

# Cytokine milieu, immune maturation and the risk of blood stream infection in extremely low birth weight infants

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# Disclosure Statement

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- No financial relationships to disclose

# Every Baby Has a Unique Story...



# Infections in newborns

- **Nosocomial infections in hospitalized neonates**
  - Nearly one-half of the smallest infants will become infected
  - Cause significant morbidity and mortality
  - Are costly

# These vulnerable infants need our help

- Immature cutaneous and mucus membrane barriers
- Developing host defenses
- Instrumentation





# GBS Sepsis

- Shock
- Multiple -organ system failure

# Infections in newborns

- **Contributes to adverse neurodevelopmental outcome**

- Damage cerebral white matter (Dammann, 2002; Hagberg, 2005; Dammann, 2004)
- 4-fold increase in CP (Wheater, 2000 560)
- Lead to long-term disability in ELBW infants. (O'Shea, 1998)

# Infection in Newborns

- Preventing disability in this vulnerable population is a major challenge



# Developing Host Immunity



**Immune quiescence appears  
necessary for fetal survival in  
the normally sterile  
intrauterine environment**



*22 weeks gestation*

# Developing Host Immunity



18 weeks gestation

**Immune quiescence appears  
necessary for fetal survival in  
the normally sterile  
intrauterine environment**

**Relative immunodeficiency of  
infancy**



22 weeks gestation

# Host Immunity

## Innate

- Neutrophils
- Complement
- GI Mucosal Barrier
- Skin

## Adaptive

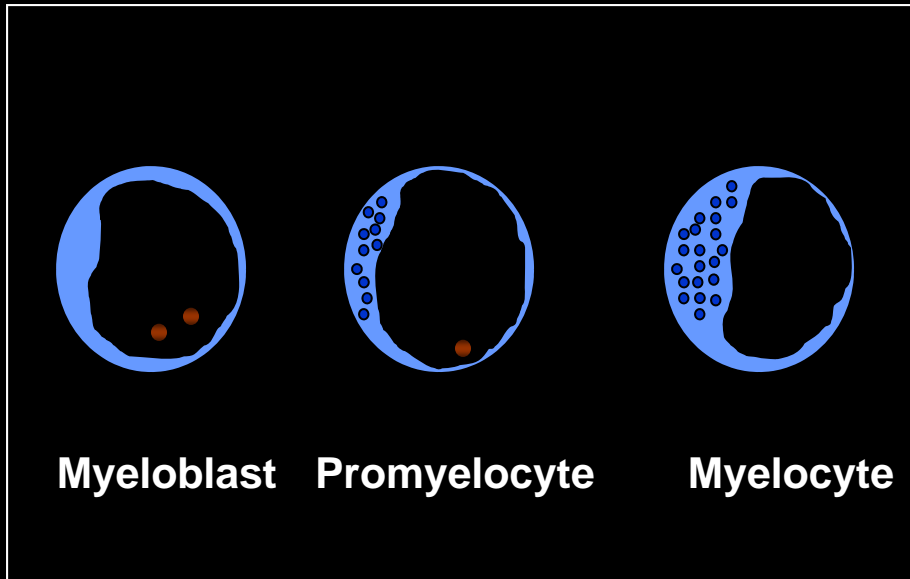
- Antigen recognition  
T and B cell interaction
- Antigen-specific antibody\_

# Innate Immunity

- Preexisting first line of defense against infection
  - Components: epithelial barriers, phagocytic cells, natural killer cells, complement and cytokines
- Phylogenetically oldest mechanism of defense
  - Present in all multicellular organisms including plants and insects

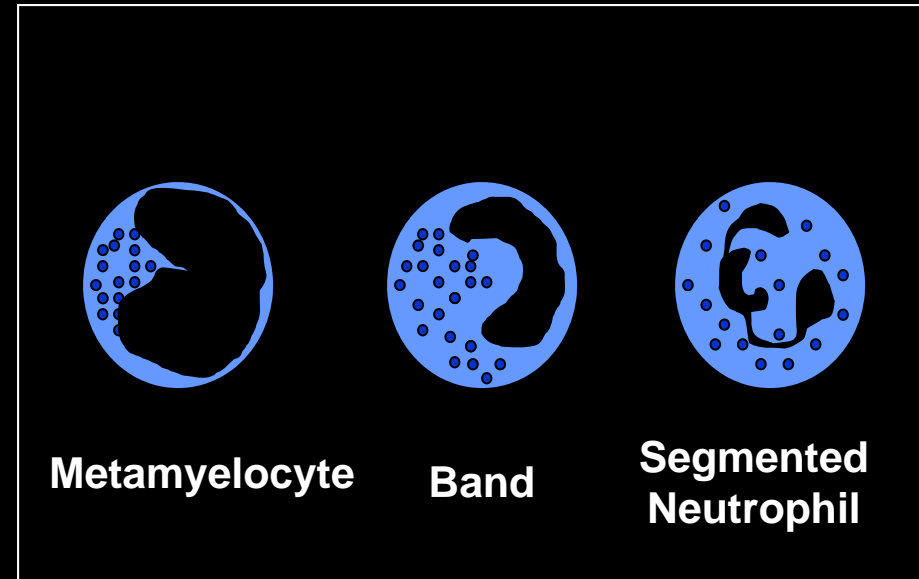
# Term and preterm neutrophils have less storage pool reserve

## Neutrophil Proliferative Pool



Expanded precursor cell populations and basal proliferative rate is near maximal (Christensen 1984, 1989)

