Ontogeny of the Immune System: Implications for the response to vaccination

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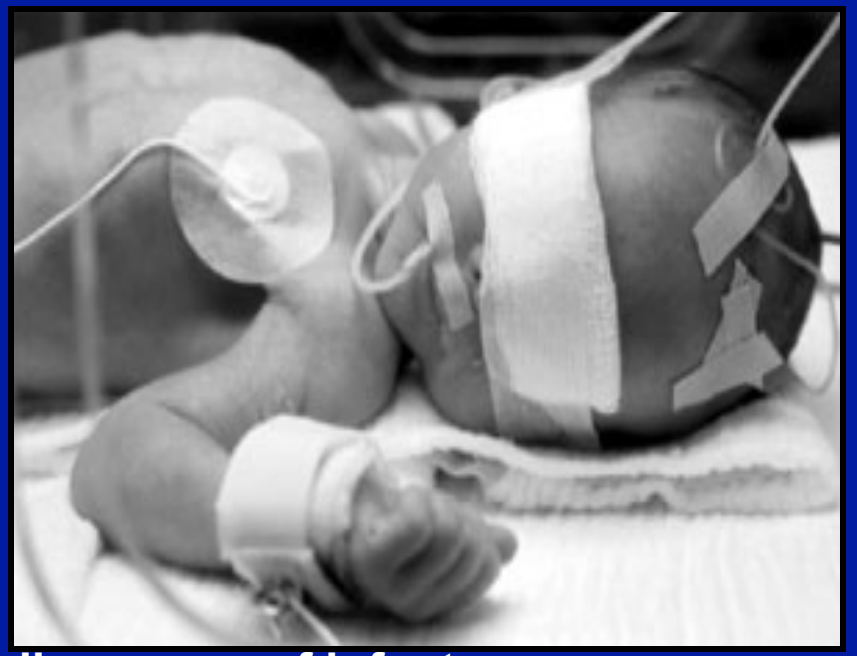
Newborns & Young Infants Have Increased Risk of Invasive Microbial Infection

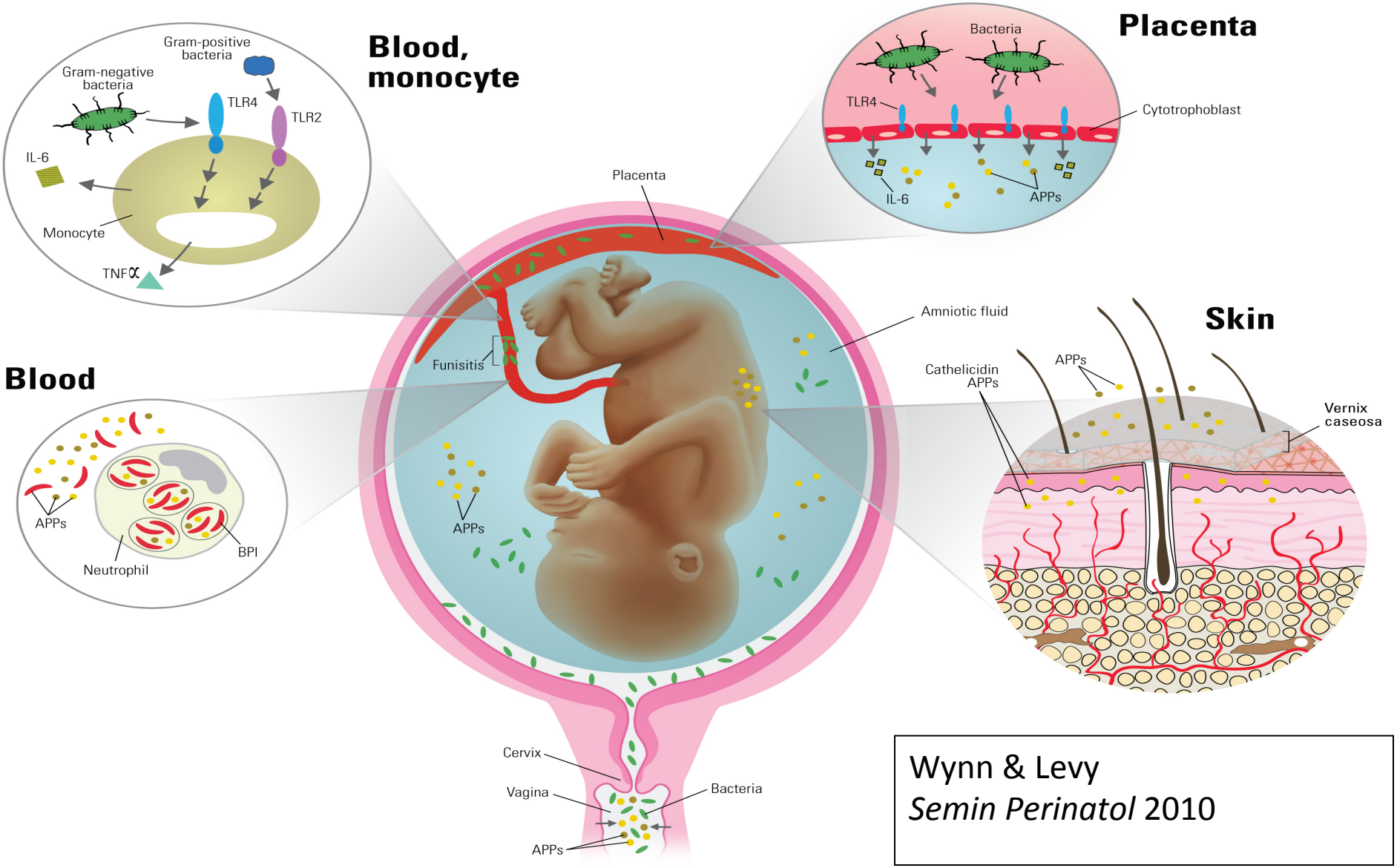
>2,000,000 deaths to infection/year in those < 6 mo



- Bacteria
  - Gram-positive
    - Group B Streptococcus
    - *S. pneumoniae* (~10^6 deaths/yr)
  - Gram-negative
    - *Haemophilus, E. coli, etc.*
    - *B. pertussis (~3x10^5 deaths/yr)*
- Viral Infection
  - Herpes Simplex Virus (HSV)
  - Respiratory syncitial virus (RSV)- leading cause of infant hospitalization in U.S.
  - Dengue (more severe in infants)
- Diarrheal diseases:
  - Rotavirus 440,000 deaths/yr

Unmet medical need: prevention of microbial infection early in life

Innate immune factors limit ascending infection *in utero*



Wynn & Levy
Semin Perinatol 2010

Ontogeny of plasma Ig levels

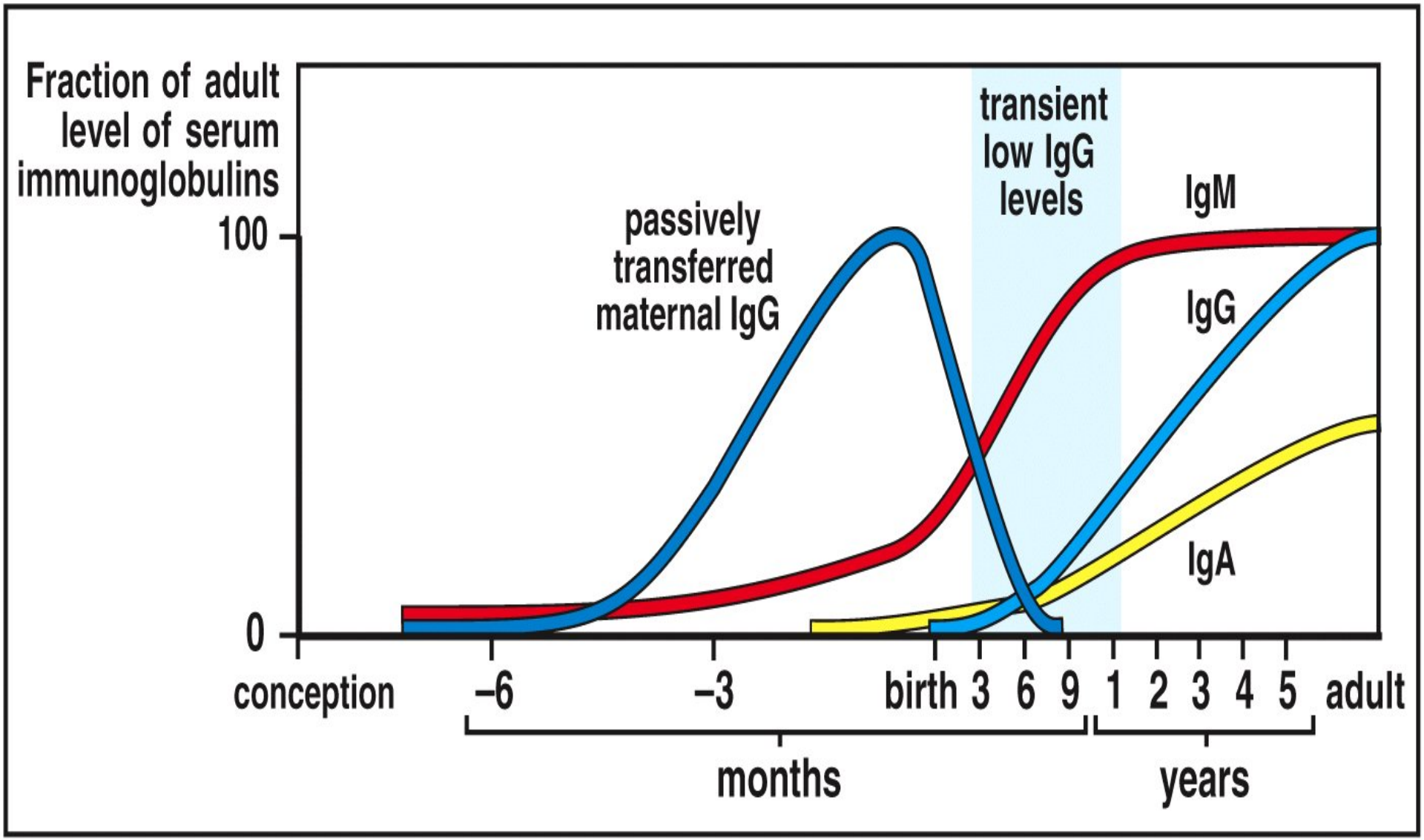


Figure 11-11 Immunobiology, 6/e. (© Garland Science 2005)

