy, the possibility of improving the health of pregnant women would be enhanced. Thus, participation in this study and the benefits gained from that will far outweigh the risks.

## F. Vertebrate Animals

None used

**G. Literature Cited**

1. Rowley DL. Research issues in the study of very low birthweight and preterm delivery among African-American women. *Journal of the National Medical Association*. 1994;86:761-4.
2. Starfield B, Shapiro S, Weiss J, et al. Race, family income, and low birth weight [see comments]. *American Journal of Epidemiology* 1991;134:1167-74.
3. Virji SK, Cottington E. Risk factors associated with preterm deliveries among racial groups in a national sample of married mothers. *American Journal of Perinatology* 1991;8:347-53.
4. Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Risk factors accounting for racial differences in the rate of premature birth. *New England Journal of Medicine* 1987;317:743-8.
5. McGrady GA, Sung JF, Rowley DL, Hogue CJ. Preterm delivery and low birth weight among first-born infants of black and white college graduates. *American Journal of Epidemiology* 1992;136:266-76.
6. Adams MM, Elam-Evans LD, Wilson HG, Gilbertz DA. Rates of and factors associated with recurrence of preterm delivery. *Jama* 2000;283:1591-6.
7. Alexander GR, Tompkins ME, Altekruze JM, Hornung CA. Racial differences in the relation of birth weight and gestational age to neonatal mortality. *Public Health Reports* 1985;100:539-47.
8. Gardner MO, Goldenberg RL. The influence of race and previous pregnancy outcome on outcomes in the current pregnancy. *Seminars in Perinatology* 1995;19:191-6.
9. Hessol NA, Fuentes-Afflick E, Bacchetti P. Risk of low birth weight infants among black and white parents. *Obstetrics & Gynecology*. 1998;92:814-22.
10. Kogan MD, Kotelchuck M, Alexander GR, Johnson WE. Racial disparities in reported prenatal care advice from health care providers [see comments]. *American Journal of Public Health* 1994;84:82-8.
11. Stancil TR, Hertz-Picciotto I, Schramm M, Watt-Morse M. Stress and pregnancy among African-American women. *Paediatric and Perinatal Epidemiology*. 2000;14:127-35.
12. Zhang J, Bowes WA, Jr. Birth-weight-for-gestational-age patterns by race, sex, and parity in the United States population. *Obstetrics & Gynecology*. 1995;86:200-8.
13. David RJ, Collins JW, Jr. Differing birth weight among infants of U.S.-born blacks, African-born blacks, and U.S.-born whites.[see comment]. *New England Journal of Medicine*. 1997;337:1209-14.
14. Palermo GD, Neri QV, Hariprashad JJ, Davis OK, Veeck LL, Rosenwaks Z. ICSI and its outcome. *Seminars in Reproductive Medicine* 2000;18:161-9.
15. Kramer MS, Goulet L, Lydon J, et al. Socio-economic disparities in preterm birth: causal pathways and mechanisms. *Paediatric and Perinatal Epidemiology*. 2001;15:104-23.
16. Lockwood CJ. Stress-associated preterm delivery: the role of corticotropin-releasing hormone. *American Journal of Obstetrics & Gynecology*. 1999;180:S264-6.
17. Majzoub JA, McGregor JA, Lockwood CJ, Smith R, Taggart MS, Schulkin J. A central theory of preterm and term labor: putative role for corticotropin-releasing hormone. *American Journal of Obstetrics & Gynecology*. 1999;180:S232-41.
18. Wadhwa PD, Sandman CA, Porto M, Dunkel-Schetter C, Garite TJ. The association between prenatal stress and infant birth weight and gestational age at birth: a prospective investigation. *American Journal of Obstetrics & Gynecology*. 1993;169:858-65.
19. Wadhwa PD, Porto M, Garite TJ, Chicx-DeMet A, Sandman CA. Maternal corticotropin-releasing hormone levels in the early third trimester predict length of gestation in human pregnancy. *American Journal of Obstetrics & Gynecology*. 1998;179:1079-85.
20. Wadhwa PD, Culhane JF, Rauh V, et al. Stress, infection and preterm birth: a biobehavioural perspective. *Paediatric and Perinatal Epidemiology*. 2001;15:17-29.
21. Field T, Sandberg D, Quetel TA, Garcia R, Rosario M. Effects of ultrasound feedback on pregnancy anxiety, fetal activity, and neonatal outcome. *Obstetrics & Gynecology*. 1985;66:525-8.

