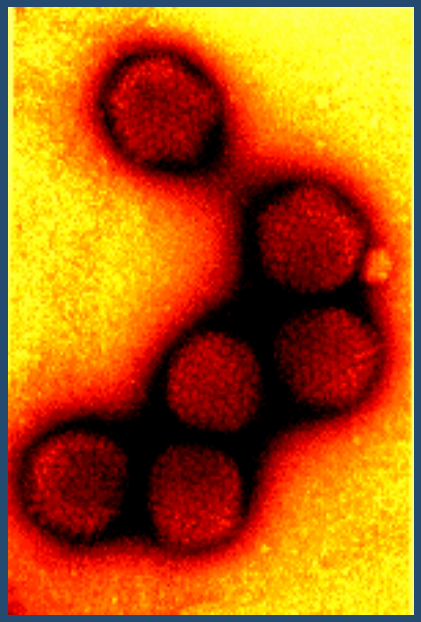

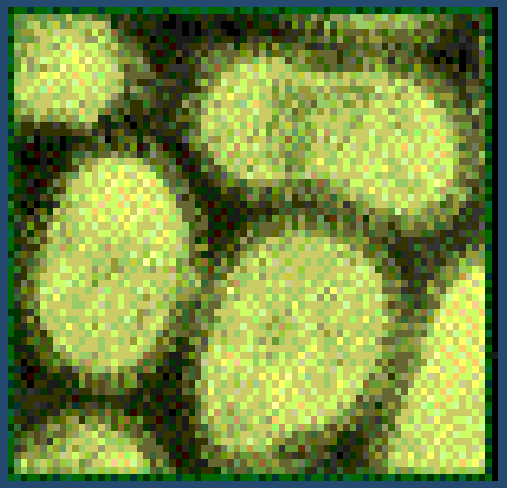

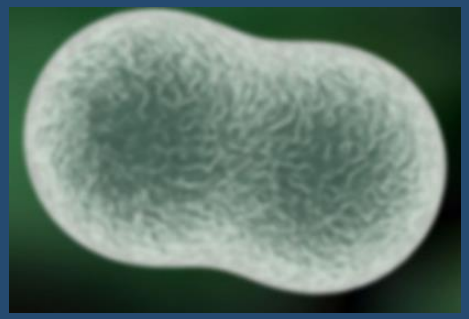

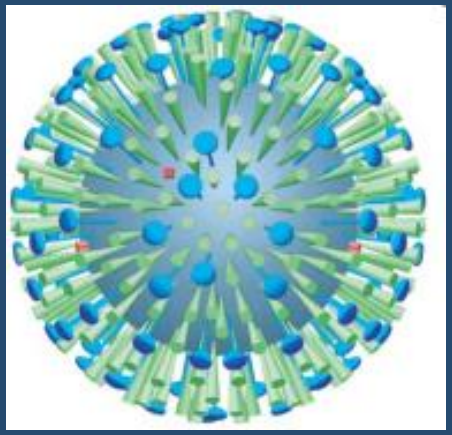

**> 2 million annual infant deaths from infections <u>between 1 and 6 months of age</u>**

**Diarrhoeal diseases**
- Viral (rotavirus)
- Bacterial (salmonella, shigella)

**Acute respiratory infections**
- Viral (RSV, influenza)
- Bacterial (*B. pertussis*, *Hib* *S. pneumoniae*)

*Vaccine-preventable causes of deaths worldwide - J. Clemens et al, Nature Immunol 2010*

# Age-specific patterns of infections in early life

**Neonates (28 d):**

- **fulminant severe infections** *(most pathogens)*
- **higher rates of chronicity** *(HBV, CMV, etc.)*

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- **increased vulnerability to specific bacteria** *(pertussis, encapsulated bacteria…)*

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**Toddlers:**

- **adult-like resistance to viruses**
- **progressive resistance to encaps. bact.**

# Age-specific patterns of infections in early life

| Neonates (28 d): | Infants (1-12 mo): | Toddlers: |
|---|---|---|

- Progressive maturation of the immune system

OR

- Age-specific patterns of immune responses

↔ distinct developmental stages?

# Comparing cord blood to adult peripheral blood

**Decades of studies ↔ numerous differences in B cell, T cell and DC surface markers and *in vitro* response capacity !**

**Table 1**
Summary of reported functional characteristics of cells of neonatal immune systems.

| Cell type | Species | Tissue | Age | Characteristic | Reference |
|---|---|---|---|---|---|
| Dendritic cells | Human | Umbilical cord blood | Birth | Neonatal DC are ineffective in mediating T cell responses to allogeneic or mitogenic stimuli | [54] |
| Dendritic cells | Human | Umbilical cord blood | Birth | Decreased IFNα response to CpG by plasmacytoid DC | [55] |
| T cells | Human | Umbilical cord blood | Birth | Neonatal T cells activated by anti-CD3 antibodies were less effective than adult T cells in inducing Ig secretion by B cells | [63] |
| T cells | Human | Umbilical cord blood | Birth | Neonatal T cells are activated by anti-CD3 antibodies, but unlike adult T cells, are not activated by phorbol myrisate or ionomycin, to express CD40-L | [64] |
| T cells | Human | Umbilical cord blood | Birth | Lower proportion of mononuclear cells are CD3 +ve and density of CD3 on T cells is lower | [65] |
| B cells | Human | Umbilical cord blood | Birth | Neonatal B cells secrete Ig less effectively than adult B cells when stimulated by adult T cell factors | [63] |
| B cells | Human | Umbilical cord blood | Birth | 76% of B cells are CD21 +ve | [68] |
| B cells | Human | Umbilical cord blood | Birth | Higher intensity of expression of IgM on neonatal B cells than adult | [69] |
| | | | | Lower intensity of expression of CD32 on neonatal B cells than adult | |
| B cells | Human | Umbilical cord blood | Birth | Lower expression of CD62L (L-selectin) and CCR7 (chemokine receptor 7) by B cells than for adults | [70] |
| | | | | neonatal cord and adult peripheral B cells respond to BCR cross-linking and CpG with proliferation and upregulation of MHC class II, & CD86 | |

*Adapted from Hodgins DC, Vaccine 2012*

# Neonatal monocytes respond differently to TLR ligands than adult cells

Table 1 | **Polarization of TLR-mediated cytokine responses of neonatal cord-blood-derived monocytes and antigen-presenting cells**

| Cytokine | Relative expression in newborns | Effect of cAMP on cytokine production | General function | Comment | References |
|---|---|---|---|---|---|
| TNF | ↓ | ↓ | Pro-inflammatory; activates neutrophils; $T_H1$-cell response | TNF associated with spontaneous abortion and pre-term labour | 71 |
| IFNα | ↓ | ↓ | Antiviral; contributes to vaccine responses | Important for MHC class I expression | 108,163 |
| IFNγ | ↓ | ↓ | Activation of macrophages; induction of IL-12; $T_H1$-cell response | Newborns have impaired killing of intracellular pathogens | 11 |
| IL-12 | ↓ | ↓ | p40–p35 heterodimer activates cell-mediated immunity; $T_H1$-cell response | Neonatal defect in p35 promoter nucleosome remodelling | 126 |
| IL-1β | ↓ | ↓ | Endothelial adhesion; fever; acute-phase response | Newborn febrile response is blunted | 164 |

*Levy O, Nature Rev Immunol 2007*

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| IL-6 | ↑ | ↑ | Acute-phase response; inhibits tissue neutrophilia; inhibits $T_{Reg}$ cells and promotes $T_H17$ cells | May contribute to acute-phase response at birth | 12,165 |
| IL-8 | ↑ | ↔ | Neutrophil chemoattractant | Expression increased during hypoxia; role in parturition | 166 |
| IL-10 | ↑ | ↑ | Anti-inflammatory; inhibits TNF, IL-1 and IFNγ production | Blocking IL-10 can restore IL-1 production | 14,167 |
| IL-23 | ↑ | ↑ | p40–p19 heterodimer promotes $T_H17$ cells | IL-17 enhances epithelial expression of antimicrobial peptides | 13,125 |

↑, increased; ↓, decreased; ↔, unchanged; cAMP, cyclic AMP; IFN, interferon; IL, interleukin; $T_H$, T helper; TLR, Toll-like receptor; TNF, tumour-necrosis factor; $T_{Reg}$, T regulatory.