Innate and adaptive immunity change with age

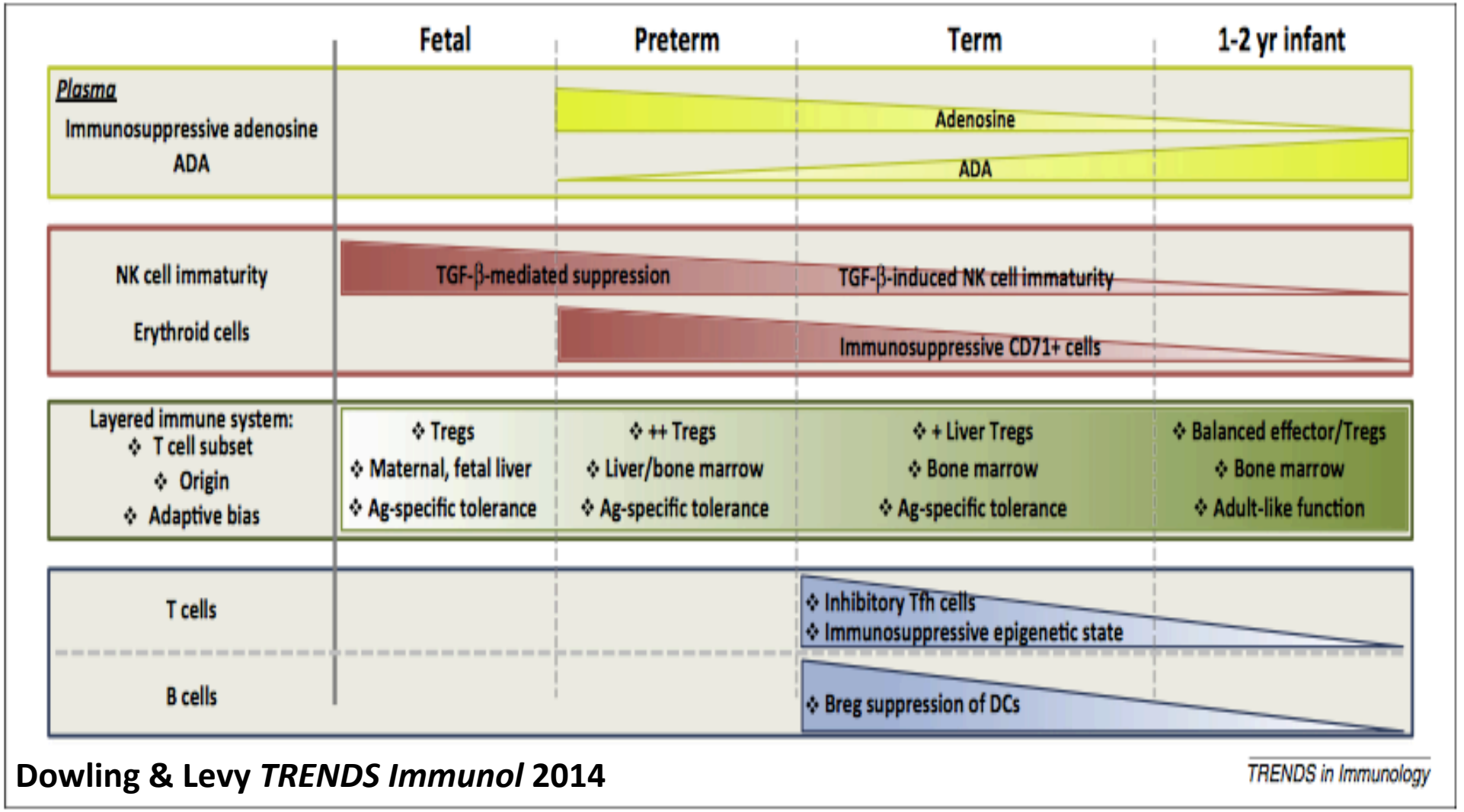
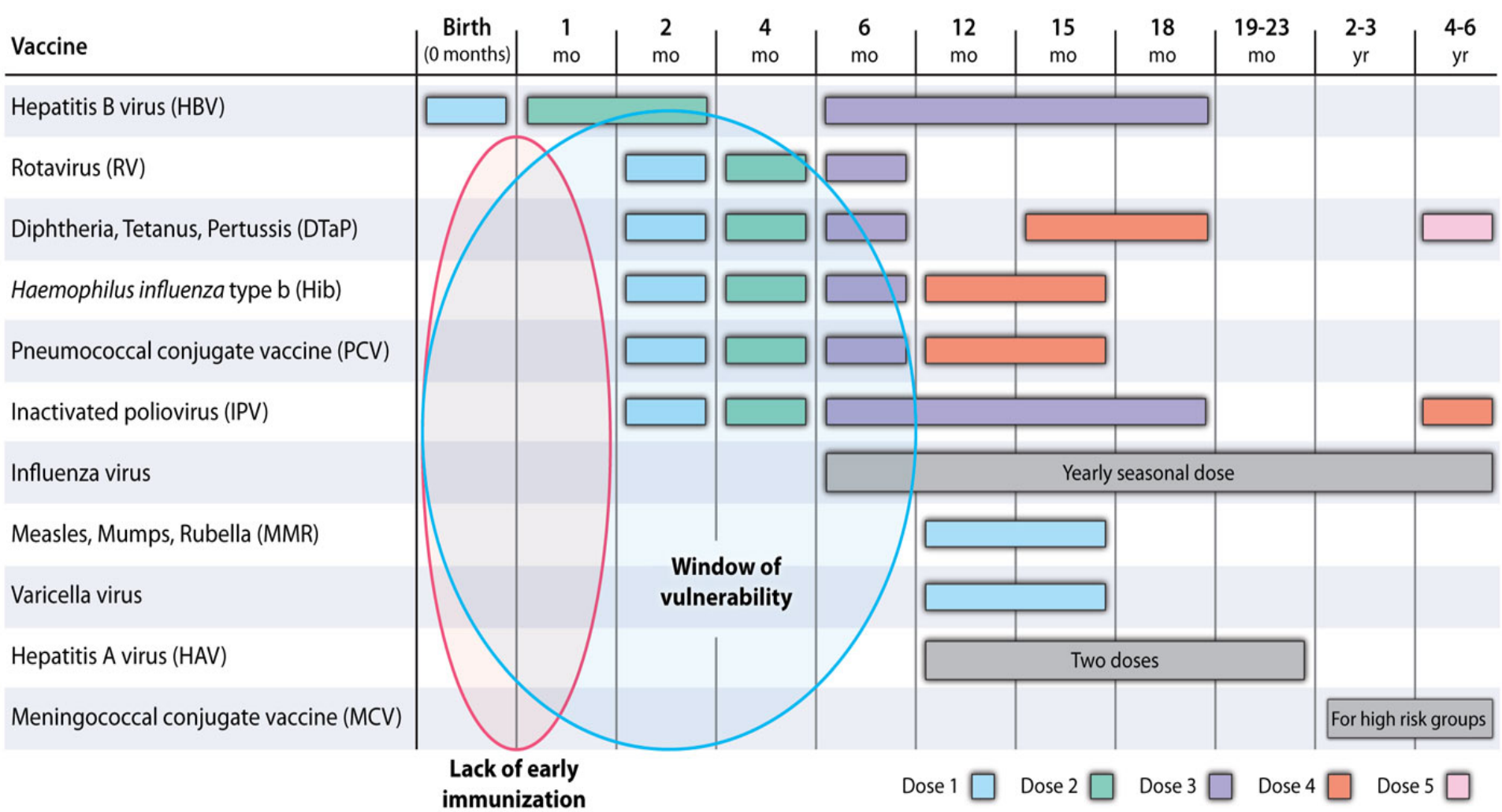