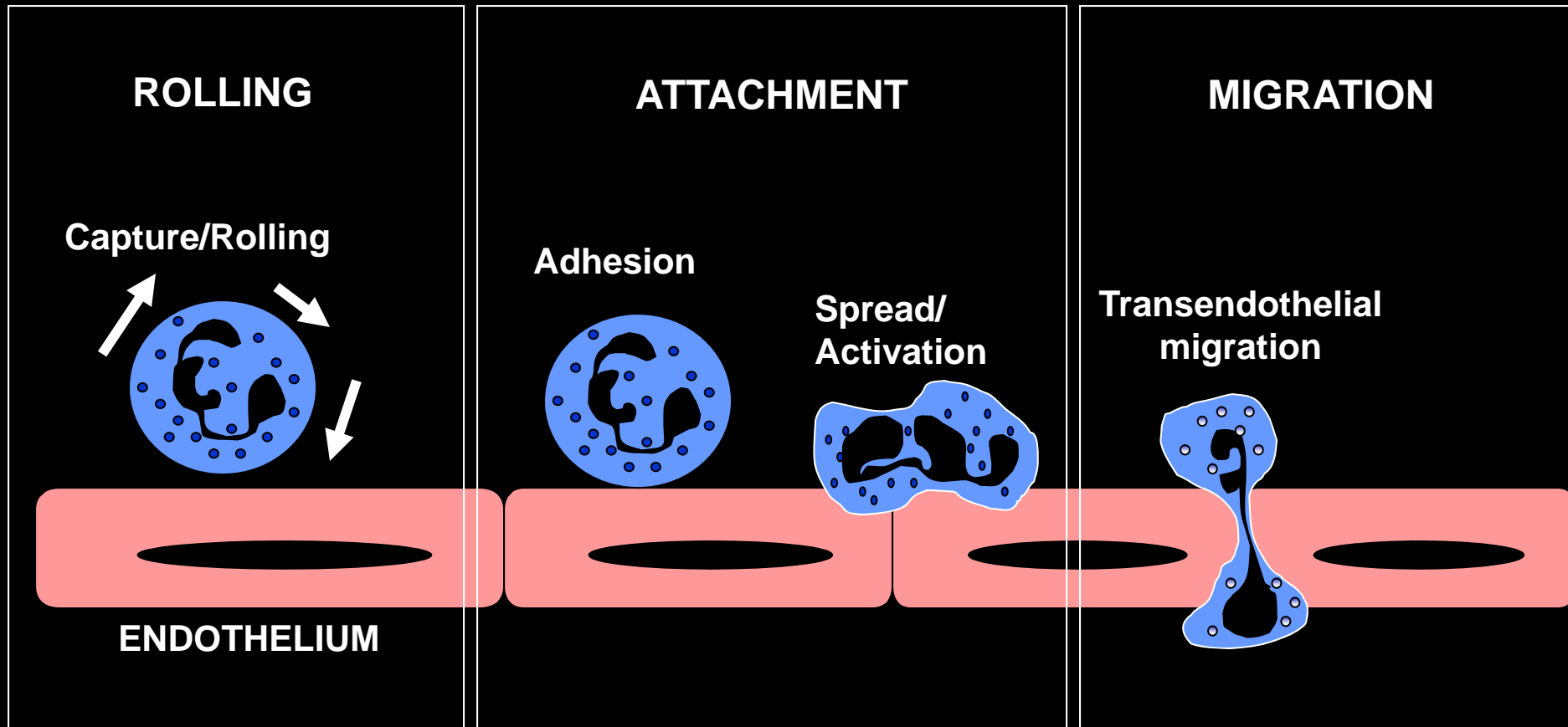
## Neutrophil Storage Pool



↓ storage pool size and may be exhausted in sepsis (Christensen 1980 and 1982)

# Term and preterm neutrophils have impaired chemotaxis

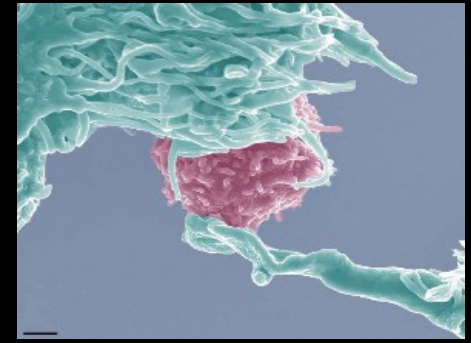
migrate at about half the speed of adult cells



↓ expression and shedding of L-selectin  
• reduces rolling  
• rate limiting step in tissue recruitment of neutrophils

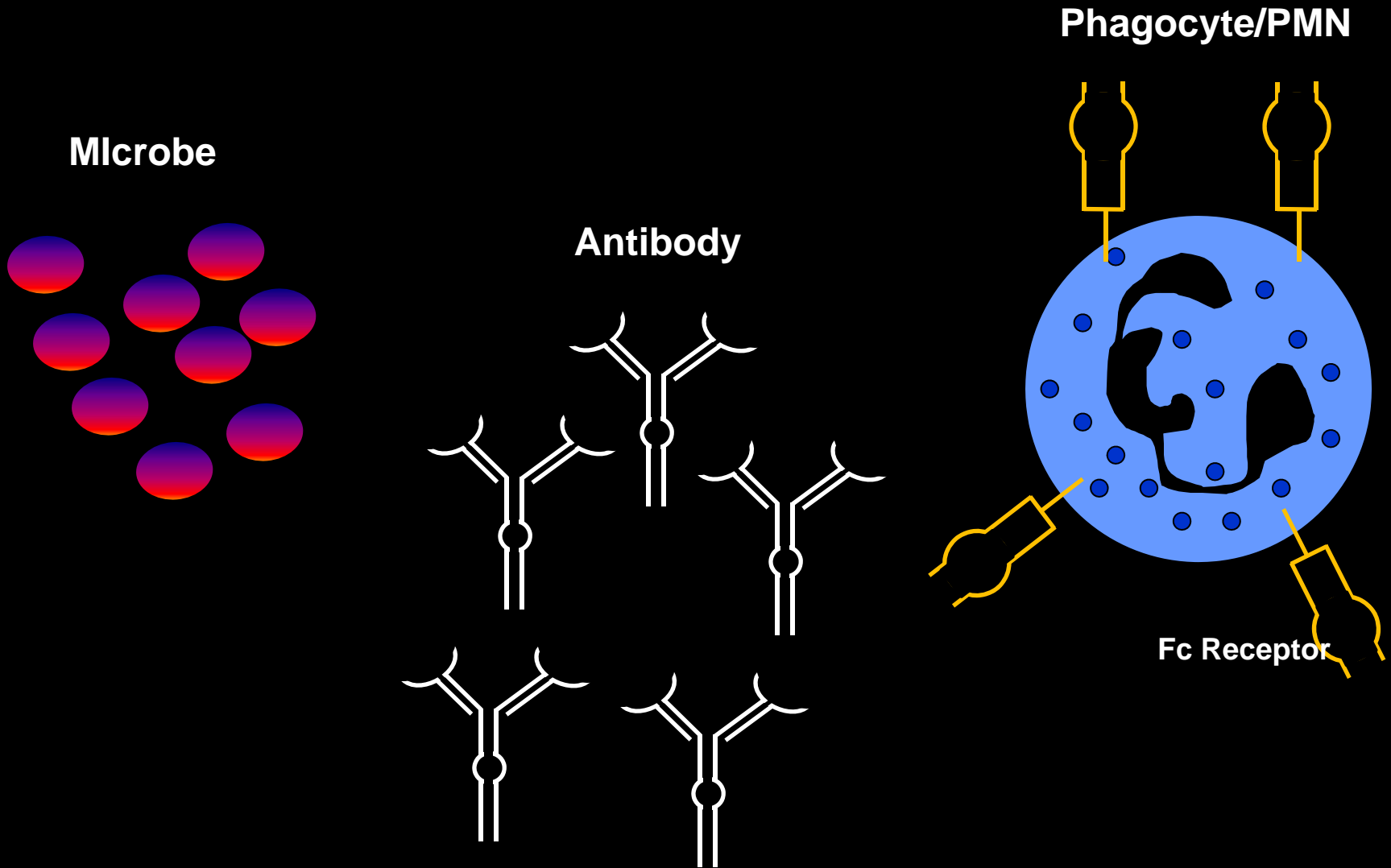
↓ expression of beta<sub>2</sub>-integrin Mac-1 (CD18/CD11b)  
• reduced neutrophil-endothelial adherence and transmigration