*Levy O, Nature Rev Immunol 2007*

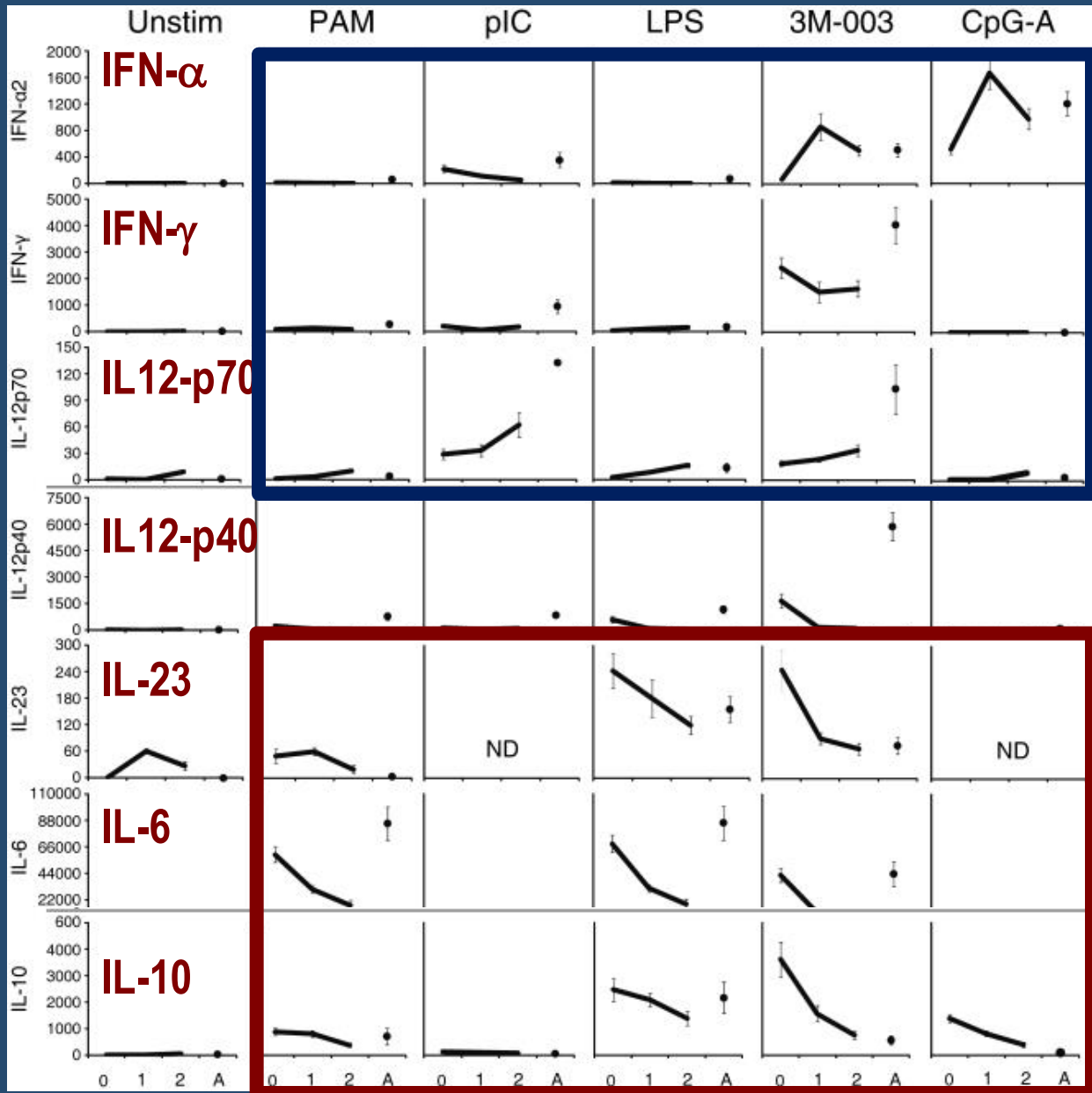
***Neither deficient nor immature… but <u>different</u> !***

# Comparing neonatal and infant responses

**Longitudinal study of monocyte responses to TRL stimulation at birth, 1 and 2 yrs**

- **Slow increase** of **IFN-α** (1 year), **IFN-γ and IL12p70** (2 years)
- Slow decline of **IL17-supporting cytokines** (IL-23, IL-6) and IL-10 (1 year)
- Stimulus-dependent TNF-α responses from 1 month of age

*Corbett NP, Plos One 2010*



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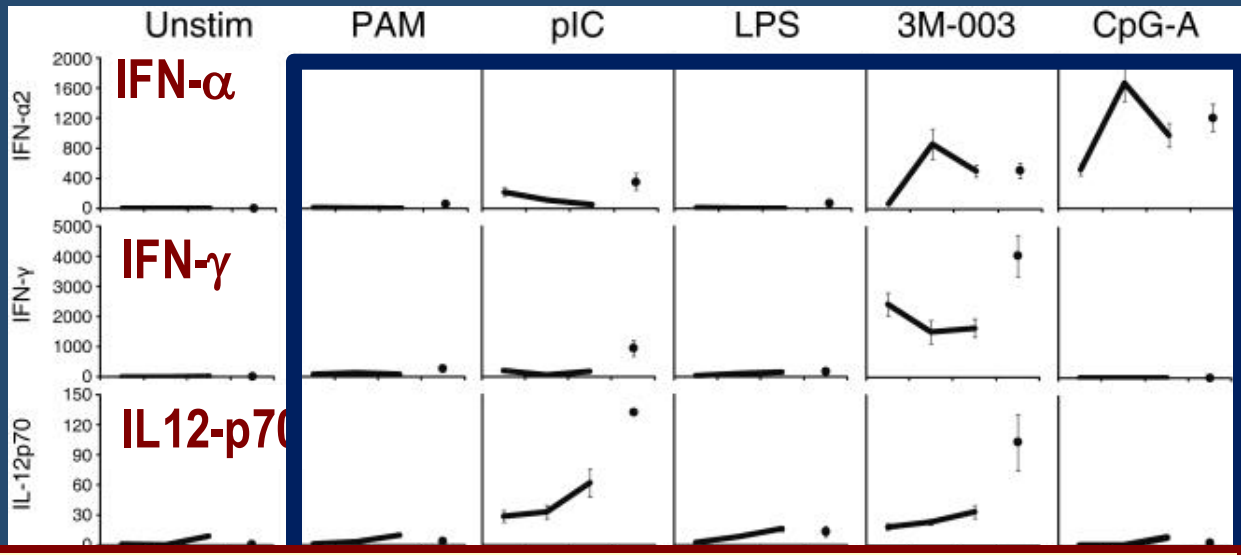


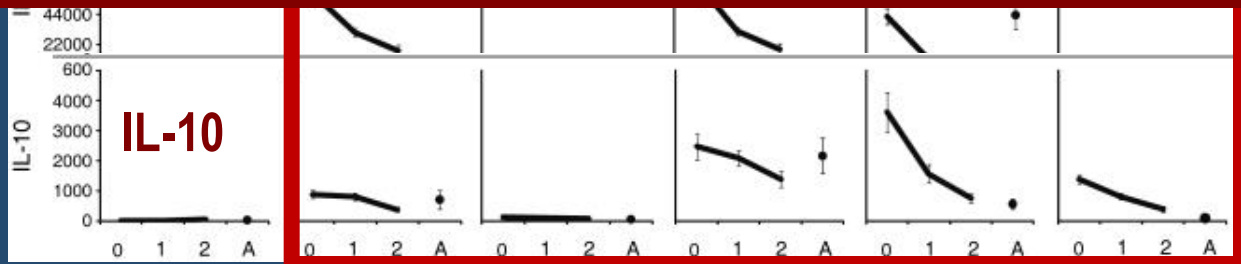

- **Slow increase** of **IFN-α** (1 year), **IFN-γ and**

**Not a smooth progression from an « immature » neonatal pattern to a « mature » adult pattern…**

**… but qualitative and quantitative age-specific differences in innate immune reactivity !**

- Stimulus-dependent TNF-α responses from 1 month of age

*Corbett NP, Plos One 2010*



# Limitations of early life T cell responses: from neonatal tolerance to early life regulation…

In early life, most antigenic stimulations induce

- limited IFN-γ responses
- lower $CD8^+$ responses

**« Neonatal immune tolerance »**

***1950 - 1990***

# Limitations of early life T cell responses:
# from neonatal tolerance to early life regulation…

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- excess Th2 responses
- lower $CD8^+$ responses

**Preferential Th2 polarization !**

*2000 - 2005*

# Limitations of early life T cell responses:
# from neonatal tolerance to early life regulation…

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- specific patterns of innate responses
- limited IFN-γ responses
- excess Th2 responses
- lower $CD8^+$ responses

**Tightly regulated process !**

***2005 – to date***

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**Similar early life immune regulation patterns in human and mice** (distinct kinetics!)