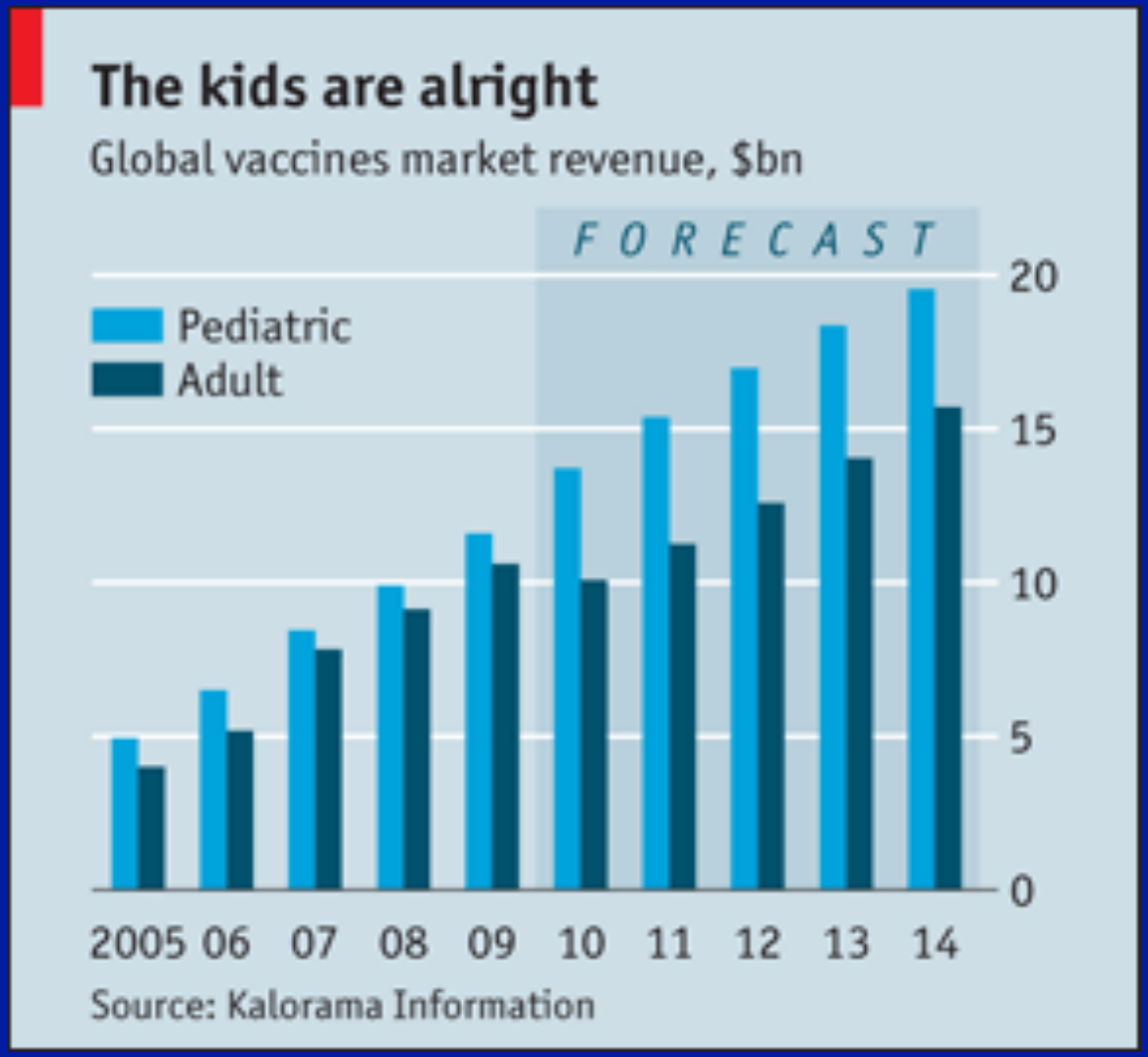
Figure 1. Ontogeny of early life immunomodulation. Preterm (<37 weeks gestational age, GA) and full-term newborns (37–41 weeks GA). Abbreviations: ADA, adenosine deaminase; Breg, regulatory B cell; NK, natural killer; Tfh, T follicular helper; TGF-β, transforming growth factor β; Tregs, regulatory T cells.

Current Vaccine Schedule Leaves Newborns and Young Infants At Risk



Sanchez-Schmitz G , Levy O Sci Transl Med 2011

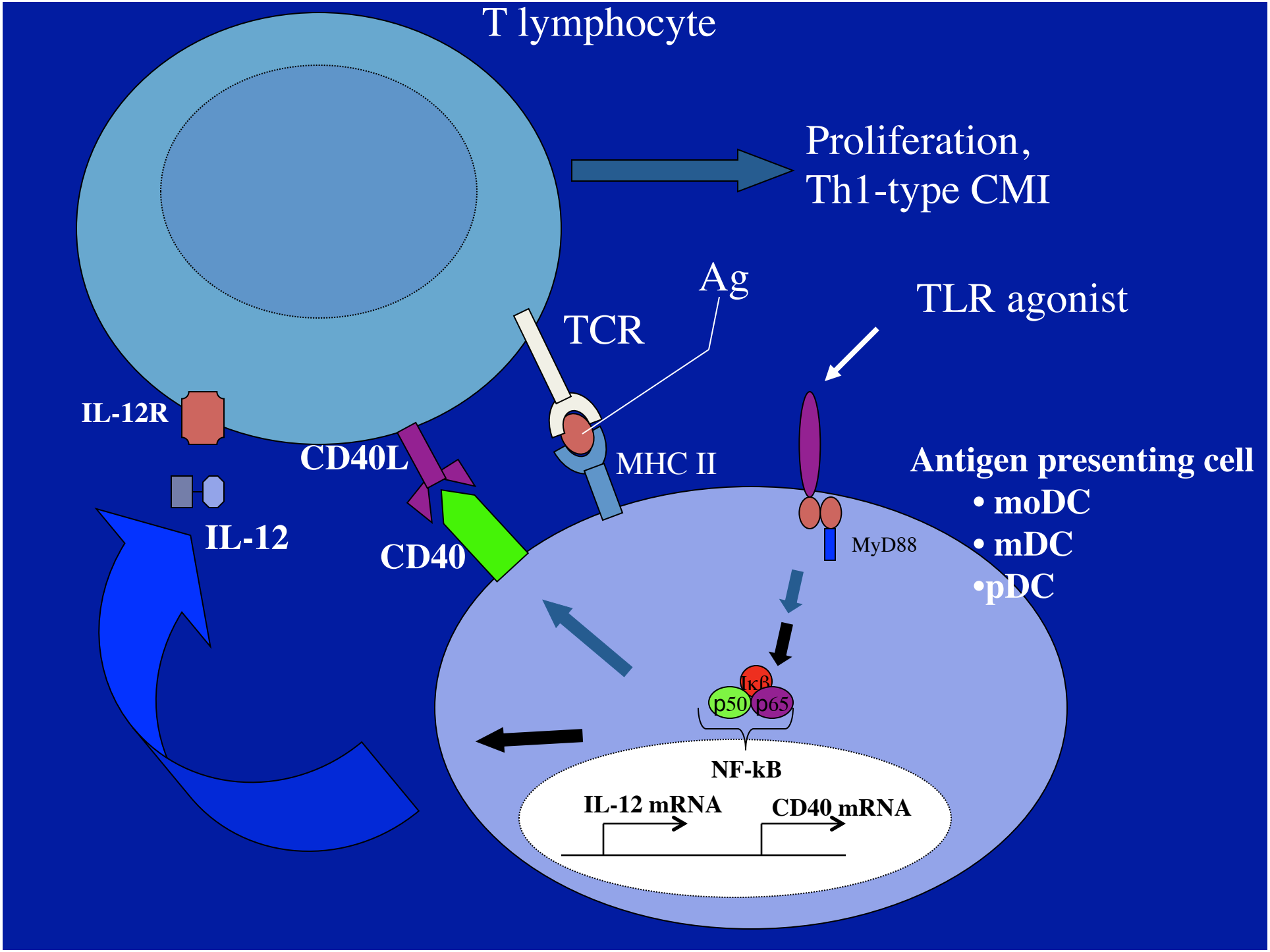
The majority of the global vaccine market is pediatric