# Adaptive Immunity

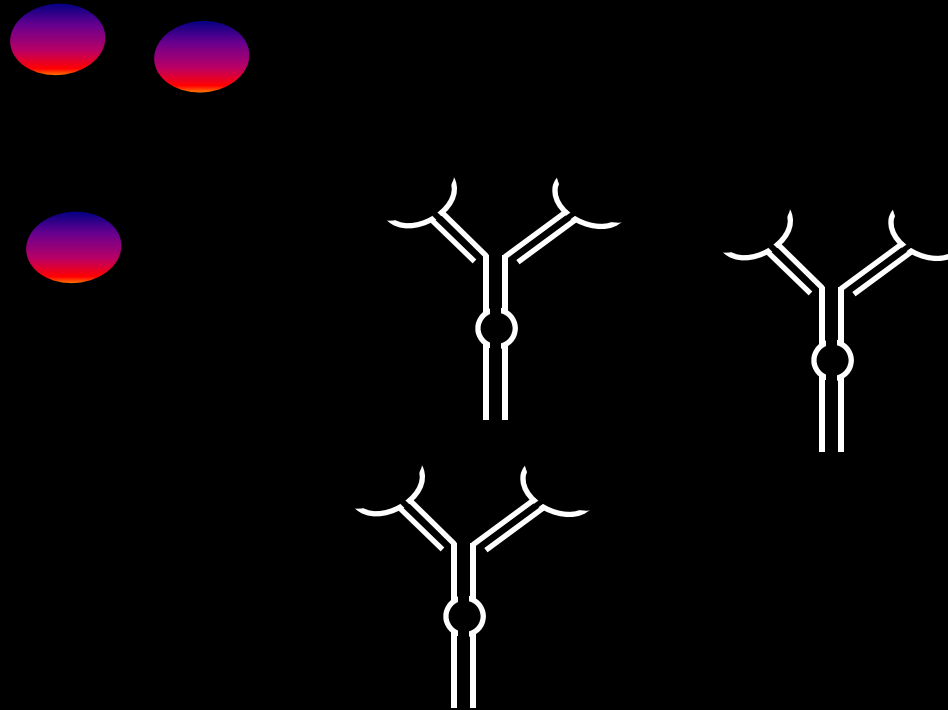


- Second line of defense against infection
  - Components: T and B lymphocytes, immunoglobulin, antigen presenting cells
- Specific antigen recognition on the basis of cell surface receptors
- Developmentally-regulated

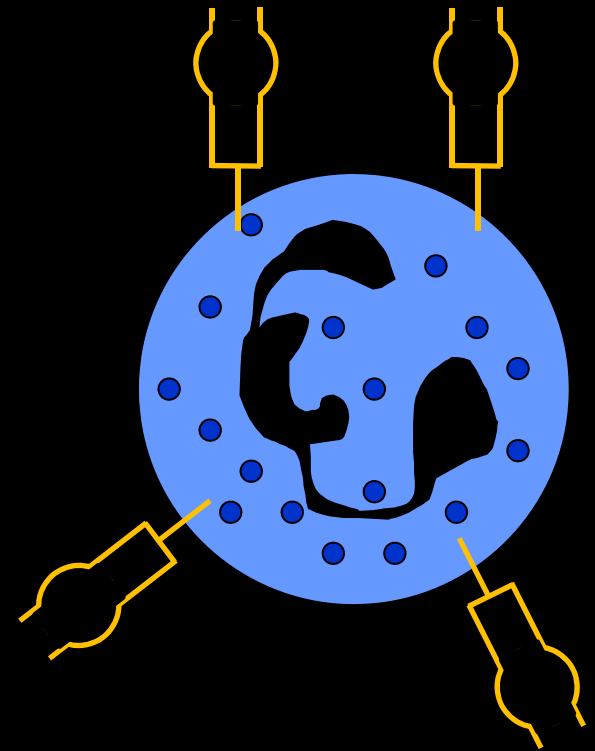
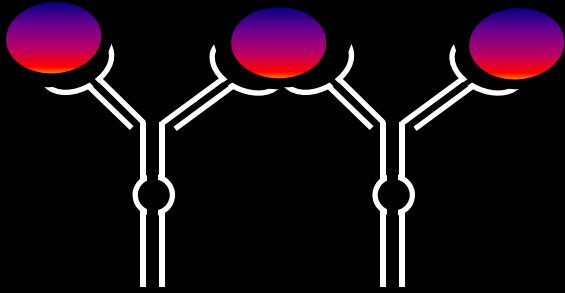
# Antibody-mediated opsonization and phagocytosis of microbes