22. Gorsuch RL, Key MK. Abnormalities of pregnancy as a function of anxiety and life stress. *Psychosomatic Medicine*. 1974;36:352-62.
23. Barglow P, Hatcher R, Berndt D, Phelps R. Psychosocial childbearing stress and metabolic control in pregnant diabetics. *Journal of Nervous & Mental Disease*. 1985;173:615-20.
24. Sandler IN, Lakey B. Locus of control as a stress moderator: the role of control perceptions and social support. *American Journal of Community Psychology*. 1982;10:65-80.
25. Newton RW, Hunt LP. Psychosocial stress in pregnancy and its relation to low birth weight. *British Medical Journal Clinical Research Ed.*. 1984;288:1191-4.
26. Sandman CA, Wadhwa PD, Chicz-DeMet A, Dunkel-Schetter C, Porto M. Maternal stress, HPA activity, and fetal/infant outcome. *Annals of the New York Academy of Sciences*. 1997;814:266-75.
27. Crandon AJ. Maternal anxiety and neonatal wellbeing. *Journal of Psychosomatic Research*. 1979;23:113-5.
28. Crandon AJ. Maternal anxiety and obstetric complications. *Journal of Psychosomatic Research*. 1979;23:109-11.
29. Sikkema JM, Robles de Medina PG, Schaad RR, et al. Salivary cortisol levels and anxiety are not increased in women destined to develop preeclampsia. *Journal of Psychosomatic Research*. 2001;50:45-9.
30. Lobel M, DeVincent CJ, Kaminer A, Meyer BA. The impact of prenatal maternal stress and optimistic disposition on birth outcomes in medically high-risk women. *Health Psychology*. 2000;19:544-53.
31. Schell LM, Ravenscroft J, Czerwinski SA, Stark AD, Grattan WA, Gordon M. Social support and adverse pregnancy outcome in a high-risk population. *Journal of Public Health Management & Practice*. 1997;3:13-26.
32. Schell LM. Culture as a stressor: a revised model of biocultural interaction. *American Journal of Physical Anthropology*. 1997;102:67-77.
33. Ratliff CJ, Kane J. Predictors for altering caffeine consumption during stress. *Addiction Behavior* 1995;20:509-516.
34. Steptoe A, Wardle J, Pollard TM, Canaan L, Davies GJ. Stress, social support and health-related behavior: a study of smoking, alcohol consumption and physical exercise. *Journal of Psychosomatic Research*. 1996;41:171-80.
35. Verlander LA, Benedict JO, Hanson DP. Stress and sleep patterns of college students. *Perceptual & Motor Skills*. 1999;88:893-8.
36. Pagel MD, Smilkstein G, Regen H, Montano D. Psychosocial influences on new born outcomes: a controlled prospective study. *Social Science & Medicine*. 1990;30:597-604.
37. DaCosta D, Brender W, Larouche J. A prospective study of the impact of psychosocial and lifestyle variables on pregnancy complications. *Journal of Psychosomatic Obstetrics and Gynaecology* 1998;19:28-47.
38. Moynihan JA. Mechanisms of stress-induced modulation of immunity. *Brain, Behavior, & Immunity*. 2003;17:S11-6.
39. Pruett SB. Quantitative aspects of stress-induced immunomodulation. *International Immunopharmacology*. 2001;1:507-20.
40. Wadhwa PD, Culhane JF, Rauh V, Barve SS. Stress and preterm birth: neuroendocrine, immune/inflammatory, and vascular mechanisms. *Maternal & Child Health Journal*. 2001;5:119-25.
41. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis.[erratum appears in JAMA 1992 Jul 8;268(2):200]. *Jama*. 1992;267:1244-52.
42. Challis JRG, Matthews SG, Gibb W, Lye SJ. Endocrine and paracrine regulation of birth at term and preterm. *Endocrine Reviews* 2000;21:514-50.
43. Thorburn GD, Challis JR. Endocrine control of parturition. *Physiological Reviews*. 1979;59:863-918.
44. Gu W, Jones CT, Parer JT. Metabolic and cardiovascular effects on fetal sheep of sustained reduction of uterine blood flow. *Journal of Physiology*. 1985;368:109-29.

45. Powell LH, Lovallo WR, Matthews KA, et al. Physiologic markers of chronic stress in premenopausal, middle-aged women. *Psychosomatic Medicine*. 2002;64:502-9.