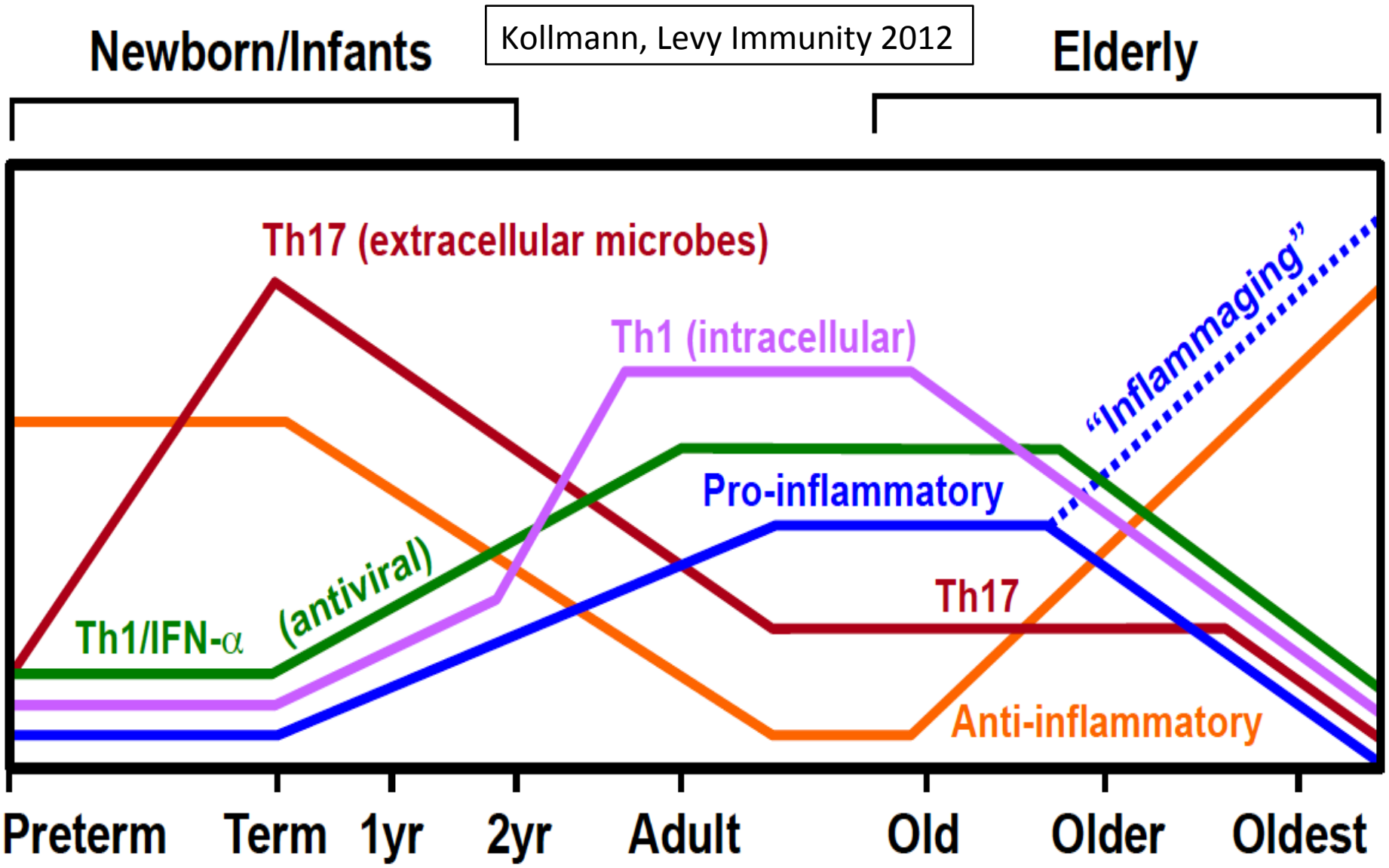
The Economist Oct 14, 2010

Potential hurdles to neonatal immunization

- Maternal Ab Modulation (interference/enhancement)
  - General inverse assoc passive vs vaccine-ind active Ab titers
  - Depends on ratio MatAb titers to Vacc Ag
  - For live vaccines: MatAb can decrease effective mass of Ag by reducing viral replication
  - Soluble protein Ag MatAb may increase immunogenicity (increase uptake or incr efficiency of T/B cell stim?)
  - APC uptake and T cell responses unaffected (eg, HibPS-TT conjugate).
  - Can be overcome
- Tolerance
  - Pertussis imm at birth → blunting of booster responses (Provenzano *NEJM* 1965)
- Auto-immunity?
  - Theoretical concern, not observed c neonatal BCG, HBV or infant vaccines


T lymphocyte
Proliferation,
Th1-type CMI
Ag
TLR agonist
TCR
IL-12R
CD40L
MHC II
Antigen presenting cell
• moDC
• mDC
•pDC
IL-12
CD40
MyD88
Iκβ
p50
p65
NF-kB
IL-12 mRNA
CD40 mRNA

Ontogeny of TLR Function



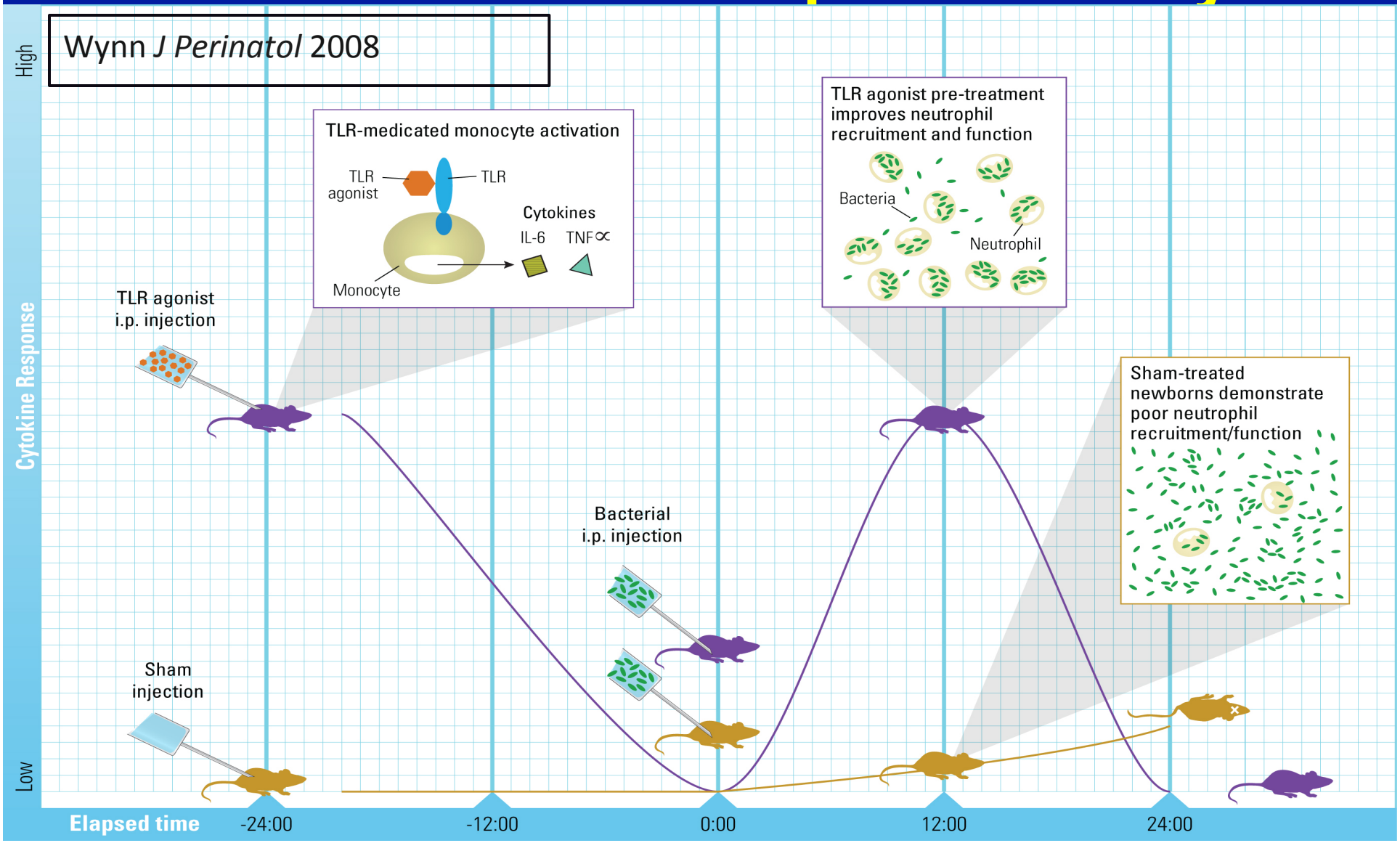
Age-specific Adjuvant Effects

Adjuvant/ vaccine	PRR	In Vitro	Effect in Newborn/ Infant	Ref
Alum	INFL (?)	• whole blood cytokine decrease c age in infancy (Papua New Guinea)	• Enhance Ab production • NSE: Th2 polarizing	Van Den Biggelaar Vaccine 2009; PLoS ONE 2012
HBV/VLP	INFL?	• Weak DC-dep lymphoprolif	• 30-50% efficacy after 1st neonatal dose	Sanchez-Schmitz AAI 2012 (Abstract & unpublished)
Oil in water (MF-59, AS03)	MyD88 & CARD/ASC	?	?	Sancho/Heikkinen NEJM 2012
OMP-C (Hib)	TLR2	• TLR2→ hi IL-10	• Newborns: poor Ab • Impaired booster responses	Ward Am Soc Micro 1992 (Abstract)
MPLA	TLR4	• whole blood, modest TNF induction	Malaria vaccine impaired	Levy J Immunol 2004; Agnandji NEJM 2012
ssRNA (Yellow Fever)	Multiple TLRs, incl TLR8	• TLR8 agonists induce robust Neo TNF&IL-1ß	• Safe in infants > 9 mo age	Thomas Am J Trop Med 2012
BCG	TLR2,4,8, & 9	• Robust DC-dep lymphoprolif	•Cell-mediated immunity	Sanchez-Schmitz AAI 2012

Evidence of trained immunity in early life (Levy & Netea *Ped Res* 2013)

- **Stimulation of newborn cord blood mononuclear cells *in vitro* with *Staphylococcus epidermidis* (SE) increases PRR expression (Strunk T, et al. *Pediatr Res* 2012;72:10–8)**
- **Infection of newborn mice with SE in the first 24 h of life induces selective TLR2 upregulation in liver mRNA (Kronforst K, et al *PLoS ONE* 2012;7:e43897).**
- **Intraperitoneal administration of TLR agonists (TLRAs) to newborn mice enhances subsequent cytokine- and phagocyte-based responses to bacterial infection and increases survival from polymicrobial sepsis (Wynn JL, et al. *Blood* 2008;112:1750–8).**
- **Bloodstream infection in critically ill preterm human newborns associated with enhancement of pathogen-specific mononuclear cell PRR in Gram-positive bacteremia (TLR2, MyD88) and Gram-negative bacteremia (TLR4, MD-2, and MyD88) (Zhang JP, et al. *Pediatr Res* 2010;68:479–83).**