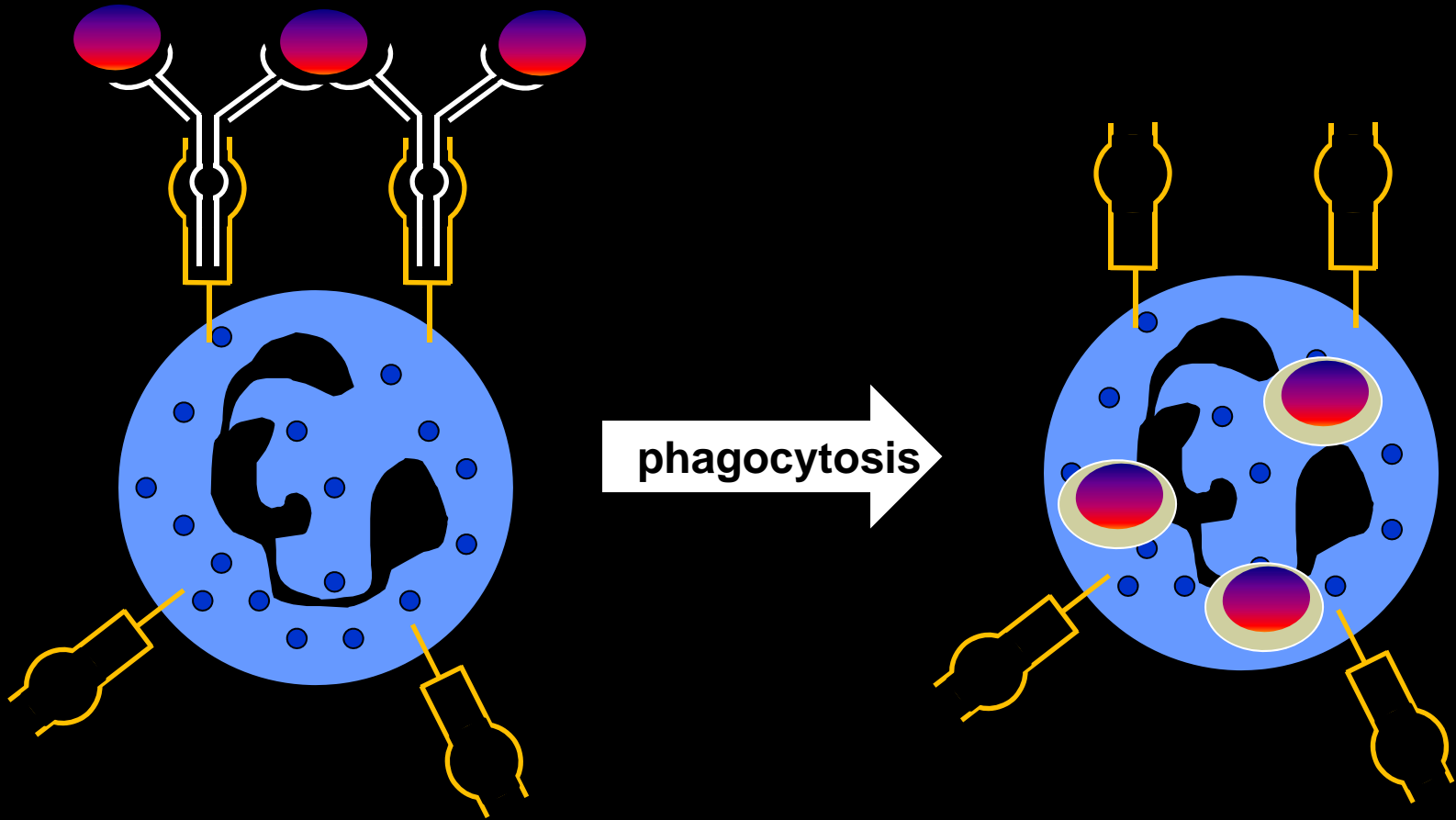
# Antibodies opsonize microbes



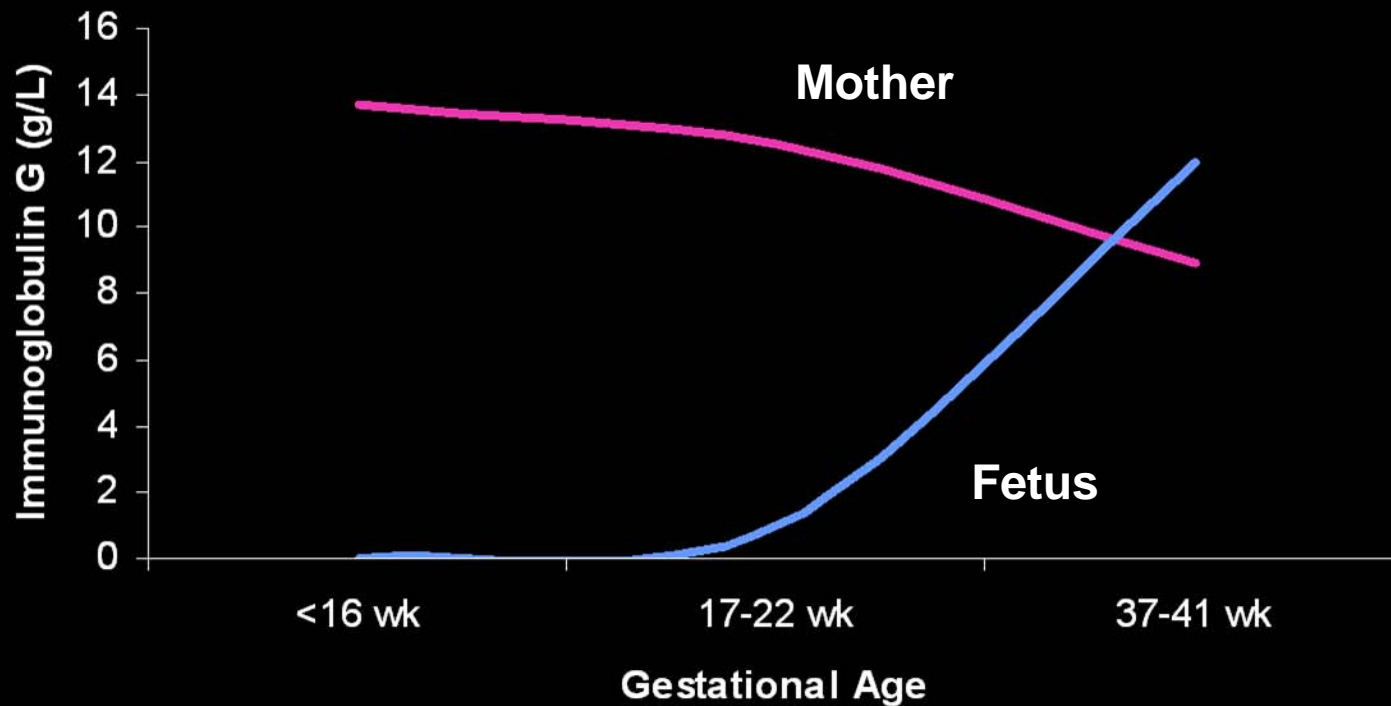
# Phagocytes recognize opsonized microbes via Fc receptors



# Microbe recognition promotes phagocytosis



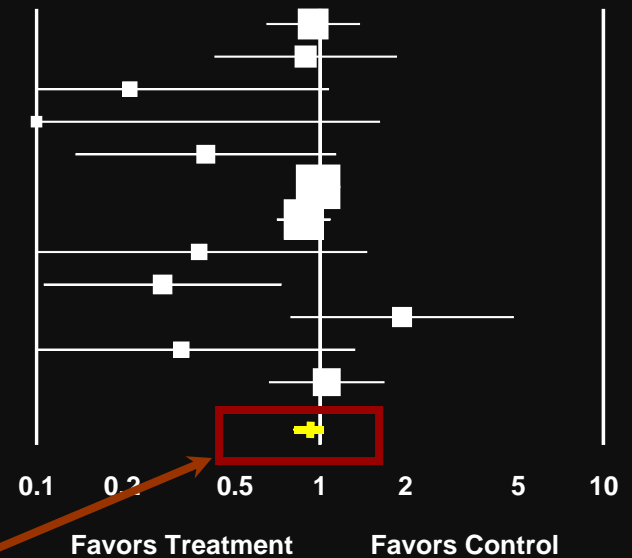
# The gift of immunoglobulin



Adapted from Malek A. *Am J Reprod Immunol.* 1996;36:248–255

# IVIg for the Prevention of Sepsis in VLBW infants

Citation	Year	Treated	Control	Effect	Lower	Upper	N	Total	P	Value
Bloom	2005	160 / 347	75 / 158	.95	.65	1.38	505		.78	
Bussel	1990	20 / 61	23 / 65	.89	.43	1.86	126		.76	
Chirico	1987	2 / 43	8 / 43	.21	.04	1.07	86		.04	
Clapp	1989	0 / 56	5 / 59	.09	.00	1.62	115		.04	
Conway	1990	8 / 34	14 / 32	.40	.14	1.14	66		.08	
DeJonge	2007	458 / 989	464 / 994	.99	.83	1.18	1983		.87	
Fanaroff	1994	186 / 1204	209 / 1212	.88	.71	1.09	2416		.23	
Hague	1986	4 / 100	5 / 50	.38	.10	1.46	150		.14	
Ratrisawadi	1991	10 / 68	13 / 34	.28	.11	.73	102		.01	
Sandberg	2000	19 / 40	13 / 41	1.95	.79	4.81	81		.15	
Tanzer	1997	3 / 40	8 / 40	.32	.08	1.33	80		.10	
Weisman	1994	40 / 372	39 / 381	1.06	.66	1.68	753		.82	
<b>Fixed Combined (12)</b>		<b>910 / 3354</b>	<b>876 / 3109</b>	<b>.91</b>	<b>.81</b>	<b>1.02</b>	<b>6463</b>		<b>.10</b>	