46. Blandini F, Martignoni E, Sances E, Bono G, Nappi G. Combined response of plasma and platelet catecholamines to different types of short-term stress. *Life Sciences*. 1995;56:1113-20.
47. Carstensen E, Ramaiya K, Denver E, Mohamed-Ali V, Yudkin JS. The contribution of the sympathoadrenomedullary system to the etiology of essential hypertension: a study using plasma and platelet catecholamine concentrations. *Journal of Clinical Endocrinology & Metabolism*. 1995;80:455-60.
48. Strobel G, Friedmann B, Jost J, Bartsch P. Plasma and platelet catecholamine and catecholamine sulfate response to various exercise tests.[erratum appears in *Am J Physiol* 1995 Jun;268(6 Pt 1):section E followi]. *American Journal of Physiology*. 1994;267:E537-43.
49. Van Faassen I, Popp-Snijders C, Nauta JJ, van Zijderveld G, van Doornen LJ, Tilders FJ. Platelet catecholamine contents as related to trait anxiety and aerobic fitness. *American Journal of Physiology*. 1992;263:E245-9.
50. Wilson AP, Smith CC, Prichard BN, Betteridge DJ. Platelet catecholamines and platelet function in normal human subjects. *Clinical Science*. 1987;73:99-103.
51. Smith CC, Curtis LD, Delamothe AP, Prichard BN, Betteridge DJ. The distribution of catecholamines between platelets and plasma in normal human subjects. *Clinical Science*. 1985;69:1-6.
52. Hobel CJ, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CP. Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. *American Journal of Obstetrics & Gynecology*. 1999;180:S257-63.
53. Palermo Neto J, Massoco CO, Favare RC. Effects of maternal stress on anxiety levels, macrophage activity, and Ehrlich tumor growth. *Neurotoxicology & Teratology* 2001;23:497-507.
54. Palermo-Neto J, de Oliveira Massoco C, Robespierre de Souza W. Effects of physical and psychological stressors on behavior, macrophage activity, and Ehrlich tumor growth. *Brain, Behavior, & Immunity*. 2003;17:43-54.
55. Coe CL, Kramer M, Kirschbaum C, Netter P, Fuchs E. Prenatal stress diminishes the cytokine response of leukocytes to endotoxin stimulation in juvenile rhesus monkeys. *Journal of Clinical Endocrinology & Metabolism*. 2002;87:675-81.
56. Keelan JA, Sato T, Mitchell MD. Interleukin (IL)-6 and IL-8 production by human amnion: regulation by cytokines, growth factors, glucocorticoids, phorbol esters, and bacterial lipopolysaccharide. *Biology of Reproduction*. 1997;57:1438-44.
57. Elenkov IJ, Chrousos GP. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Annals of the New York Academy of Sciences*. 2002;966:290-303.
58. Abraham E, Kaneko DJ, Shenkar R. Effects of endogenous and exogenous catecholamines on LPS-induced neutrophil trafficking and activation. *American Journal of Physiology*. 1999;276:L1-8.
59. Riese U, Brenner S, Docke WD, et al. Catecholamines induce IL-10 release in patients suffering from acute myocardial infarction by transactivating its promoter in monocytic but not in T-cells. *Molecular & Cellular Biochemistry*. 2000;212:45-50.
60. Cohen M, Klein E, Kuten A, Fried G, Zinder O, Pollack S. Increased emotional distress in daughters of breast cancer patients is associated with decreased natural cytotoxic activity, elevated levels of stress hormones and decreased secretion of Th1 cytokines. *International Journal of Cancer*. 2002;100:347-54.
61. Petraglia F, Aguzzoli L, Florio P, et al. Maternal plasma and placental immunoreactive corticotrophin-releasing factor concentrations in infection-associated term and pre-term delivery. *Placenta*. 1995;16:157-64.
62. Arck PC. Stress and pregnancy loss: role of immune mediators, hormones and neurotransmitters. *American Journal of Reproductive Immunology*. 2001;46:117-23.
63. Arck PC, Rose M, Hertwig K, Hagen E, Hildebrandt M, Klapp BF. Stress and immune mediators in miscarriage. *Human Reproduction*. 2001;16:1505-11.

64. Hillier SL, Krohn MA, Cassen E, Easterling TR, Rabe LK, Eschenbach DA. The role of bacterial vaginosis and vaginal bacteria in amniotic fluid infection in women in preterm labor with intact fetal membranes. *Clin Infect Dis* 1995;20 Suppl 2:S276-8.
65. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group [see comments]. *N Engl J Med* 1995;333:1737-42.
66. Din-Dzietham R, Nembhard WN, Collins R, Davis SK. Perceived stress following race-based discrimination at work is associated with hypertension in African-Americans. The metro Atlanta heart disease study, 1999-2001. *Social Science & Medicine*. 2004;58:449-61.
67. Sellers RM, Caldwell CH, Schmeelk-Cone KH, Zimmerman MA. Racial identity, racial discrimination, perceived stress, and psychological distress among African American young adults. *Journal of Health & Social Behavior*. 2003;44:302-17.
68. Clark R. Parental history of hypertension and coping responses predict blood pressure changes in black college volunteers undergoing a speaking task about perceptions of racism. *Psychosomatic Medicine*. 2003;65:1012-9.
69. Kwate NO, Valdimarsdottir HB, Guevarra JS, Bovbjerg DH. Experiences of racist events are associated with negative health consequences for African American women. *Journal of the National Medical Association*. 2003;95:450-60.
70. Collins JW, Jr., David RJ, Symons R, Handler A, Wall SN, Dwyer L. Low-income African-American mothers' perception of exposure to racial discrimination and infant birth weight.[see comment]. *Epidemiology*. 2000;11:337-9.
71. Collins JW, Jr., David RJ. Urban violence and African-American pregnancy outcome: an ecologic study. *Ethnicity & Disease*. 1997;7:184-90.
72. Zapata C, Rebolledo A, Atalah E. The influence of social and political violence on the risk of pregnancy complications. *American Journal of Public Health* 1992;82:685-90.
73. Acevedo-Garcia D, Lochner KA, Osypuk TL, Subramanian SV. Future directions in residential segregation and health research: a multilevel approach. *American Journal of Public Health*. 2003;93:215-21.
74. Acevedo-Garcia D. Zip code-level risk factors for tuberculosis: neighborhood environment and residential segregation in New Jersey, 1985-1992. *American Journal of Public Health*. 2001;91:734-41.
75. Acevedo-Garcia D. Residential segregation and the epidemiology of infectious diseases. *Social Science & Medicine*. 2000;51:1143-61.
76. Jackson SA, Anderson RT, Johnson NJ, Sorlie PD. The relation of residential segregation to all-cause mortality: a study in black and white. *American Journal of Public Health*. 2000;90:615-7.
77. Fang J, Madhavan S, Bosworth W, Alderman MH. Residential segregation and mortality in New York City. *Social Science & Medicine*. 1998;47:469-76.
78. Hart KD, Kunitz SJ, Sell RR, Mukamel DB. Metropolitan governance, residential segregation, and mortality among African Americans. *American Journal of Public Health*. 1998;88:434-8.
79. Polednak AP. Trends in US urban black infant mortality, by degree of residential segregation. *American Journal of Public Health*. 1996;86:723-6.
80. van Deventer SJ. Cytokine and cytokine receptor polymorphisms in infectious disease. *Intensive Care Medicine* 2000;26:S98-102.
81. Suthanthiran M. The importance of genetic polymorphisms in renal transplantation. *Current Opinion in Urology* 2000;10:71-5.
82. Hutchinson IV, Pravica V, Perrey C, Sinnott P. Cytokine gene polymorphisms and relevance to forms of rejection. *Transplantation Proceedings* 1999;31:734-6.
83. Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1999;1:3-19.

84. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation* 1998;66:1014-20.
85. Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJ, Hutchinson IV. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. *European Journal of Immunogenetics* 1999;26:1-3.
86. John S, Turner D, Donn R, et al. Two novel biallelic polymorphisms in the IL-2 gene. *European Journal of Immunogenetics* 1998;25:419-20.
87. Hajeer AH, Lazarus M, Turner D, et al. IL-10 gene promoter polymorphisms in rheumatoid arthritis. *Scandinavian Journal of Rheumatology* 1998;27:142-5.
88. Lazarus M, Hajeer AH, Turner D, et al. Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus [see comments]. *Journal of Rheumatology* 1997;24:2314-7.
89. Freeman RB, Jr., Tran CL, Mattoli J, et al. Tumor necrosis factor genetic polymorphisms correlate with infections after liver transplantation. NEMC TNF Study Group. *New England Medical Center Tumor Necrosis Factor* [published erratum appears in *Transplantation* 1999 Dec 15;68(11):1823]. *Transplantation* 1999;67:1005-10.