TLR agonist pre-treatment enhances subsequent innate responses and reduces neonatal sepsis mortality



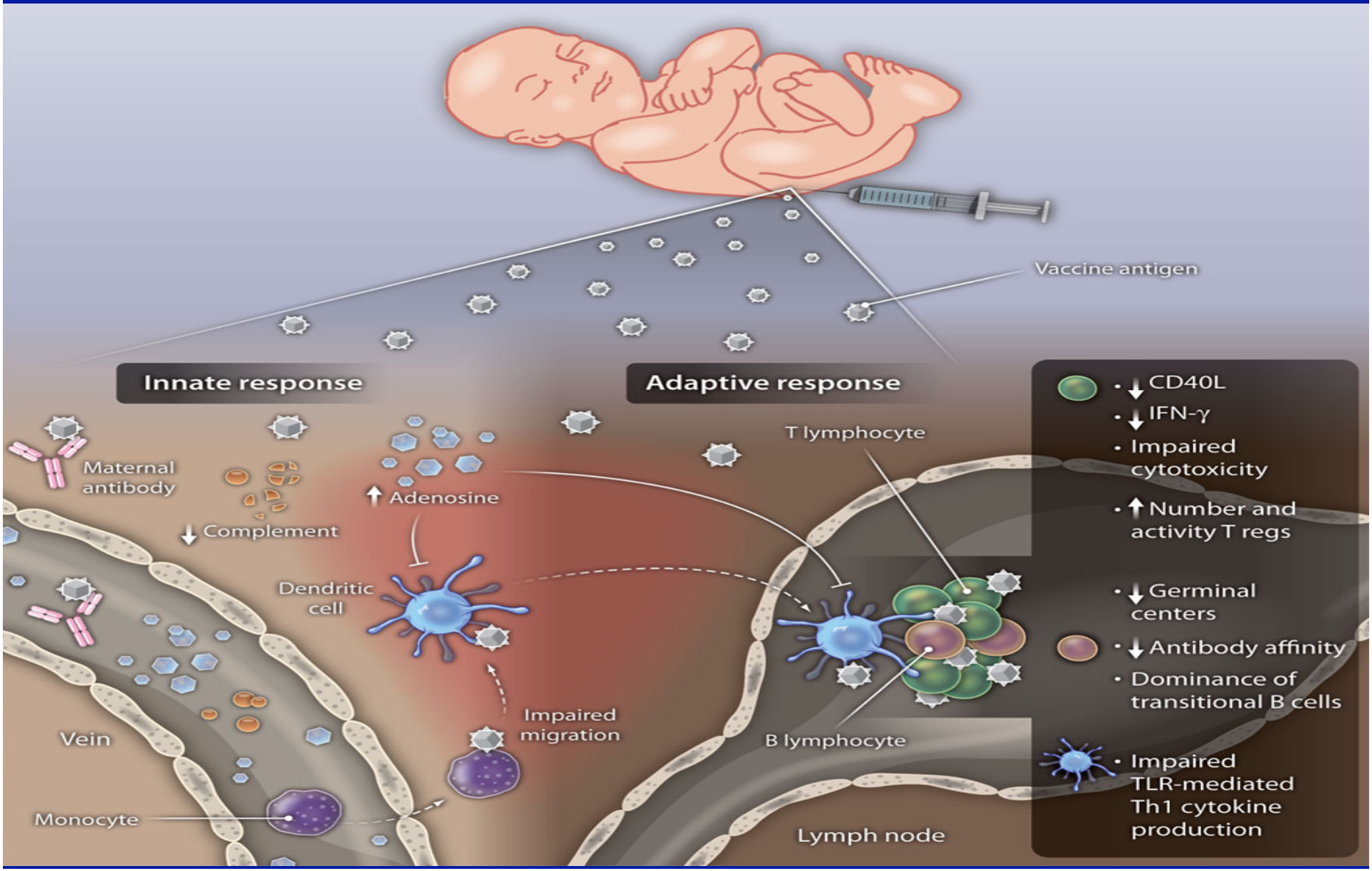
Clinical inflammation/infection may reduce late onset sepsis

- Histologic chorioamnionitis is associated with increased TLR expression in the chorioamniotic membranes (36) and a reduced risk of late onset bacterial sepsis, including a lower risk of S. epidermidis bacteremia (Strunk T et al. *Pediatrics* 2012;129:e134–41).
- Among the smallest, most premature infants, early onset sepsis (i.e., that within 1st 72 h of life) associated with diminished risk of late onset sepsis (i.e., occurring >72 h of life) (Wynn JL, *J Pediatr* 2013;162:942–8.e1–3).
- As early onset sepsis and late onset sepsis can be caused by distinct bacteria, these observations raise the possibility that innate immune engagement during early onset sepsis enhances innate immune responses such that the risk of late onset sepsis is diminished.

Live attenuated BCG activates TLRs and reduces all cause mortality in early life

- Immunization of human newborns can also trigger trained immunity.
- Live vaccines such as BCG (*Mycobacterium bovis)* are self-adjuvanted , activates multiple TLRs and nucleotide- binding oligomerization domain 2, triggers trained immunity (Kleinnijenhuis J, et al. *Clin Dev Immunol* 2011).
- BCG is associated with reduced all cause mortality, largely due to infections other than tuberculosis, in the 1st month of life (Aaby et al *Nat Rev Immunol* 2014).

Modeling neonatal immune responses must take into account humoral and cellular differences

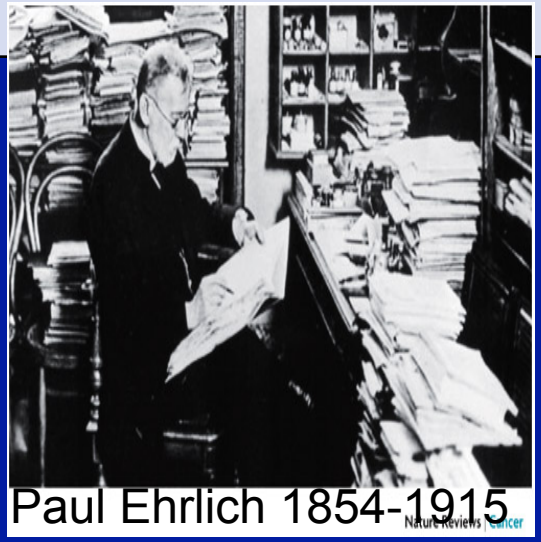


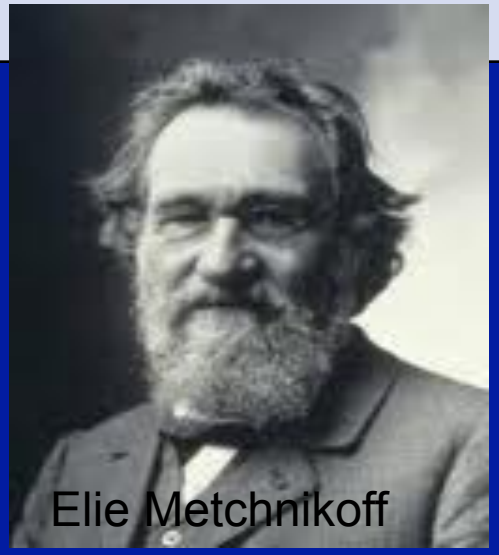
Rationale for modeling human neonatal and infant immunity in vitro

- Majority of vaccine market is pediatric
- Animal models are costly
- Animal models reflect species-specific biology (re adjuvants: innate immune system is hyper-variable between species!)
- In vitro modeling of vaccine safety & efficacy could reduce risk & increase yield in vaccine development
- Approach: Age-specific
  - Use autologous human plasma
  - Primary leukocytes (genetic diversity, epigenetic programs)
  - No exogenous cytokines: autonomous APC development
  - No heat-treated conditions
  - Completely human system