**Effect 0.91 (CI-0.81-1.02) p=0.1**

# Host Immunity

## Innate

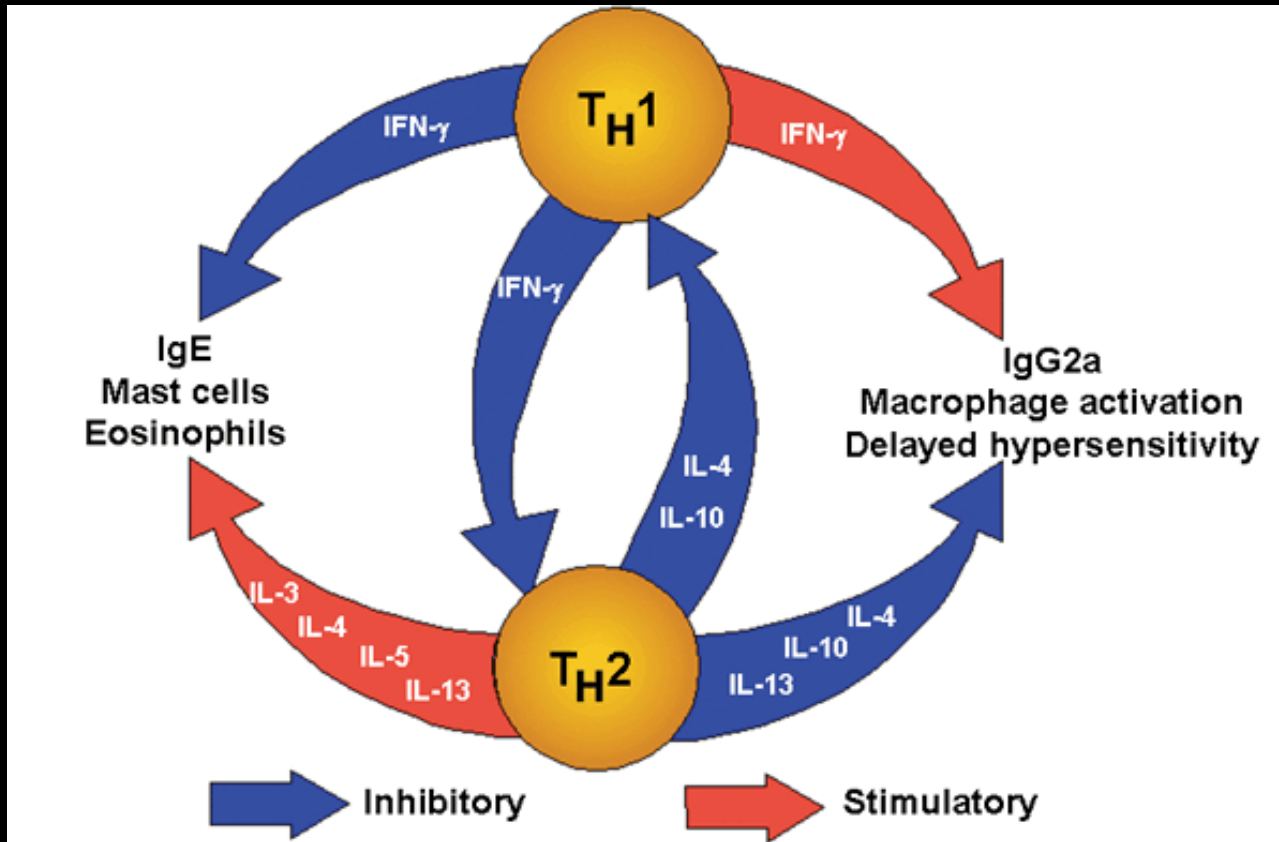
- Complement
- GI Mucosal Barrier
- Neutrophils
- Skin

- Acquired immunoglobulin
- Cytokines
- Chemokines
- Macrophages/APCs
- Microbiota
- Natural Killer Cells
- Pattern Recognition Receptors  
TLRs
- Regulatory T cells

## Adaptive

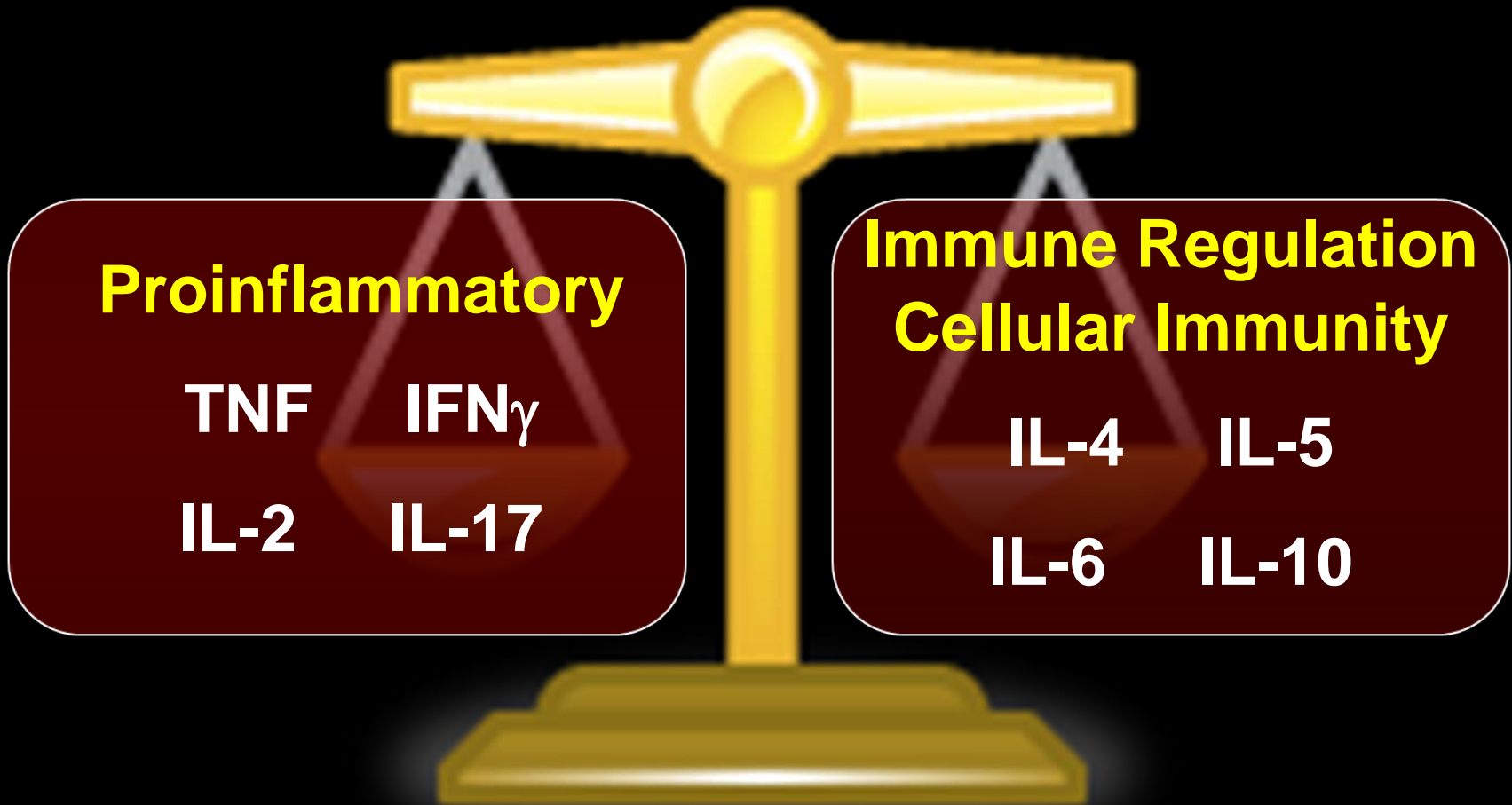
- Antigen recognition  
T and B cell interaction
- Antigen-specific antibody

# Helper T cells subsets



- Distinguished by cytokines secreted after activation
- Mediate different regulatory and effector functions

# A delicate balance



# Hypothesis

- ELBW infants who fail to down-regulate immune suppressive cytokines and/or fail to up-regulate pro-inflammatory cytokines have a greater risk of developing late onset sepsis and/or meningitis

# Methods

- ELBW infants (401-1000g) enrolled at birth
  - Exclusions
    - Death in the first 7 days of age
    - Major congenital malformations
    - Early onset sepsis
    - Blood spots on filter paper obtained on days 0 ( $\leq 4$  hrs),  $3 \pm 1$ ,  $7 \pm 1$ ,  $14 \pm 3$  and  $21 \pm 3$
- Blood Samples were dried and frozen
- Cytokines were analyzed by Luminex multiplex assay