# Why would neonatal responses be similarly regulated in several species ?


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| IL-10 | ↑ |
| IL-23 | ↑ |

↑, increased; ↓, decreased; ↔, unchanged; cAMP, cyclic AMP; IFN, interferon; IL, interleukin; $T_H$, T helper; TLR, toll-like receptor; TNF, tumour necrosis factor;

**To avoid potent inflammatory responses:**

- limits the risks of alloimmune reactions between mother and fetus
- limits the risks of premature labor (TNF, IL1β)
- limits inflammatory responses at sites of intense neonatal colonization *(skin, gut…)*
- favors tolerance induction to common or self Ag ↔ reduction of the risk of autoimmunity
- avoids potentially harmful immune-mediated responses to common viral pathogens

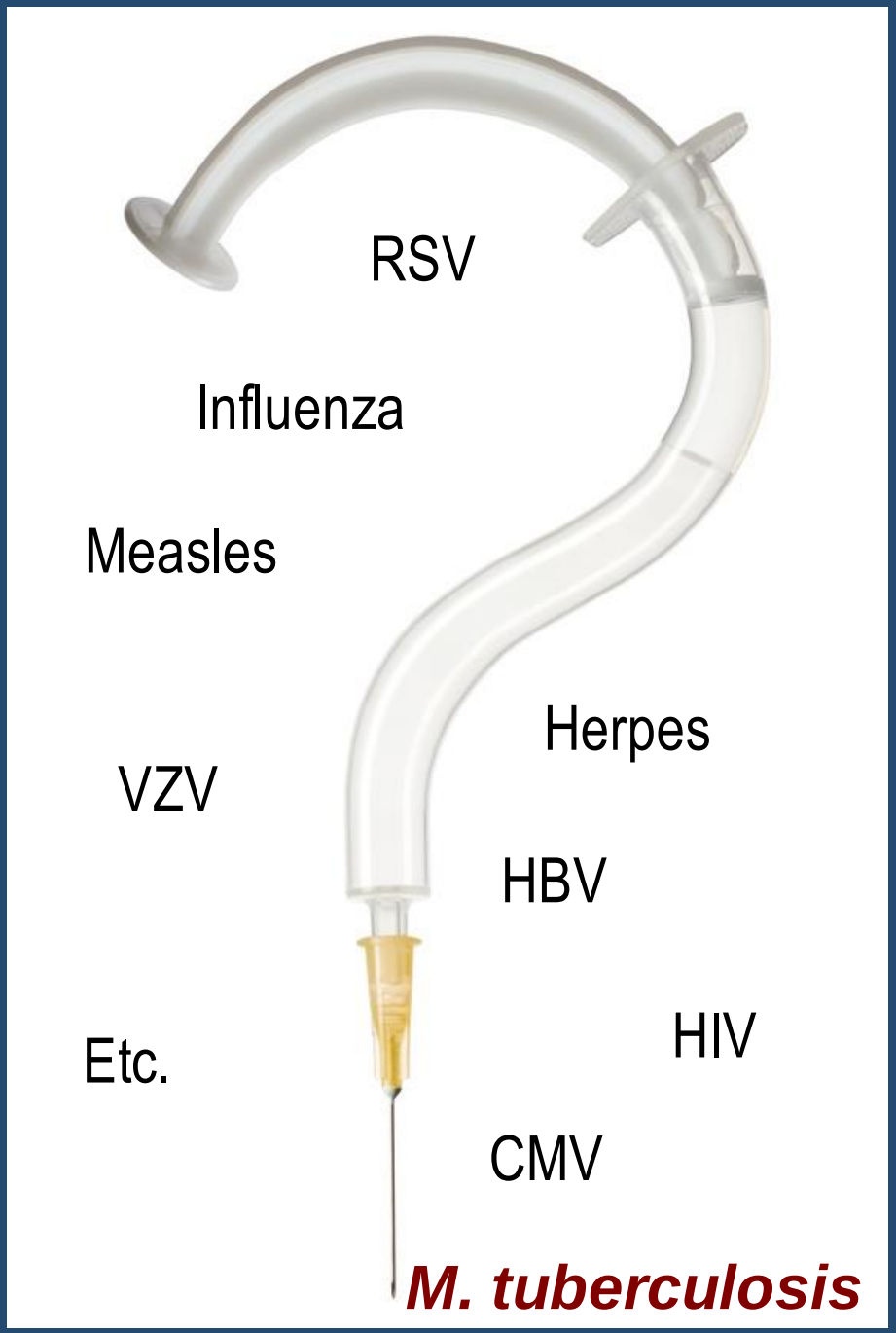
~~Deficiencies~~ → Adaptation !

# Age-specific patterns of infection in early life

| Neonates (28 d): | Infants (1-12 mo): | Toddlers: |
|---|---|---|

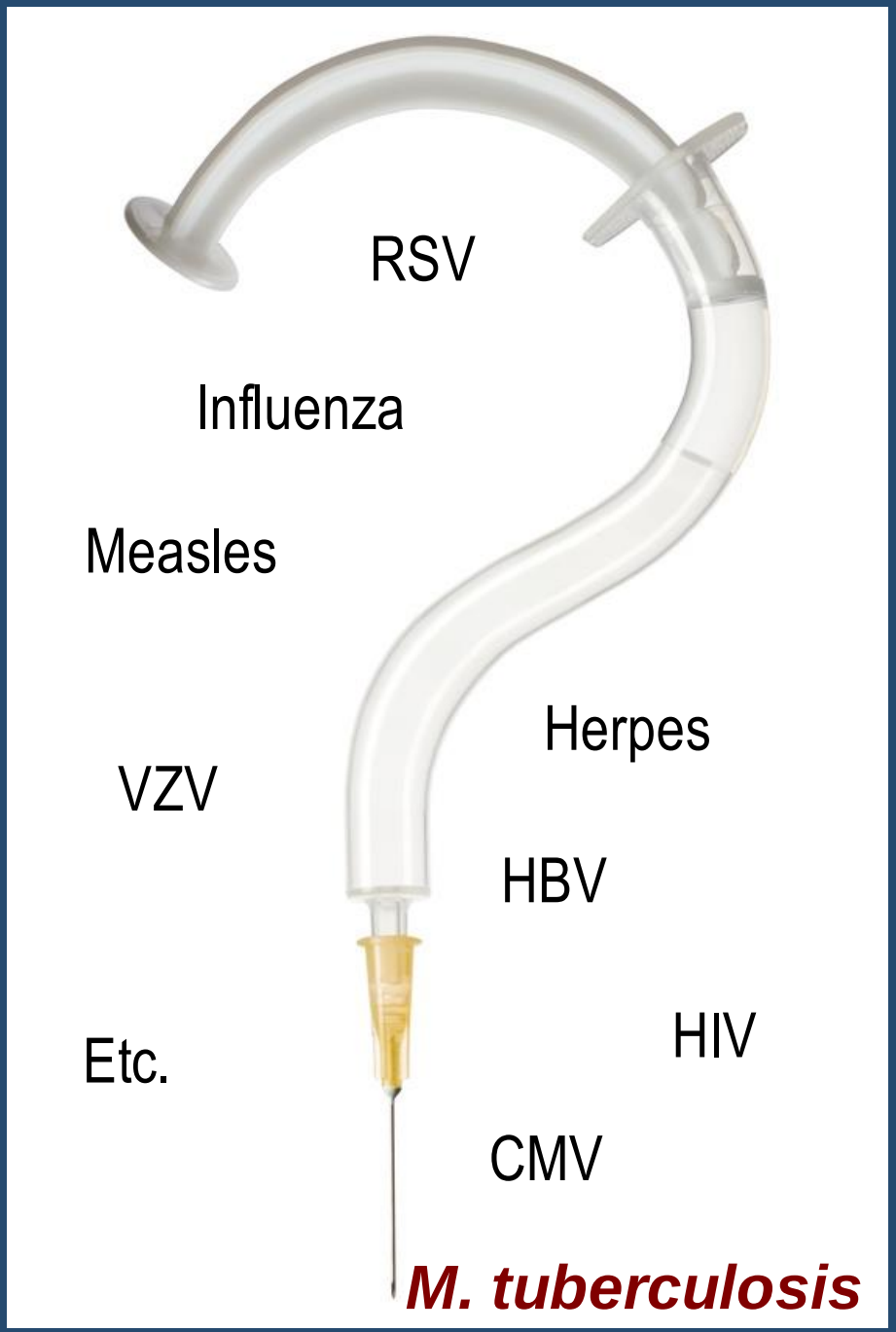
**Age-specific patterns of innate immune responses ↔ various developmental stages**

**Regulation of adaptative responses?**




# Inducing Th1 and CD8+ T cell responses in an immune system adapted against such responses ?

**Avoiding Th2-generating anti-inflammatory signals**




# Inducing Th1 and CD8+ T cell responses in an immune system adapted against such responses ?

**Focused DC targeting**

↓

**Exquisite DC activation**

↓

**Avoiding Th2-generating anti-inflammatory signals**

↓

**Potent Th1 and CD8+ responses**

*Kamath A, EJI 2008; Kamath A, Plos One 2008; Kamath A, Plos One 2009; Kamath A, JI 2012*

# Th1/CD8$^{+}$ neonatal T cell responses may be safely elicited under specific conditions


**In early life, most antigenic stimulations induce**

- **specific patterns of innate responses**
- **limited IFN-γ responses**
- **excess Th2 responses**