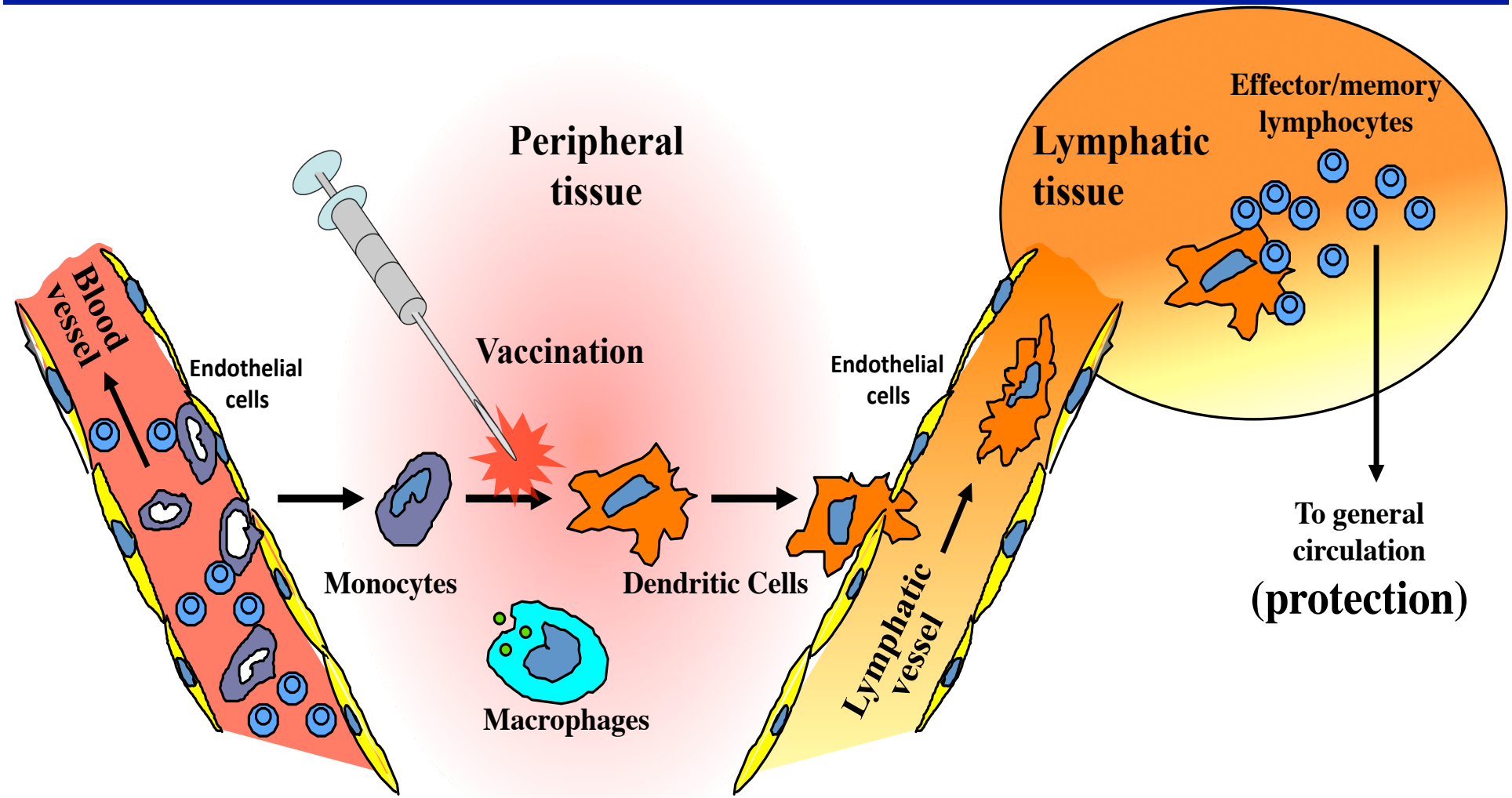
Dialectic: Humoral vs. Cellular Immunity

	Humoralists	Cellularlists
View	Proportion of bodily fluids/ serum components determine health/disease	Cells play a predominant role in diseases, incl. inflammation
Leader	Koch, Ehrlich	Metchnikoff
Nationality	German	French
HQ	Koch Institute, Berlin	Pasteur Institute, Paris

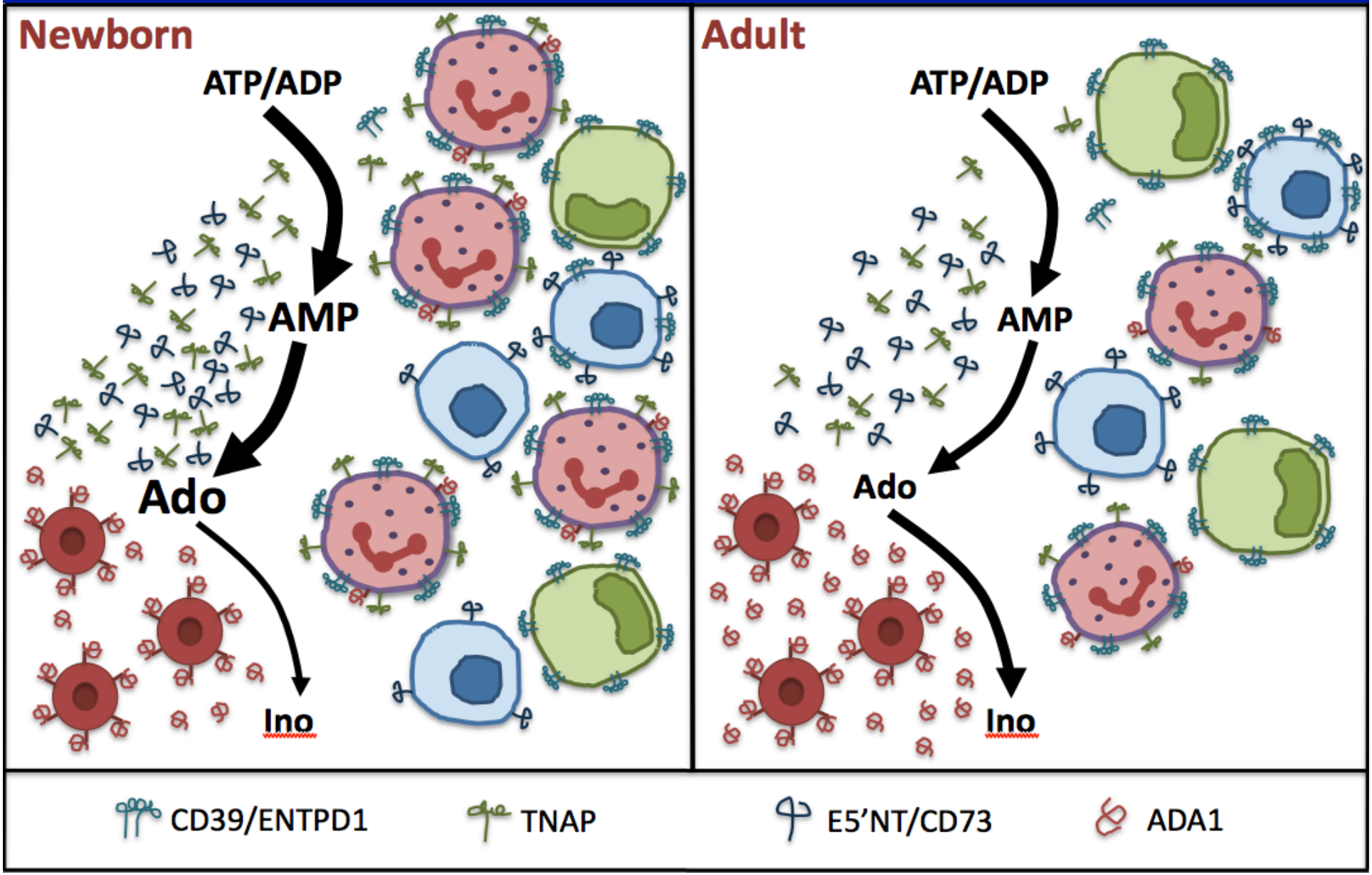

Paul Ehrlich 1854-1915


Elie Metchnikoff

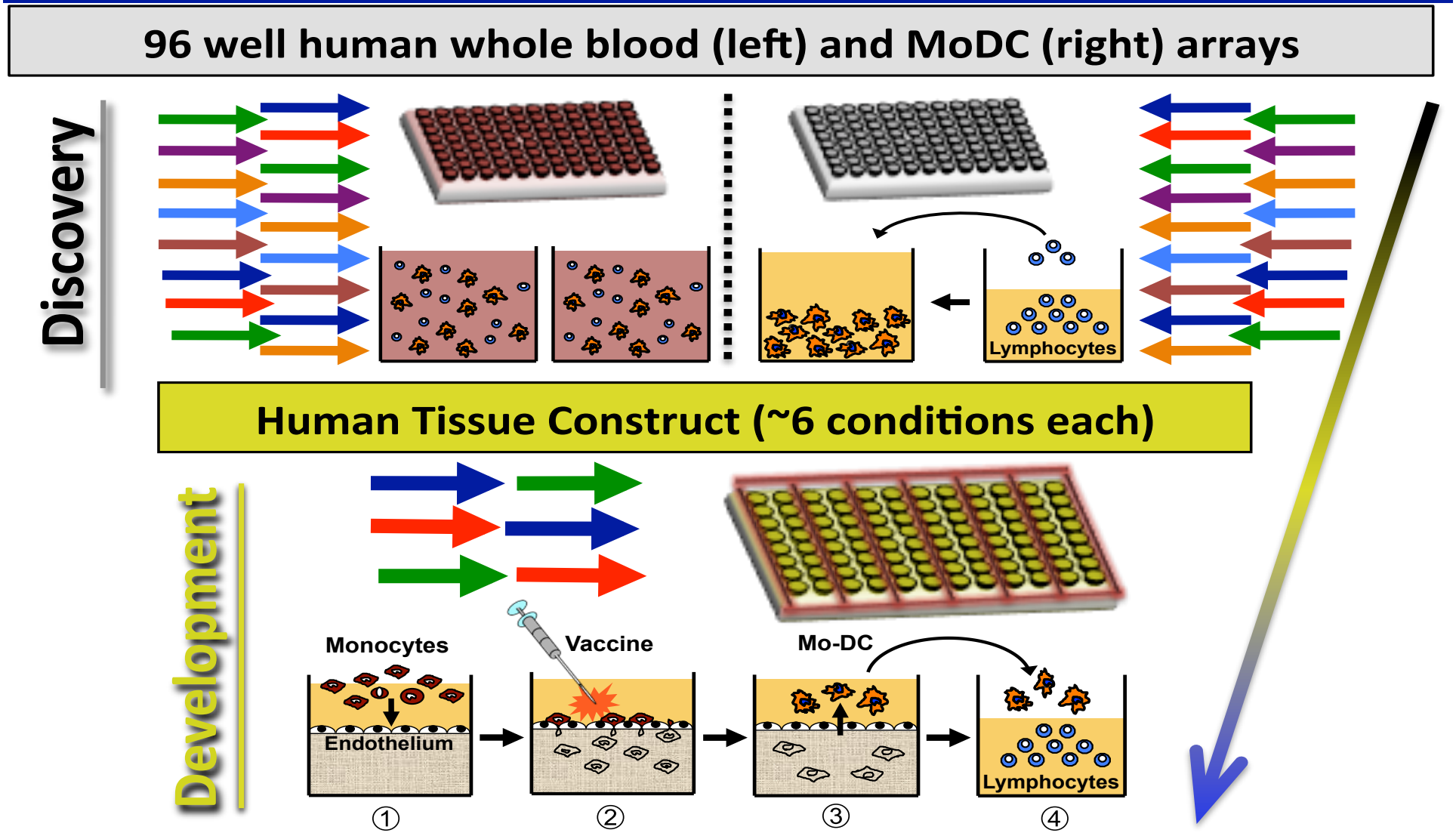
Development of microphysologic systems to model immune ontogeny