# Luminex<sup>®</sup> Multiplex assay

- 3  $\mu$ l of whole blood from stored blood spots
- <10% intra-assay and 7–23% inter-assay variation

*Skogstrand K, et al. Simultaneous measurement of 25 inflammatory markers and neurotrophins in neonatal dried blood spots by immunoassay with xMAP technology. Clin Chem 2005;51:1854–66.*

# Luminex<sup>®</sup> Multiplex assay

**Appropriate bloodspot storage does not change cytokine concentrations**

*Skogstrand K, et al. Effects of blood sample handling procedures on measurable inflammatory markers in plasma, serum and dried blood spot samples. J Immunol Methods 2008;20(336):78–84.*

# Methods

- Analyzed 4 proinflammatory and 4 immune regulatory cytokines
- Late onset sepsis (LOS) defined as blood and/or CSF culture performed after 72 hours of age and positive for bacteria or fungi
  - Predefined contaminants excluded
  - All cases of coagulase negative Staphylococcus (CONS) evaluated

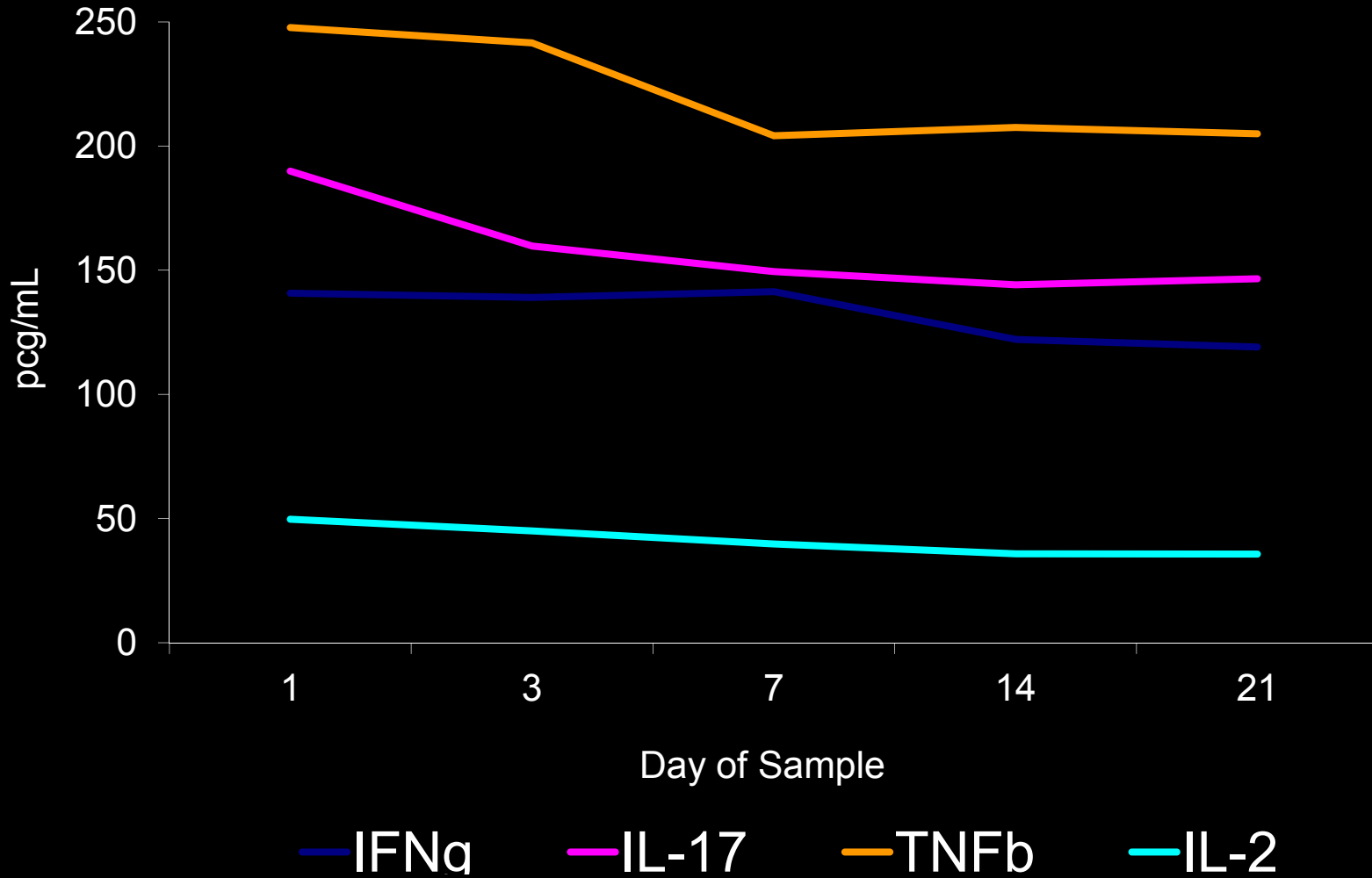
# Methods

- Multiple variable logistic regression for LOS using variables selected by stepwise regression
- Cox proportional hazards models
  - Individual cytokines and ratios included in models as time-dependent covariates