**Vaccine-type dependent !**

*Live vectors, targeted adjuvants, etc.*

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- excess Th2 responses

**Vaccine-type dependent !**

*BCG, live vectors, LAIV (≥ 6 mo), …*


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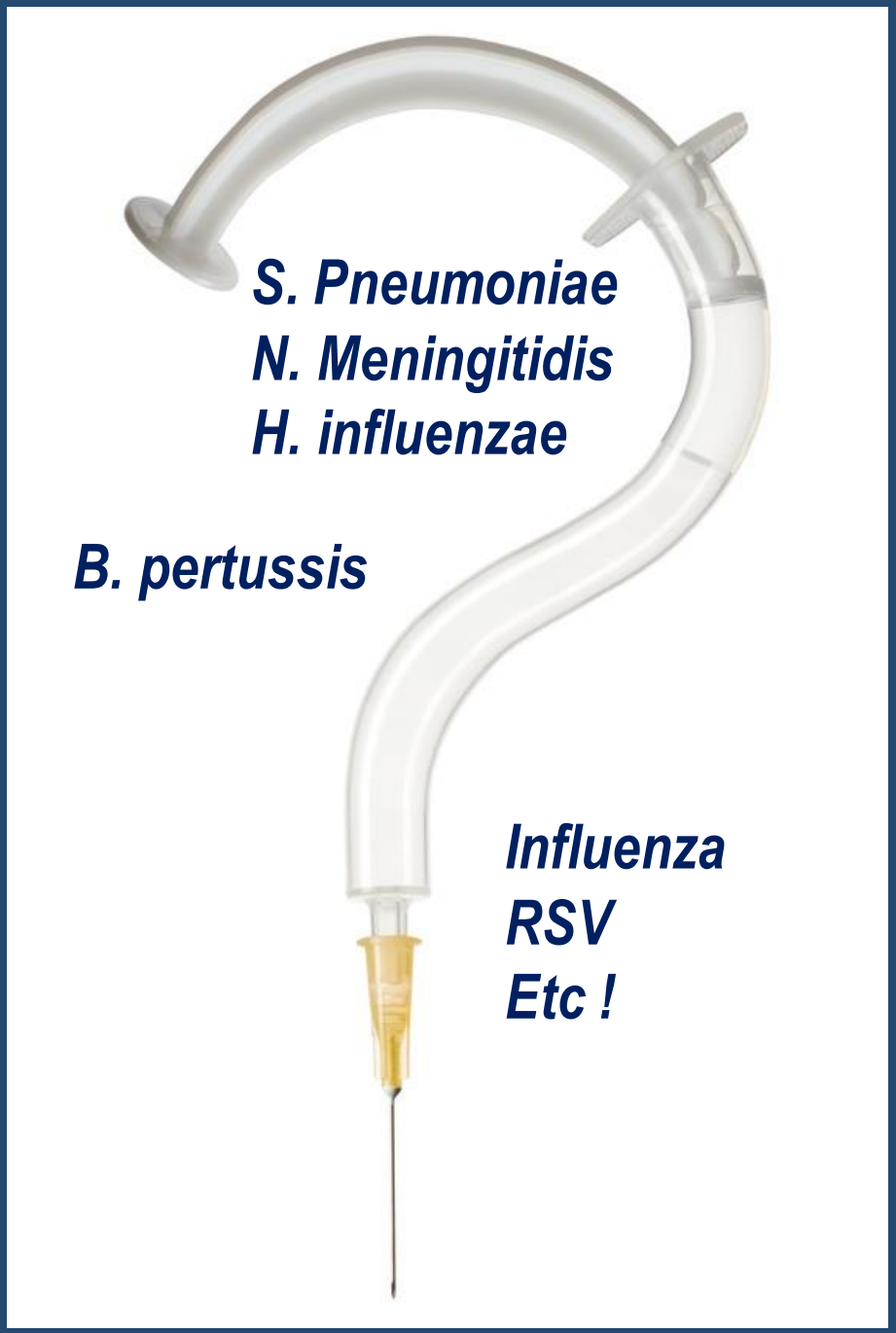
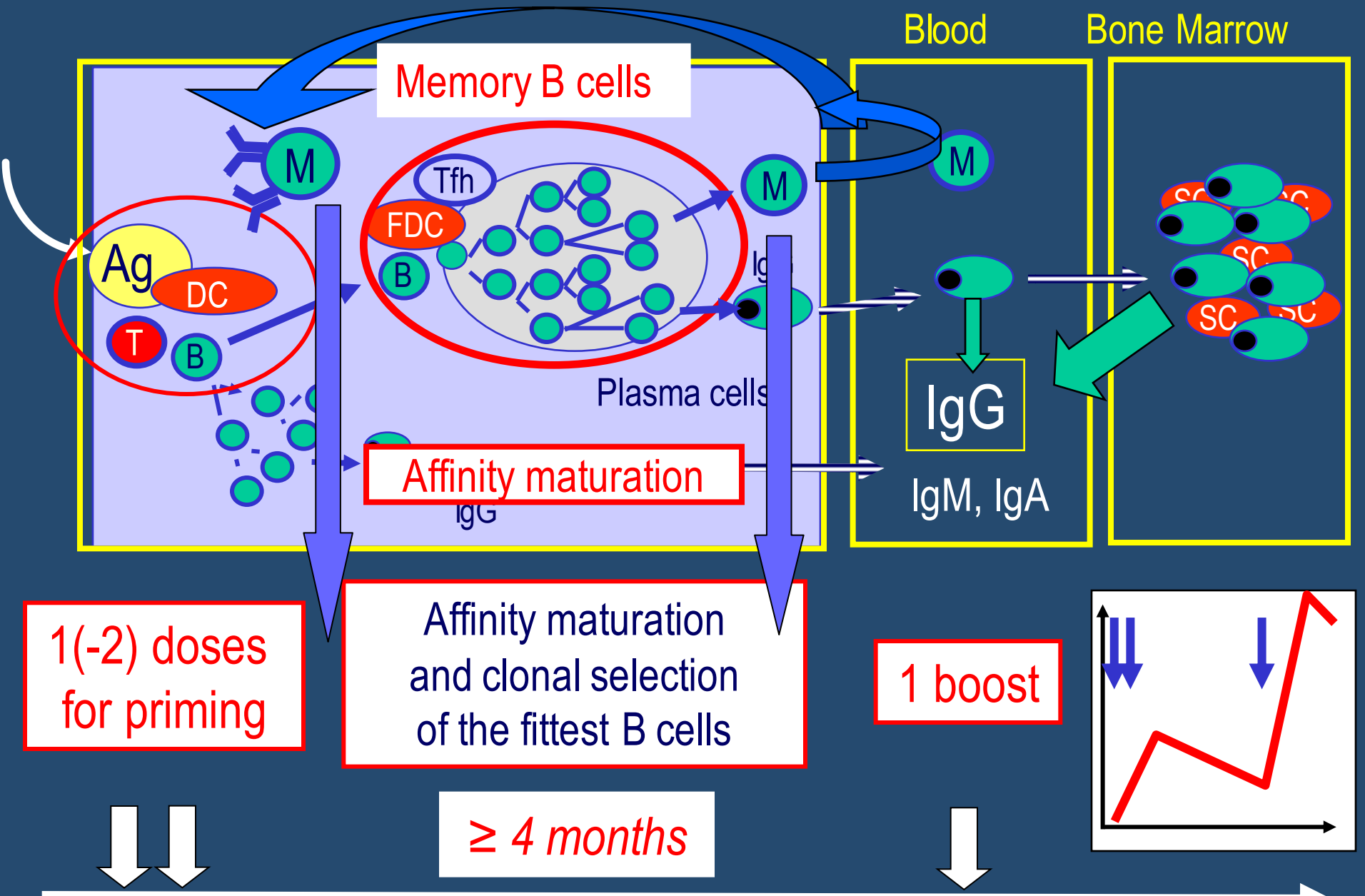
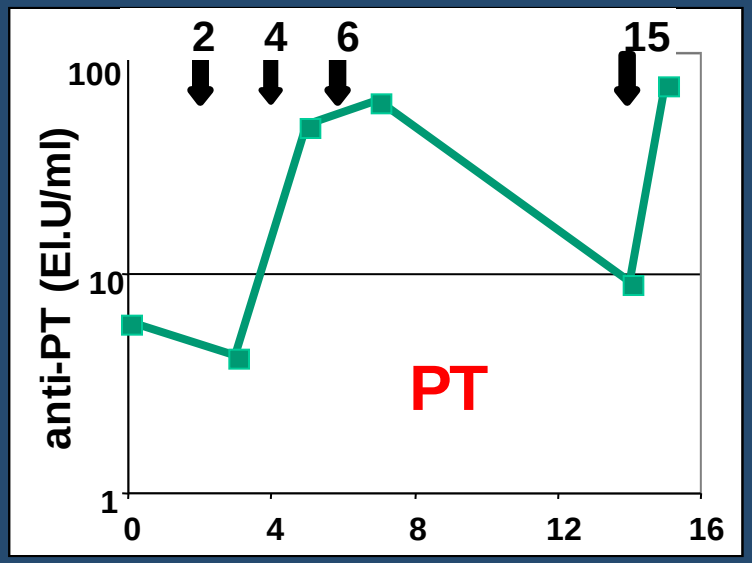