High Alk Phosphatase & sCD73 activity in human neonatal plasma coupled with low ADA results in high Adenosine concentrations



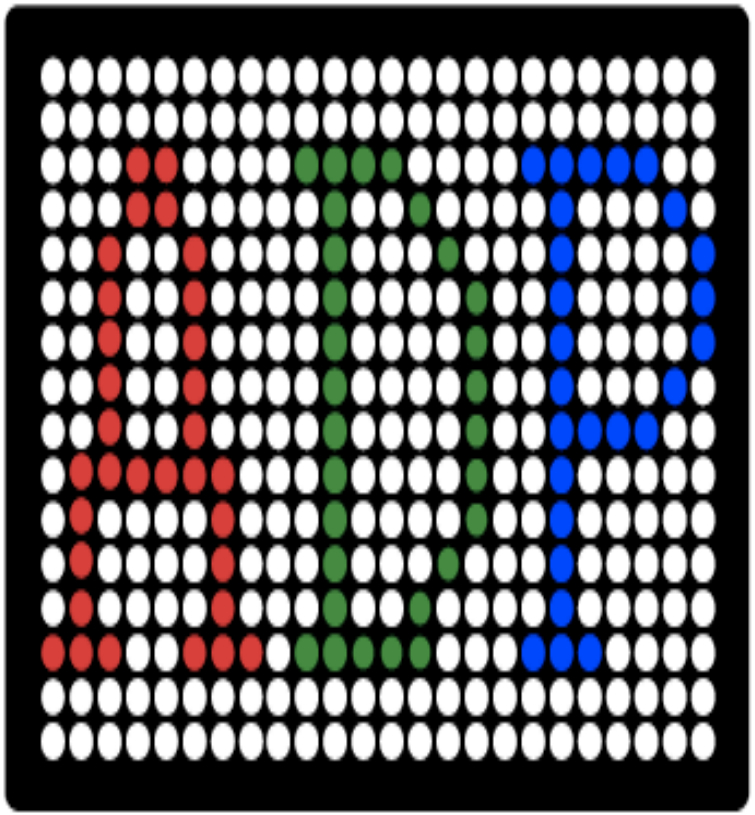
Levy Lab Sequential Approach to Adjuvanted Vaccine Development



National Institute of Allergy and Infectious Diseases

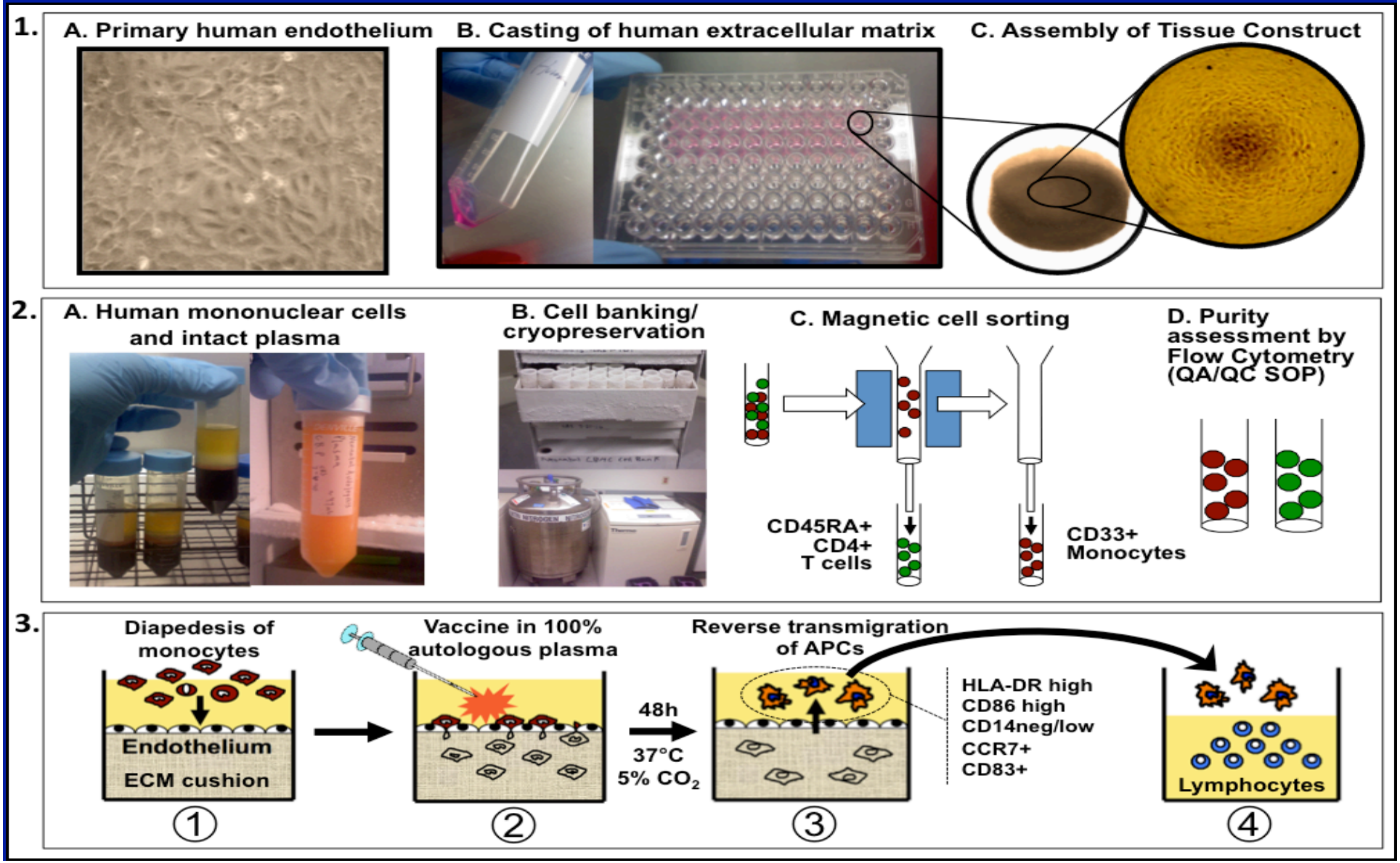
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

Levy Lab, Boston Children's Hospital

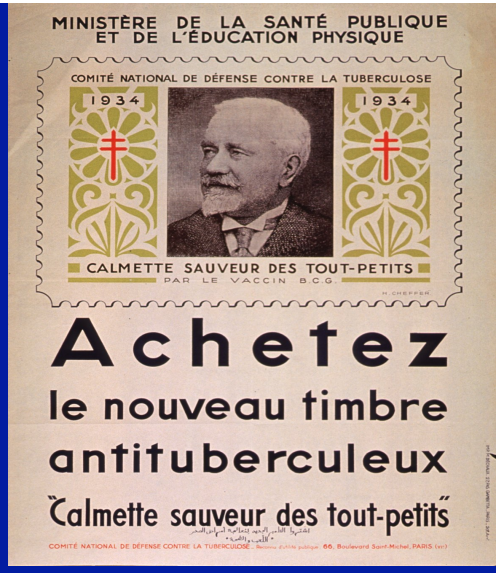


Adjuvant Discovery Program

Creation of 3-dimensional microphysiologic tissue constructs



Neonatal immunization: BCG as proof of concept



- Live attenuated *Mycobacterium bovis*
- Most commonly administered vaccine
- >3 billion doses given (!)
- Efficacy vs disseminated dz/meningitis.
- Potential beneficial heterologous ("non-specific") effects
- Apparently reduces all cause mortality in 1st month of life (Aaby, P *J Infect Dis* 204:245)
- Mechanism: NOD2-dependent epigenetic re-programming of monocytes (Kleinnijenhuis, *PNAS* 2012)