90. Turner DM, Grant SC, Lamb WR, et al. A genetic marker of high TNF-alpha production in heart transplant recipients. *Transplantation* 1995;60:1113-7.
91. Sankaran D, Asderakis A, Ashraf S, et al. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. *Kidney International* 1999;56:281-8.
92. Wilson AG, di Giovine FS, Duff GW. Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. *Journal of Inflammation* 1995;45:1-12.
93. Danis VA, Millington M, Hyland V, Lawford R, Huang Q, Grennan D. Increased frequency of the uncommon allele of a tumour necrosis factor alpha gene polymorphism in rheumatoid arthritis and systemic lupus erythematosus. *Disease Markers* 1995;12:127-33.
94. Dizon-Townson DS, Major H, Varner M, Ward K. A promoter mutation that increases transcription of the tumor necrosis factor-alpha gene is not associated with preterm delivery. *American Journal of Obstetrics & Gynecology* 1997;177:810-3.
95. Simhan HN, Krohn MA, Zeevi A, Daftary A, Harger G, Caritis SN. Tumor necrosis factor-[alpha] promoter gene polymorphism -308 and chorioamnionitis. *Obstetrics & Gynecology* 2003;102:162-166.
96. Simhan HN, Krohn MA, Roberts JM, Zeevi A, Caritis SN. Interleukin-6 promoter -174 polymorphism and spontaneous preterm birth. *American Journal of Obstetrics & Gynecology* October 2003;189:915-918.
97. Simhan HN, Caritis SN, Krohn MA, Martinez de Tejada B, Landers DV, Hillier SL. Decreased cervical proinflammatory cytokines permit subsequent upper genital tract infection during pregnancy. *American Journal of Obstetrics & Gynecology*. 2003;189:560-7.
98. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of Clinical Microbiology* 1991;29:297-301.
99. Simhan HN, Caritis SN, Krohn MA, Hillier SL. Elevated vaginal pH and neutrophils are associated strongly with early spontaneous preterm birth. *American Journal of Obstetrics & Gynecology* October 2003;189:1150-1154.
100. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. *N Engl J Med* 1988;319:972-8.
101. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. *Obstet Gynecol* 1986;67:229-37.
102. Hitti J, Hillier SL, Agnew KJ, Krohn MA, Reisner DP, Eschenbach DA. Vaginal indicators of amniotic fluid infection in preterm labor. *Obstetrics & Gynecology* 2001;97:211-9.

103. Hillier SL, Witkin SS, Krohn MA, Watts DH, Kiviat NB, Eschenbach DA. The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection. *Obstet Gynecol* 1993;81:941-8.
104. Roberts AK, Monzon-Bordonaba F, Van Deerlin PG, et al. Association of polymorphism within the promoter of the tumor necrosis factor alpha gene with increased risk of preterm premature rupture of the fetal membranes. *American Journal of Obstetrics & Gynecology* 1999;180:1297-302.
105. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. *Annals of Internal Medicine* 1998;128:127-37.
106. El-Bastawissi AY, Williams MA, Riley DE, Hitti J, Krieger JN. Amniotic fluid interleukin-6 and preterm delivery: a review. *Obstetrics & Gynecology* 2000;95:1056-64.
107. Weiyan Z, Li W. Study of interleukin-6 and tumor necrosis factor-alpha levels in maternal serum and amniotic fluid of patients with premature rupture of membranes. *Journal of Perinatal Medicine* 1998;26:491-4.
108. Rizzo G, Capponi A, Vlachopoulou A, Angelini E, Grassi C, Romanini C. Interleukin-6 concentrations in cervical secretions in the prediction of intrauterine infection in preterm premature rupture of the membranes. *Gynecologic & Obstetric Investigation* 1998;46:91-5.
109. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. *Journal of Clinical Investigation* 1990;85:1392-400.
110. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *Journal of Clinical Investigation* 1998;102:1369-76.
111. Cox ED, Hoffmann SC, DiMercurio BS, et al. Cytokine polymorphic analyses indicate ethnic differences in the allelic distribution of interleukin-2 and interleukin-6. *Transplantation* 2001;72:720-6.
112. Tsang ML, Weatherbee JA. Cytokine assays and their limitations. *Alimentary Pharmacology & Therapeutics*. 1996;10:55-61; discussion 62.