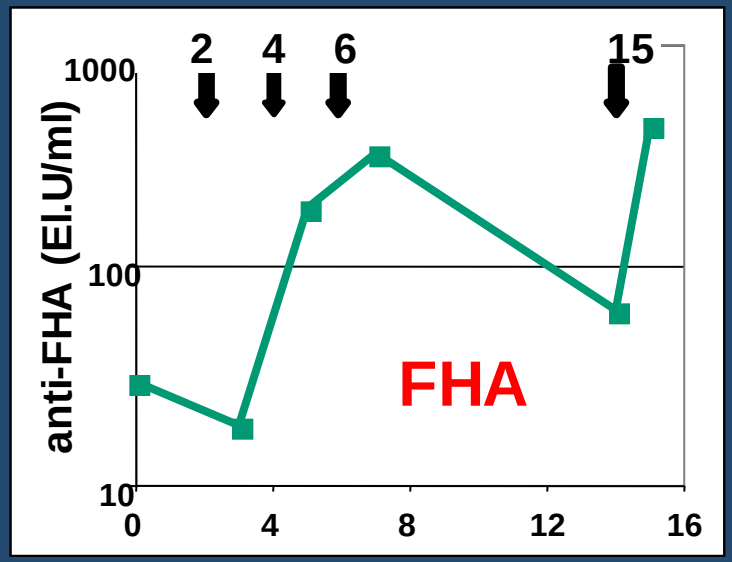

# Early life B cell responses

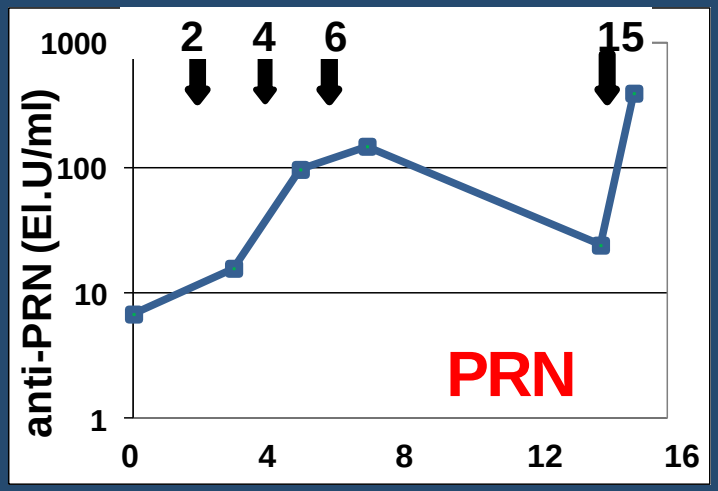
# 0 -1- 6 : the “classical” immunization schedule !



# Early life antibody vaccine responses: acellular pertussis as an example



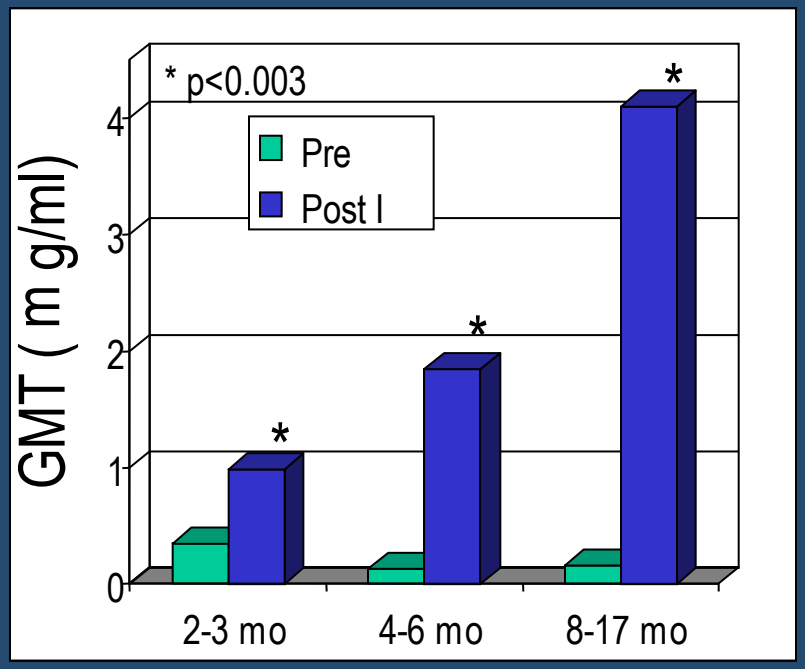

DTaP at 2-4-6 months

- 1st dose: very low responses !
- 2nd dose: marked increase
- 3rd dose: small increase
- Rapid Ab waning (< 12 mo)!!
- Effective boosting

*Knuf et al J Ped 2008; J Ped 2010*

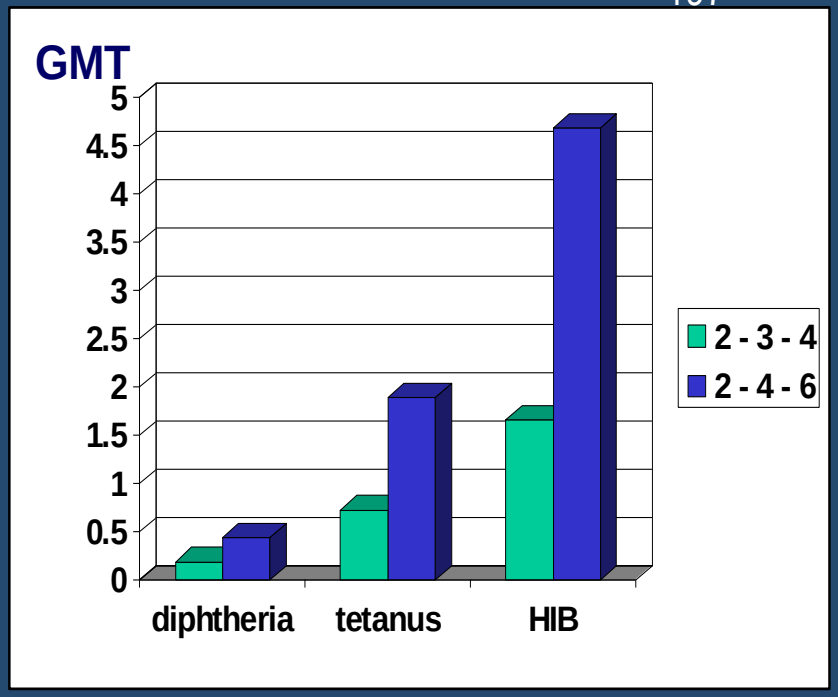
# Vaccine antibody responses slowly increase with age

PRP-OMC, anti-Hib




*Einhorn Lancet. 1986*

DTP, PRP-TT or PRP-CRM$_{197}$




**Glycoconjugate vaccines :**

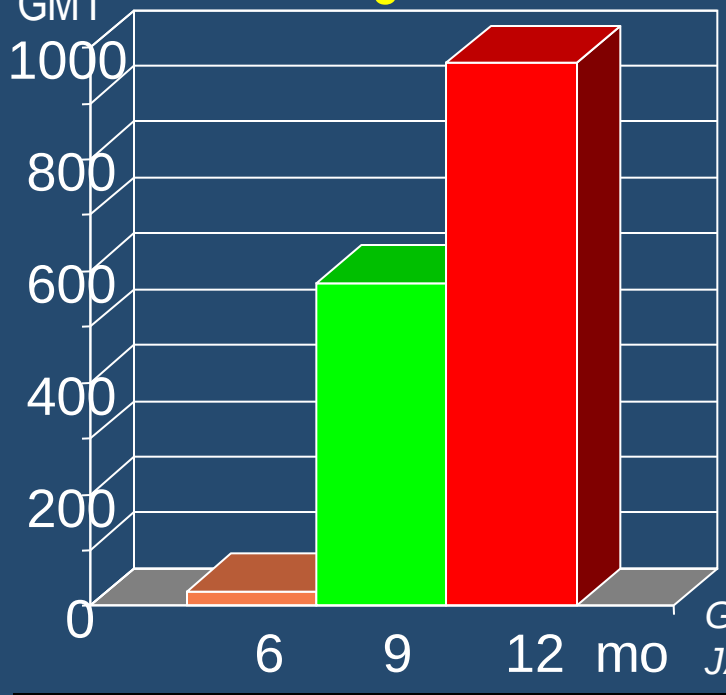
**< 6 months : 3-4 doses**

**≥ 12 months : 1 dose**

- 6 – 10 – 14 weeks
- 2 - 3 - 4 months
- 2 - 4 - 6 months
- 3 - 5 - 12 months

# Vaccine antibody responses slowly increase with age

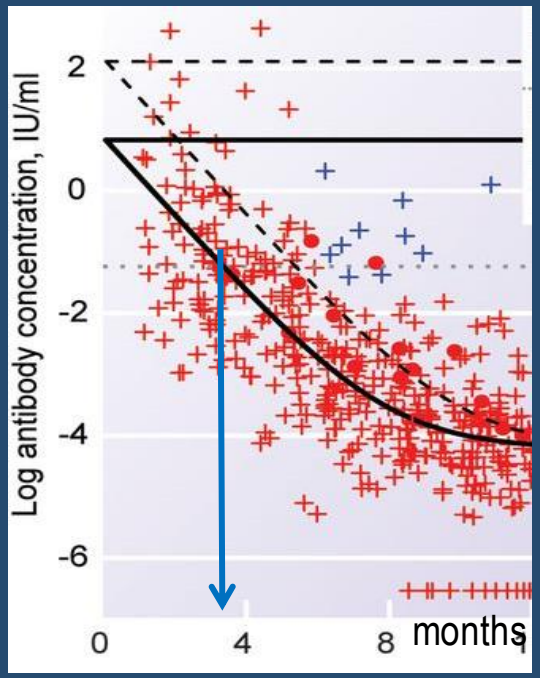
Measles responses
in seronegative infants