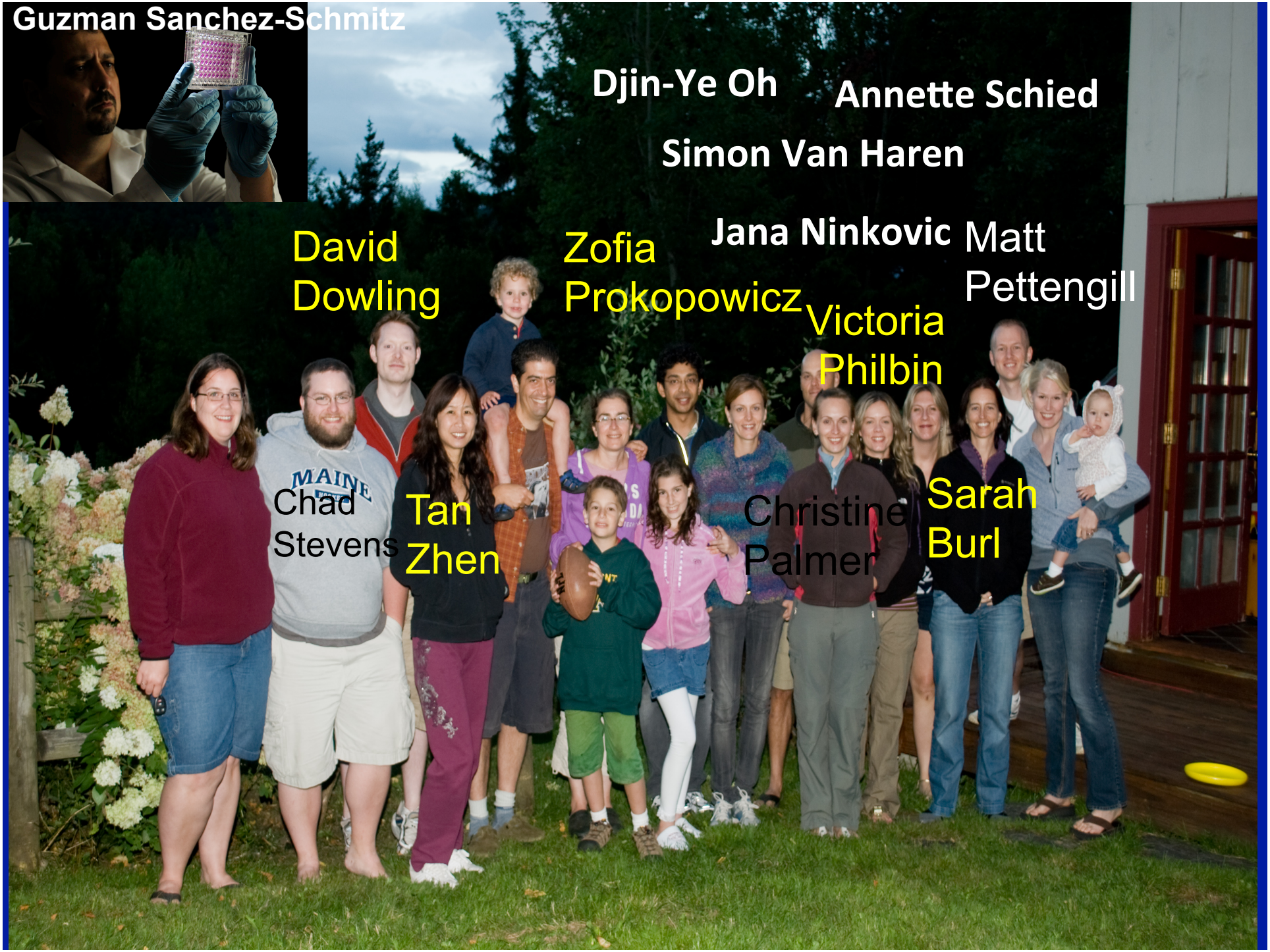

children all agree
one shot is better than three

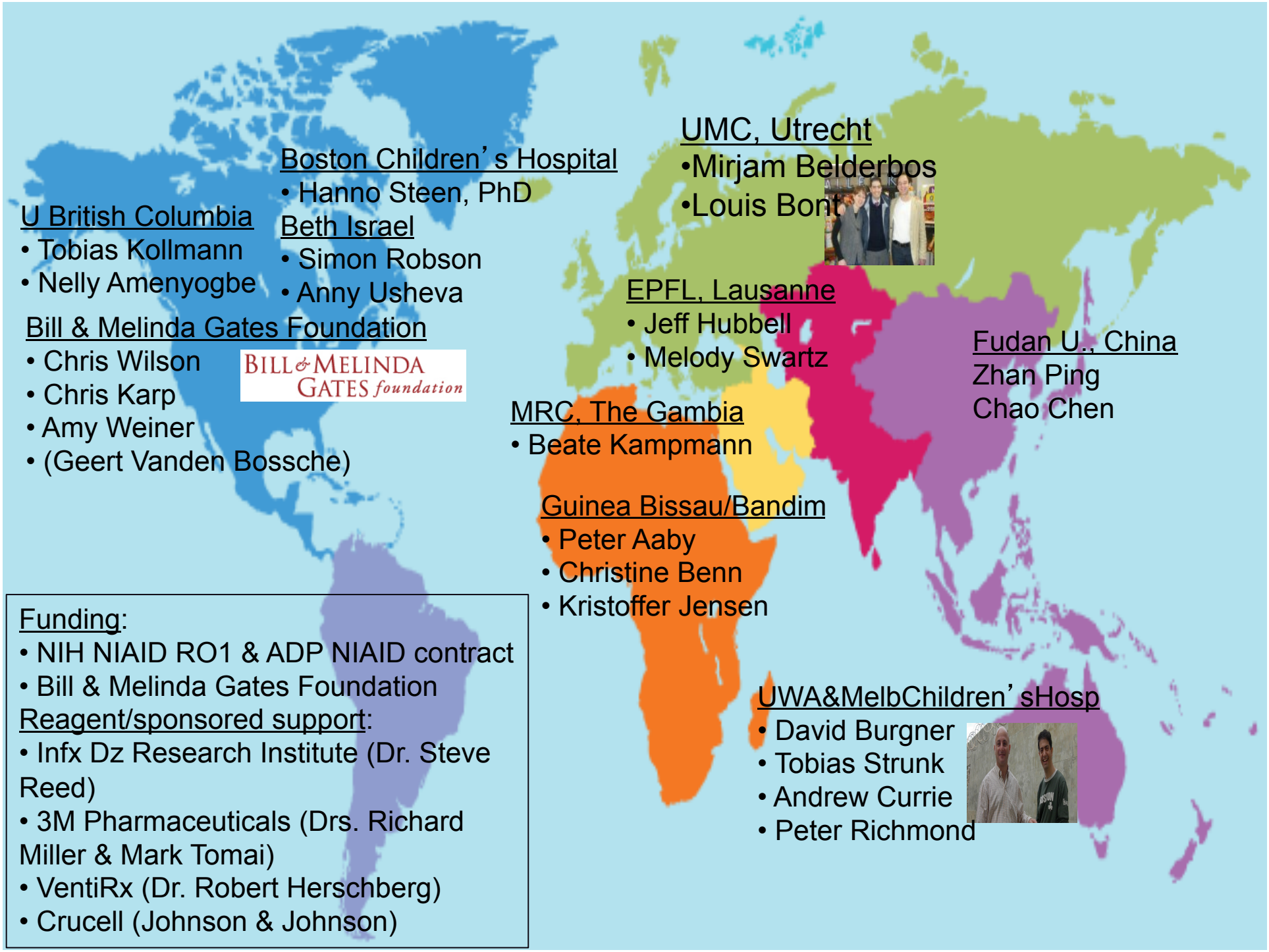
Conclusions

- **Unmet need vaccine formulations to protect those at extremes of life: newborns/infants and elderly.**
- **Human neonatal immune responses are not predictable from those of adults, nor rodent models**
- **Early life responses to inflammation, infection and live vaccines include heterologous (off target/non-specific) effects that may broadly protect vs diverse pathogens**
- **BCG, a live attenuated strain of *Mycobacterium bovis*, activates multiple TLRs is safe and effective and is associated with beneficial heterologous effects at birth.**
- **In vitro systems that model soluble (plasma) and cellular age-specific human immune responses enable:**
  - Identification of age-specific adjuvants to inform pediatric vaccine development- eg, TLR7/8 agonists ID'd in vitro effective in newborn NHPs in vivo
  - Benchmarking new vs. licensed vaccines to accelerate age-specific vaccine development

Questions guiding future efforts

- Are trained immunity/heterologous effects most marked in early life?:
  - Reliance on innate immunity, evidence from BCG, MMR
- What is the impact of immune ontogeny on trained immunity? (preterms, newborns, infants, elderly)
- What are the impact of adjuvants, including Alum and TLRAs, on subsequent immune polarization?
- Can age-specific trained immunity be modeled *in vitro* to accelerate development of vaccines with both specific and heterologous ("off target/non-specific") benefit?
- How can the research community best advocate for the needed support and funding for this highly impactful area?


Guzman Sanchez-Schmitz
Djin-Ye Oh
Annette Schied
Simon Van Haren
David Dowling
Zofia Prokopowicz
Jana Ninkovic
Matt Pettengill
Victoria Philbin
Chad Stevens
Tan Zhen
Christine Palmer
Sarah Burl



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