113. Bienvenu J, Monneret G, Fabien N, Revillard JP. The clinical usefulness of the measurement of cytokines. *Clinical Chemistry & Laboratory Medicine*. 2000;38:267-85.
114. Whiteside TL. Cytokine assays. *Biotechniques*. 2002;Suppl:4-8, 10, 12-5.
115. Vignali DA. Multiplexed particle-based flow cytometric assays. *Journal of Immunological Methods*. 2000;243:243-55.
116. Prabhakar U, Eirikis E, Davis HM. Simultaneous quantification of proinflammatory cytokines in human plasma using the LabMAP assay. *Journal of Immunological Methods*. 2002;260:207-18.
117. Pickering JW, Martins TB, Schroder MC, Hill HR. Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for quantitation of antibodies to tetanus, diphtheria, and Haemophilus influenzae Type b. *Clinical & Diagnostic Laboratory Immunology*. 2002;9:872-6.
118. Biagini RE, Sammons DL, Smith JP, et al. Comparison of a multiplexed fluorescent covalent microsphere immunoassay and an enzyme-linked immunosorbent assay for measurement of human immunoglobulin G antibodies to anthrax toxins. *Clinical & Diagnostic Laboratory Immunology*. 2004;11:50-5.
119. Nelson KB, Grether JK, Dambrosia JM, et al. Neonatal cytokines and cerebral palsy in very preterm infants.[see comment]. *Pediatric Research*. 2003;53:600-7.
120. Yudin MH, Landers DV, Meyn L, Hillier SL. Clinical and cervical cytokine response to treatment with oral or vaginal metronidazole for bacterial vaginosis during pregnancy: a randomized trial. *Obstetrics & Gynecology*. 2003;102:527-34.
121. Iams JD, Goldenberg RL, Mercer BM, et al. The Preterm Prediction Study: recurrence risk of spontaneous preterm birth. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *Am J Obstet Gynecol* 1998;178:1035-40.
122. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *Journal of Health & Social Behavior*. 1983;24:385-96.

123. Cohen S, Hamrick N, Rodriguez MS, Feldman PJ, Rabin BS, Manuck SB. Reactivity and vulnerability to stress-associated risk for upper respiratory illness. *Psychosomatic Medicine*. 2002;64:302-10.
124. Holmes TMRRH. Schedule of Recent Events. VanderPlate, C., Aral, S. O., & Magdar, L 1988;7:159-168.
125. Kanner AD, Coyne JC, Schaefer C, Lazarus RS. Comparison of two modes of stress measurement: daily hassles and uplifts versus major life events. *Journal of Behavioral Medicine*. 1981;4:1-39.
126. DeLongis A, Folkman S, Lazarus RS. The impact of daily stress on health and mood: psychological and social resources as mediators. *Journal of Personality & Social Psychology*. 1988;54:486-95.
127. Folkman S, Lazarus RS, Dunkel-Schetter C, DeLongis A, Gruen RJ. Dynamics of a stressful encounter: cognitive appraisal, coping, and encounter outcomes. *Journal of Personality & Social Psychology*. 1986;50:992-1003.
128. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale.[see comment]. *British Journal of Psychiatry*. 1987;150:782-6.
129. Johanson R, Chapman G, Murray D, Johnson I, Cox J. The North Staffordshire Maternity Hospital prospective study of pregnancy-associated depression. *Journal of Psychosomatic Obstetrics & Gynecology*. 2000;21:93-7.
130. Cox JL, Chapman G, Murray D, Jones P. Validation of the Edinburgh Postnatal Depression Scale (EPDS) in non-postnatal women. *Journal of Affective Disorders*. 1996;39:185-9.
131. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Medical Care*. 1996;34:220-33.
132. Krieger N, Sidney S. Racial discrimination and blood pressure: the CARDIA Study of young black and white adults. *American Journal of Public Health*. 1996;86:1370-8.
133. Krieger N. Racial and gender discrimination: risk factors for high blood pressure? *Social Science & Medicine*. 1990;30:1273-81.
134. Murray, PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH. *Manual of clinical microbiology*. St Louis, MO: American Society for Microbiology, 1999.
135. Hjemdahl P, Daleskog M, Kahan T. Determination of plasma catecholamines by high performance liquid chromatography with electrochemical detection: comparison with a radioenzymatic method. *Life Sciences*. 1979;25:131-8.
136. Fisher L, Van Belle, G. *Biostatistics : a methodology for the health sciences*. Hoboken, NJ: John Wiley and Sons, 1996.