*Gans HA JAMA 1998*

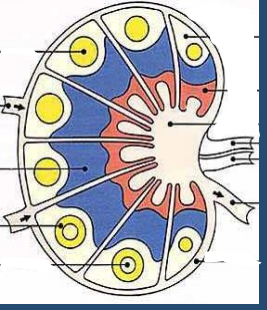
**% with neutralizing Ab titers:**
**Immunization ≥9 months : 100%**
**Immunization 6 months : 36%**

Rapid decay of MatAb in measles immunized mothers

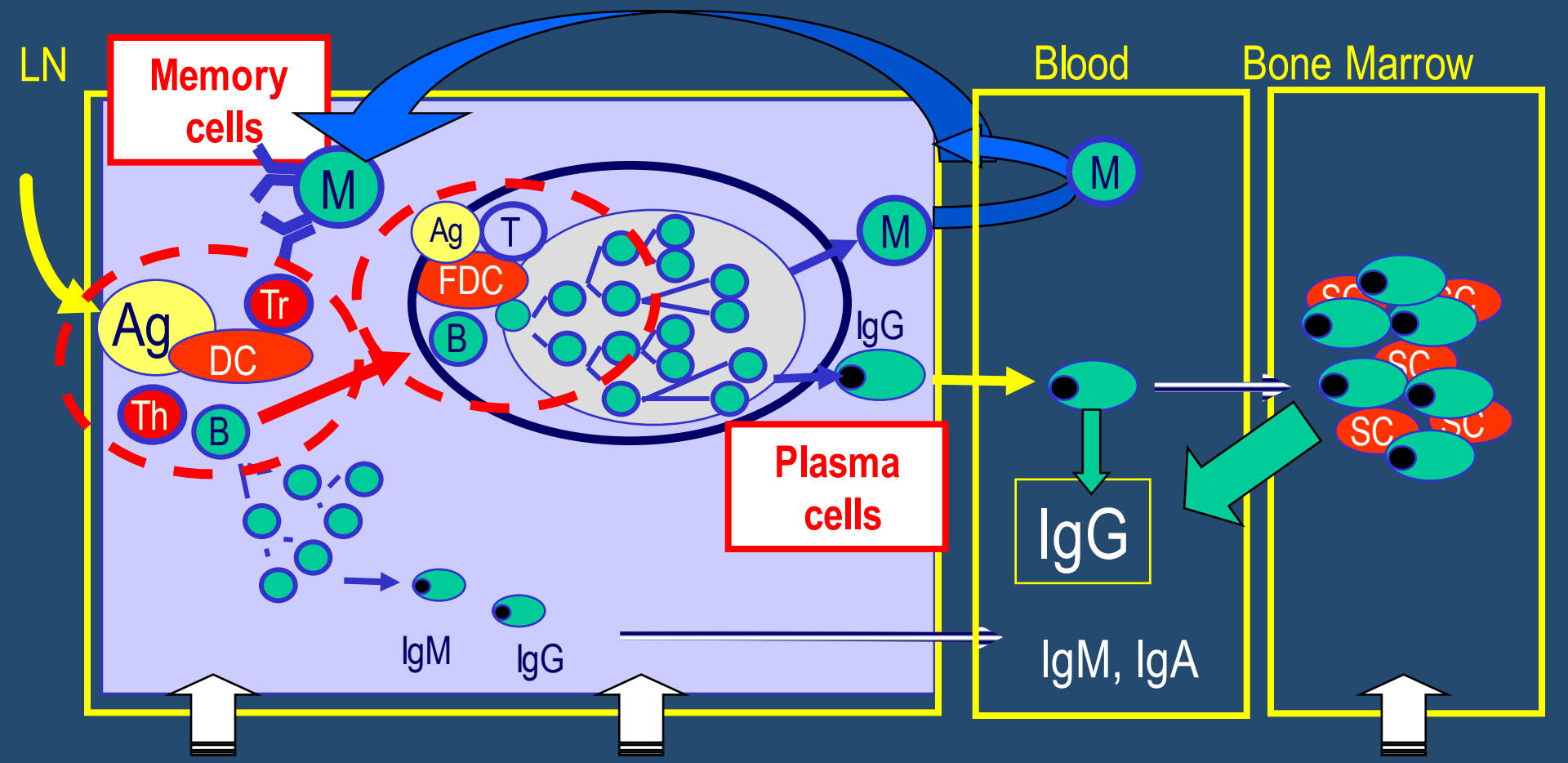


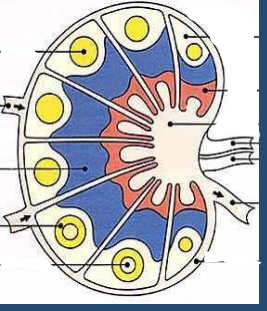

*Waaijenborg S J Infect Dis. 2013*

**Estimated duration of sero-protection: 3.3 months !**



# Why are antibody responses limited in early life ?





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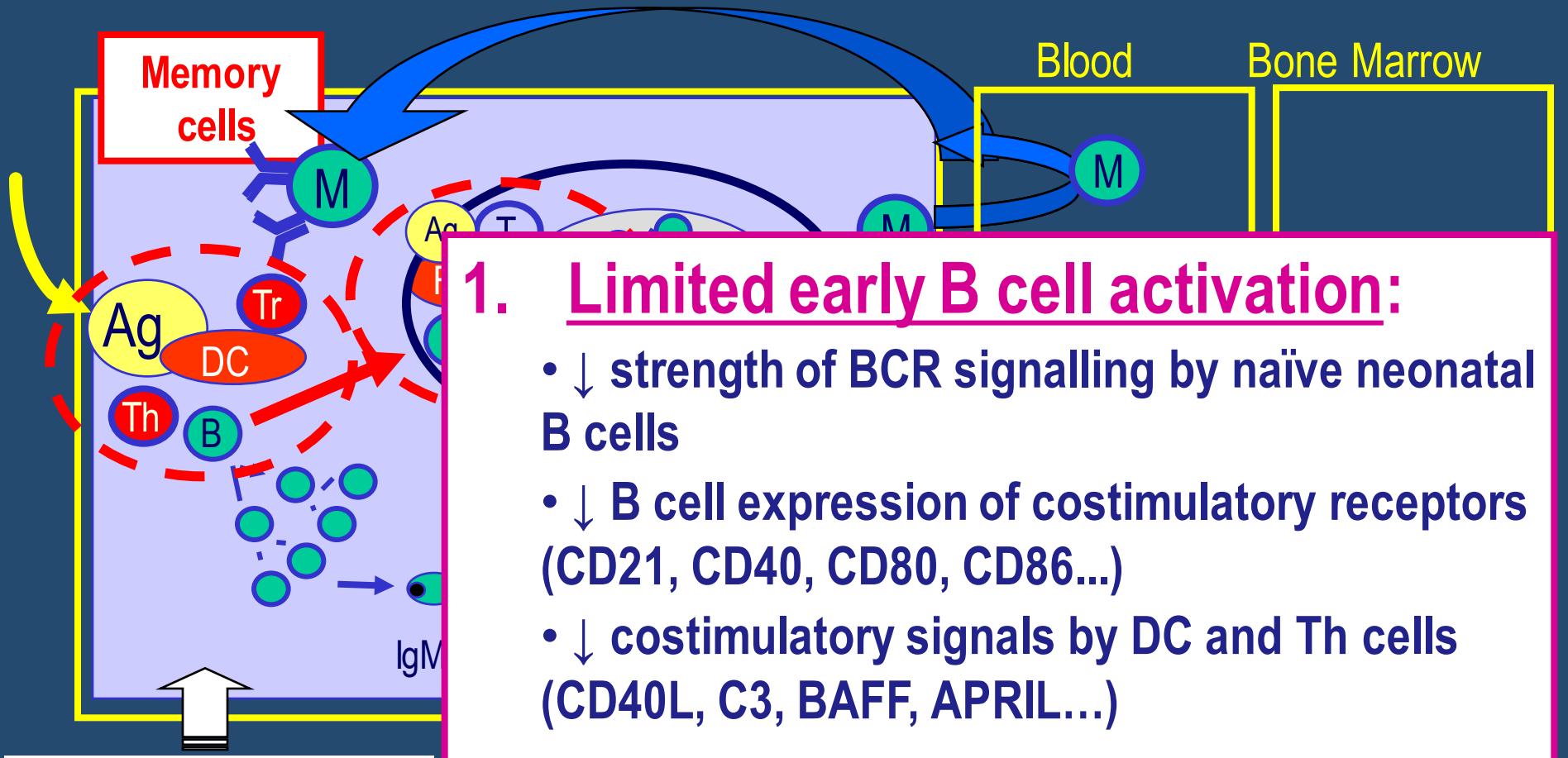
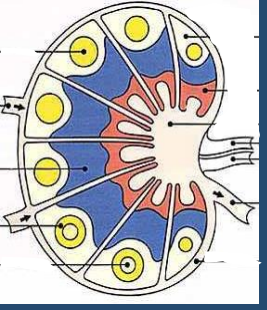



## 1. Limited early B cell activation:

- ↓ strength of BCR signalling by naïve neonatal B cells
- ↓ B cell expression of costimulatory receptors (CD21, CD40, CD80, CD86...)
- ↓ costimulatory signals by DC and Th cells (CD40L, C3, BAFF, APRIL…)
- ...