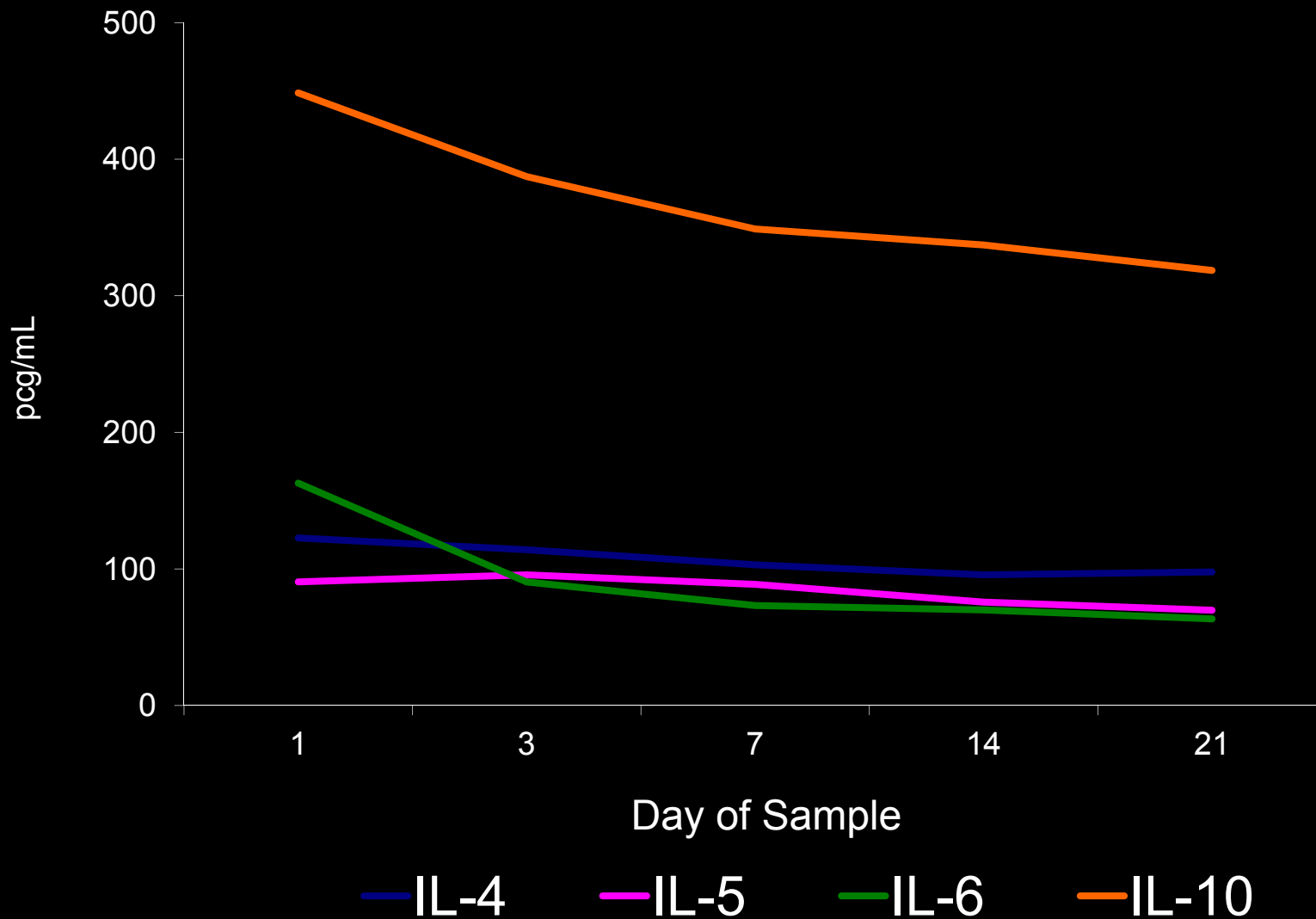
# Results

- 1071 ELBW infants in the Cytokine Study
  - 75 excluded (45 deaths in first 7 days, 16 EOS and 16 with anomalies)
- 996 infants in the analysis
- Birth weight (mean $\pm$ SD): 762  $\pm$  143g
- Gestational age: 26  $\pm$  2 weeks
- Gender: 51% male
- Race: 48% black
- Inborn: 93%
- Confirmed BSI % 436 (44%)

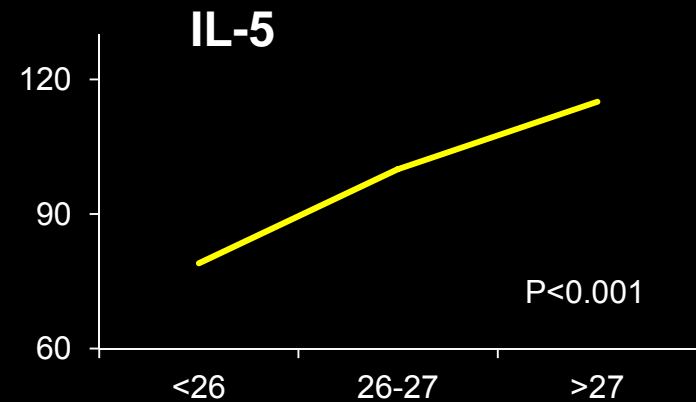
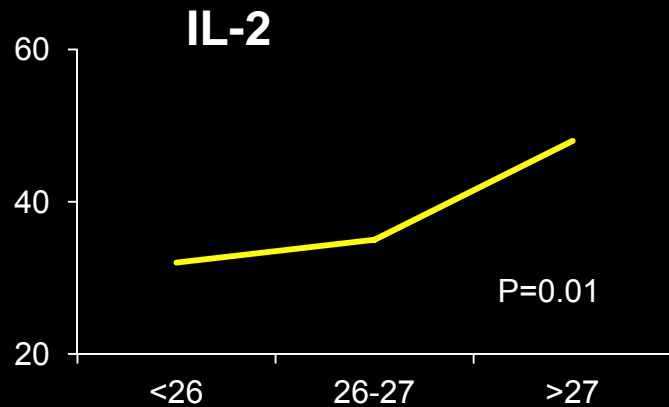
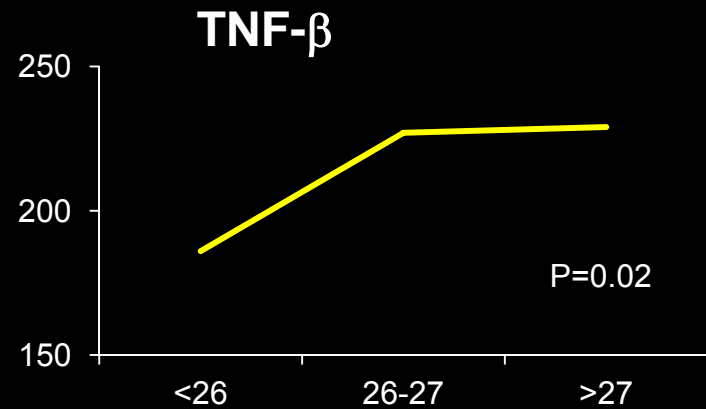
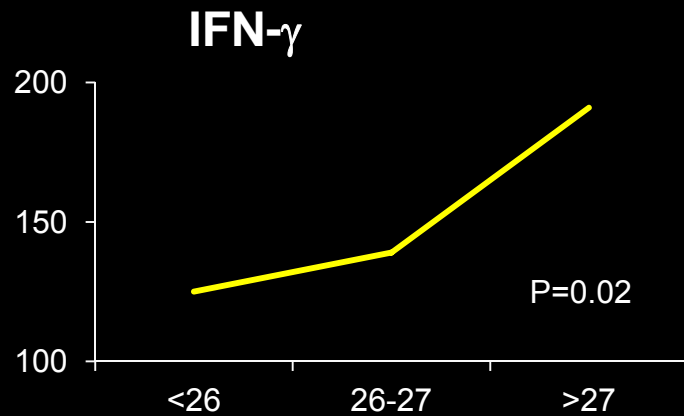
# Median Values for Th1/Th17 Cytokines