**B cell activation (DC, Th cells) ↓**

← **Regulated innate responses**



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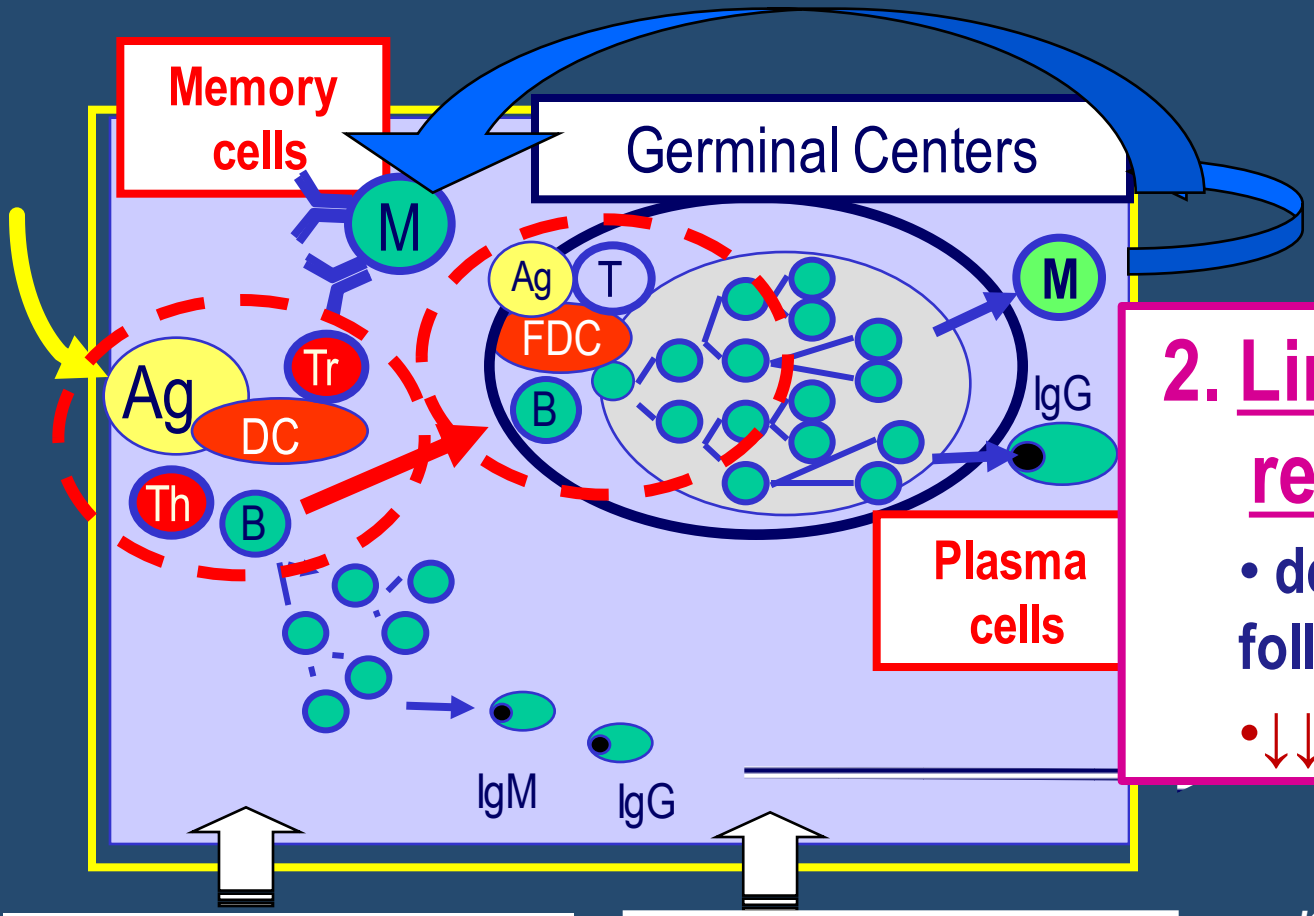
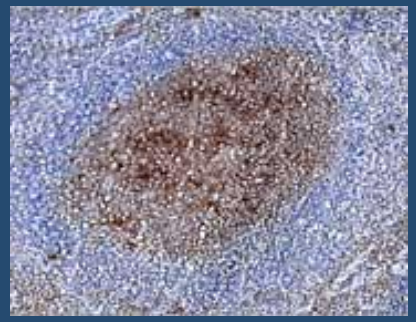


## 2. Limited GC responses:

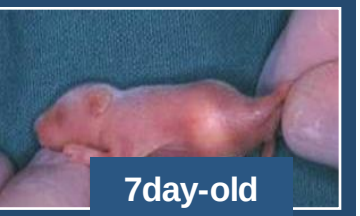
- delayed maturation of follicular DC *(mice, ♂♀?)*
- ↓↓ CD4⁺ Tfh *(mice, ♂♀?)*

**B cell activation (DC, T cells) ↓**

**Germinal center responses ↓↓**

*Kruschinski C, Hum Pathol. 2004*

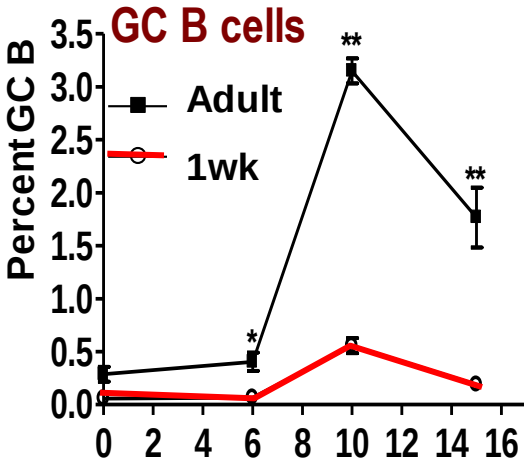
*Pihlgren M, J Immunol 2003*
*Mastelic B J Immunol 2012*

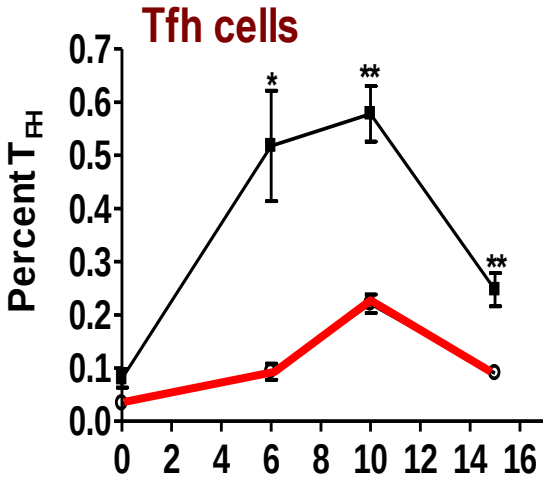


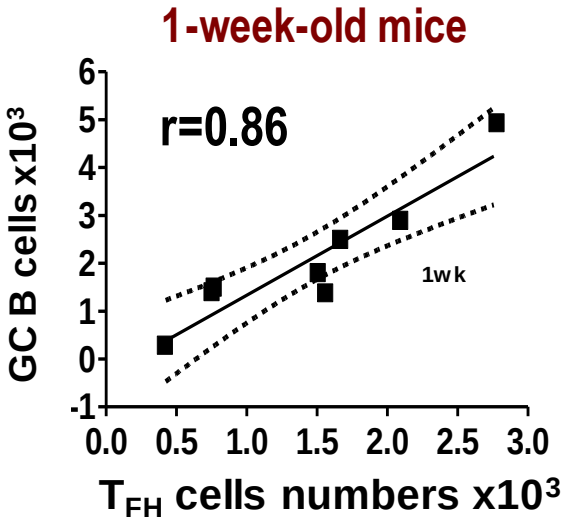

# Follicular helper T cells fail to expand following early life immunisation

**Tetanus toxoid/alum, 1 dose, Balb/c**

*Mastelic B, JI 2012*

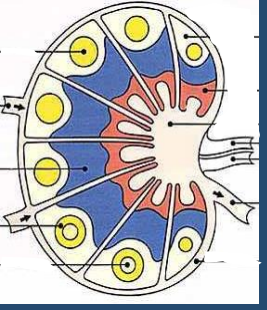



- The limited induction of GC B cells in early life results from the limited expansion of T follicular helper cells with ↓ properties)
- Strong direct correlation between early life Tfh and GC B cells