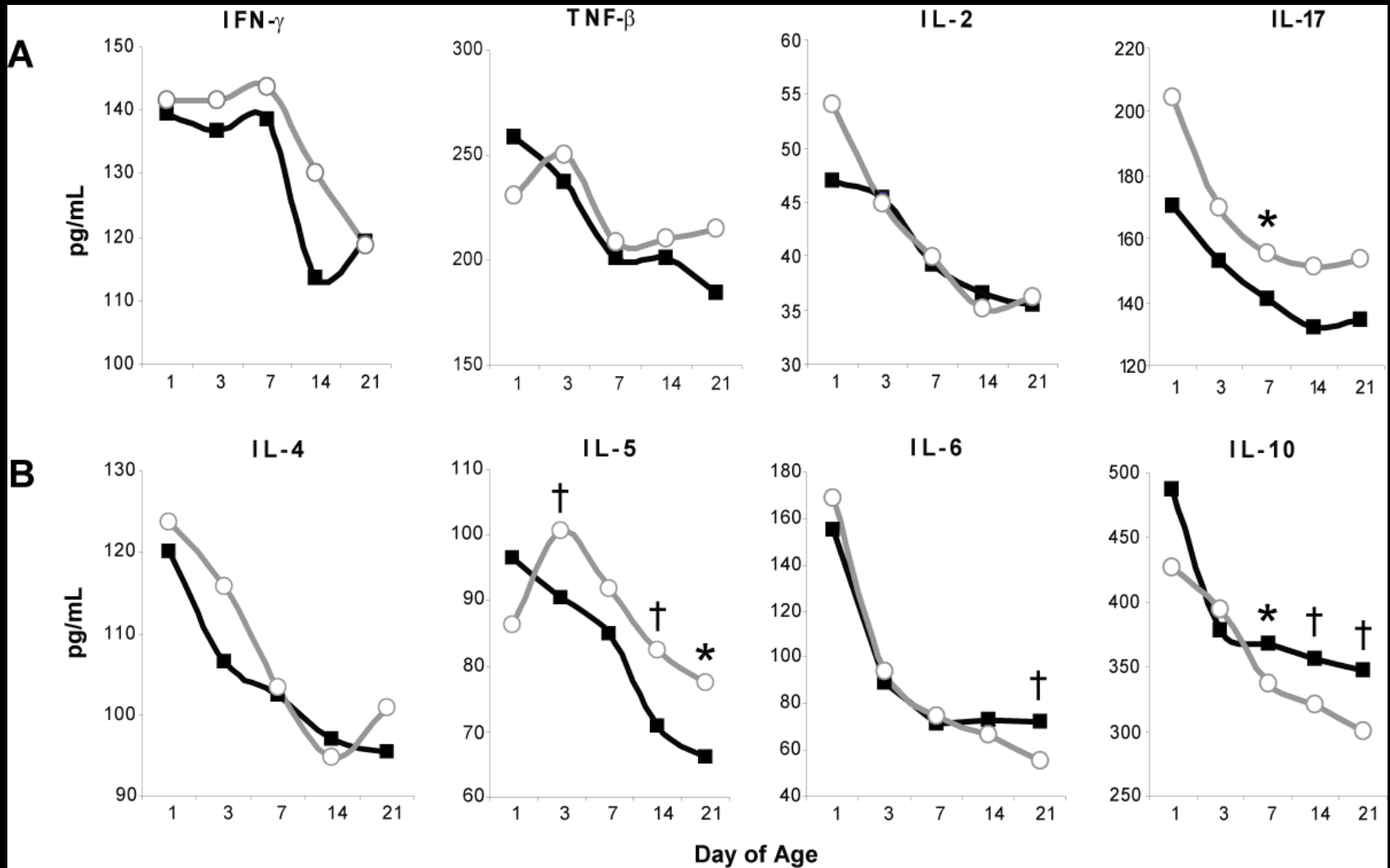
# Median Values for Th2 Cytokines



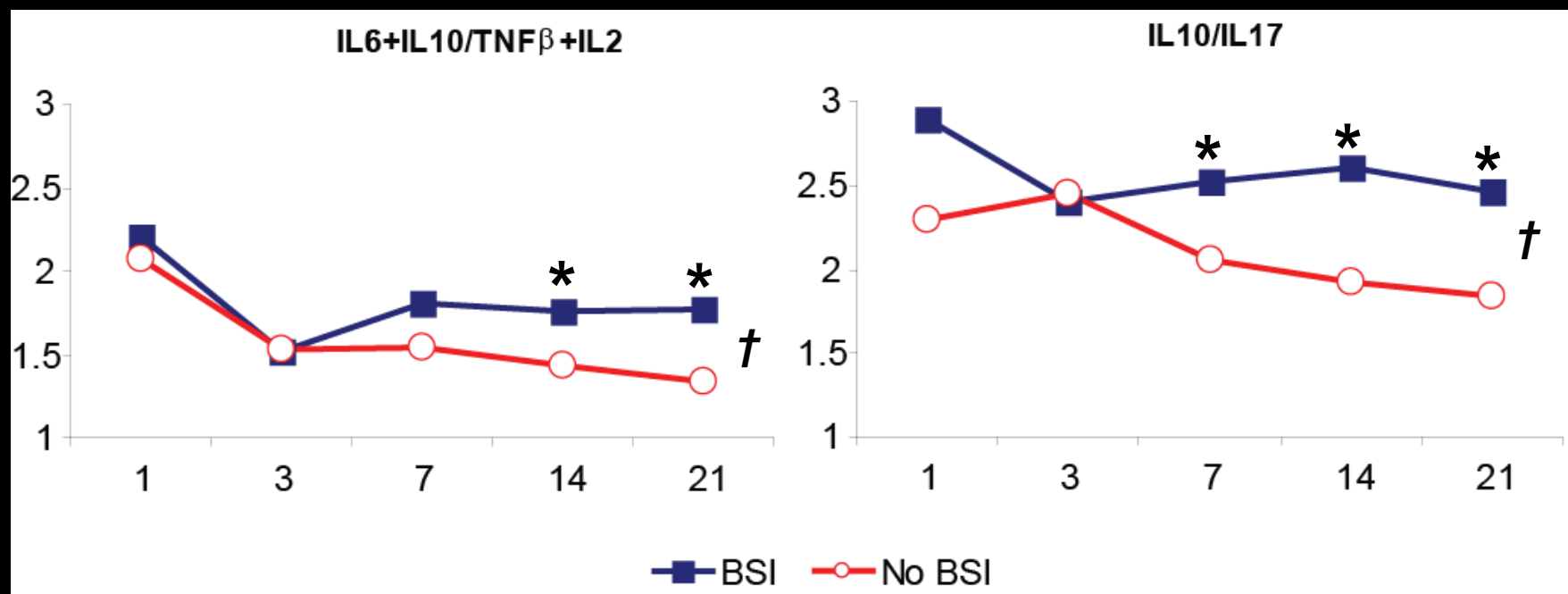
# Maturation Differences in Cytokines



# Temporal Cytokines Values for Infants with and without Bacterial Sepsis



# Ratios of regulatory and proinflammatory cytokines differ in infants with and without LOS



\*P = <0.01 Univariable analysis

†P = <0.05 Cox Proportional Hazards model using time dependent covariates

# Conclusions

- Cytokine immune regulatory bias is present in ELBW infants after birth and is associated with increased risk of LOS
- Infants who develop LOS have:
  - Higher concentrations of immune regulatory cytokines
  - Lower concentrations of proinflammatory cytokines

# Speculation



- Higher levels of cytokines favoring immune suppression may play a critical role in the functional immune deficiency of infancy
- An imbalance of pro-inflammatory and anti-inflammatory cytokines, particularly IL-10, contributes to increased risk of infection

# Speculation

- Cytokine concentrations may play a role in identifying infants at higher risk for LOS
- Infants who appear to incompletely up regulate inflammatory cytokines may be at greater risk for BSI

# Speculation

- Identification of cytokines that contribute to serious infection in ELBW infants may lead to therapeutic strategies directed to accelerate maturity of the immune system and make these infants more able to resist microbial invasion

# NICHD Neonatal Research Network Centers (1996-2006)

- Brown University
- Case Western Reserve University
- Duke University
- Emory University
- Indiana University
- Research Triangle Institute
- Stanford University
- University of Alabama – Birmingham
- University of California – San Diego
- University of Cincinnati
- University of Miami
- University of New Mexico
- University of Rochester
- University of Tennessee – Memphis
- University of Texas, Southwestern – Dallas
- University of Texas – Houston
- Wake Forest University
- Wayne State University
- Yale University



# NICHD Neonatal Research Network Centers (2006-2011)

- Brown University
- Case Western Reserve University
- Duke University
- Emory University
- Indiana University
- Research Triangle Institute
- Stanford University
- Tufts Medical Center
- University of Cincinnati
- University of Alabama – Birmingham
- University of Iowa
- University of New Mexico
- University of Texas, Southwestern – Dallas
- University of Texas – Houston
- University of Utah
- Wayne State University
- Yale University



# Best Beginnings