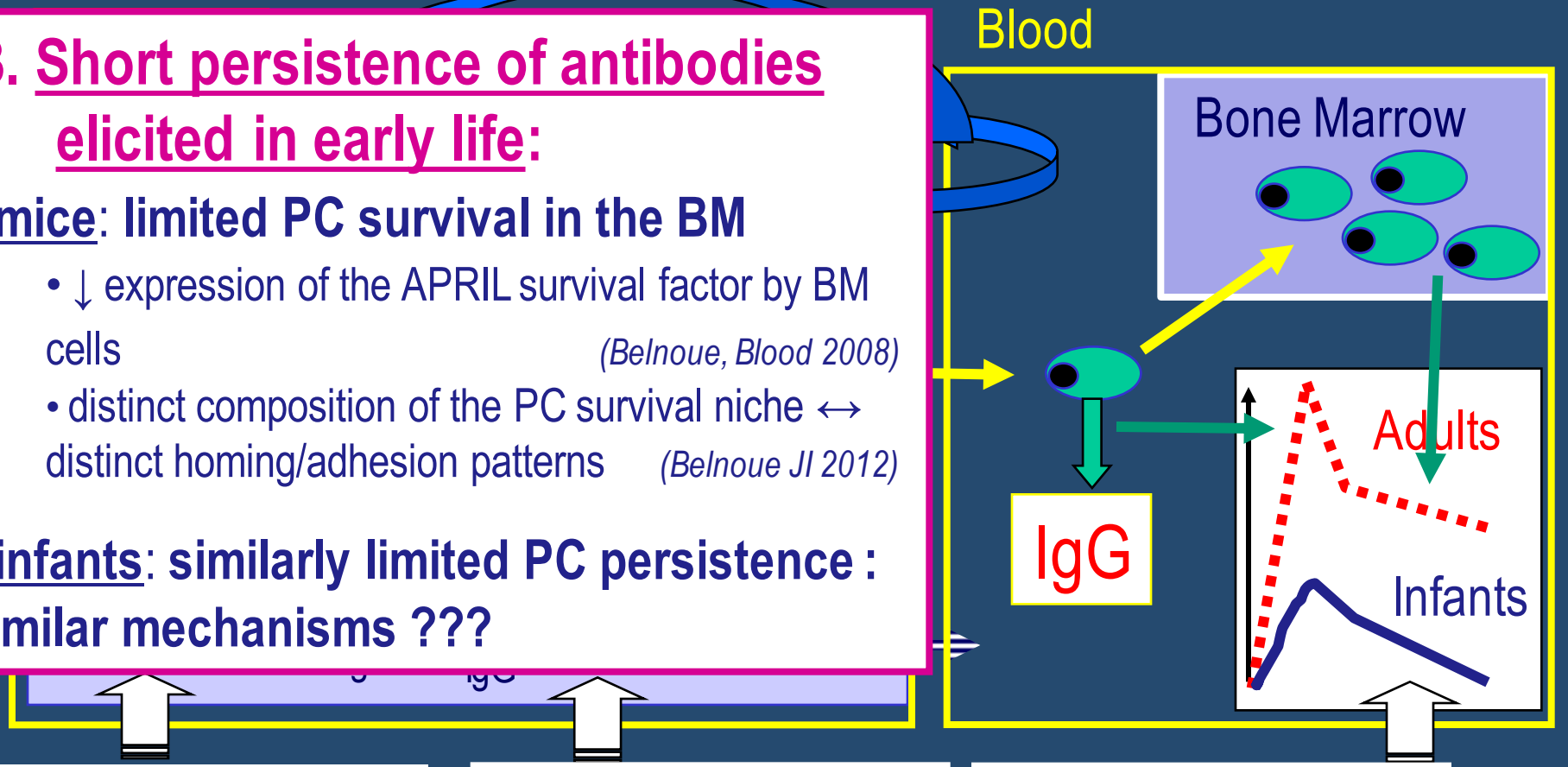
***Adjuvants supporting Tfh expansion in early life ?***



# Why are B cell responses limited in early life ?

## 3. Short persistence of antibodies elicited in early life:

- **mice: limited PC survival in the BM**
  - ↓ expression of the APRIL survival factor by BM cells *(Belnoue, Blood 2008)*
  - distinct composition of the PC survival niche ↔ distinct homing/adhesion patterns *(Belnoue JI 2012)*
- **infants: similarly limited PC persistence : similar mechanisms ???**



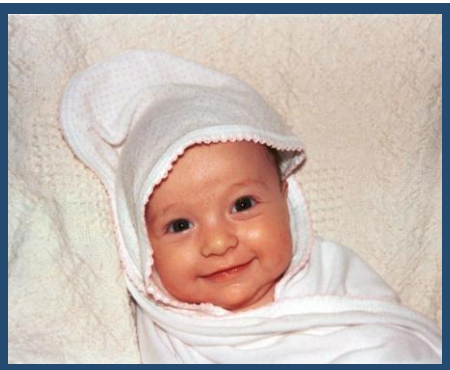

**B cell activation (DC, T cells) ↓**

**Germinal center responses ↓↓**

**Bone marrow plasma cells ↓↓**

# Early life B cell responses

***Infant B cell responses (measles, TT, DT, Pw/Pa, HBsAg, pneumo, HIV, etc…)***



**Age-dependent limitations of peak IgG / IgM responses**

**Lower resp. to most PS**

**Shorter Ab persistence**

**Inhibition by MatAb**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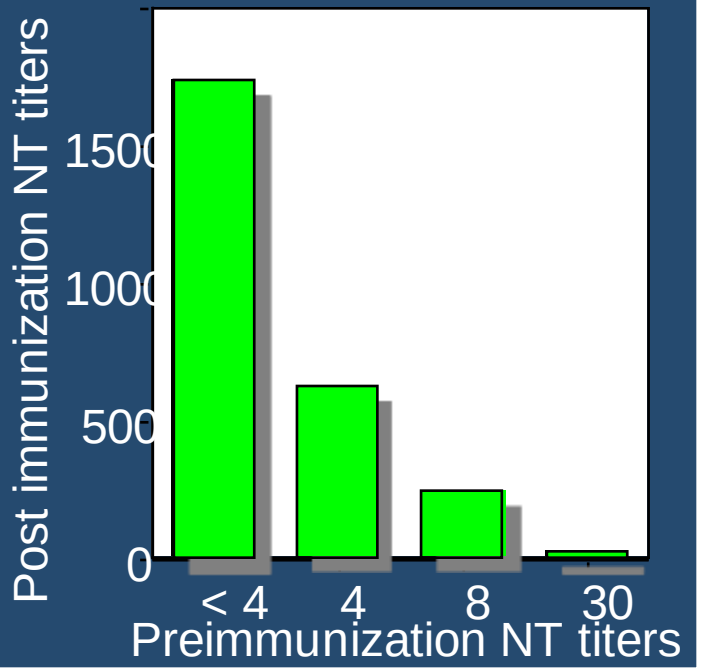
*Siegrist CA, Vaccine 2001*

*Siegrist CA Nature Rev Immunol 2009*

# Influence of maternal antibodies on infant vaccine responses

Inhibition by Maternal Ab

*Albrecht, Pediatrics 1977*




**Inhibition of Ab responses:**

- Mostly affects live vaccines *(neutralization before vaccine replication ?)*
- Affects non live vaccines at titers that depend upon MatAb / Ag ratio ↔ variable
- Is epitope-specific ↔ involves FcγR

*Siegrist CA, Vaccine 2003, 21:3406-3412*

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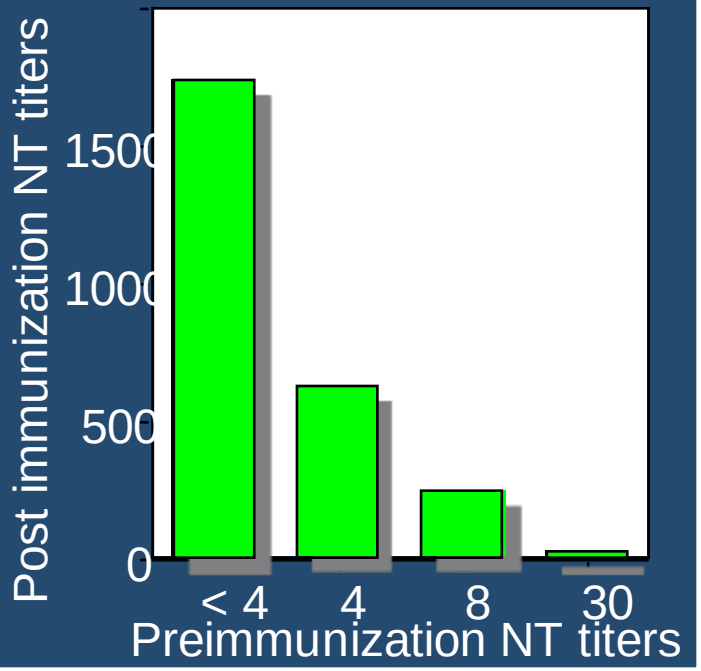


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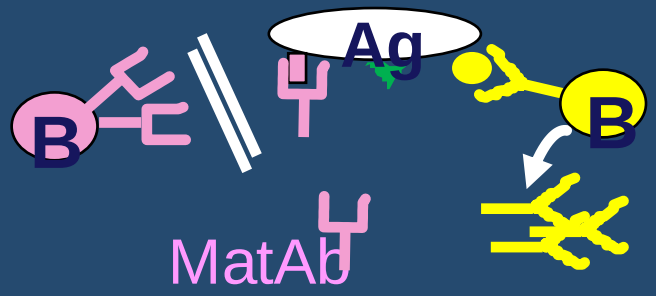
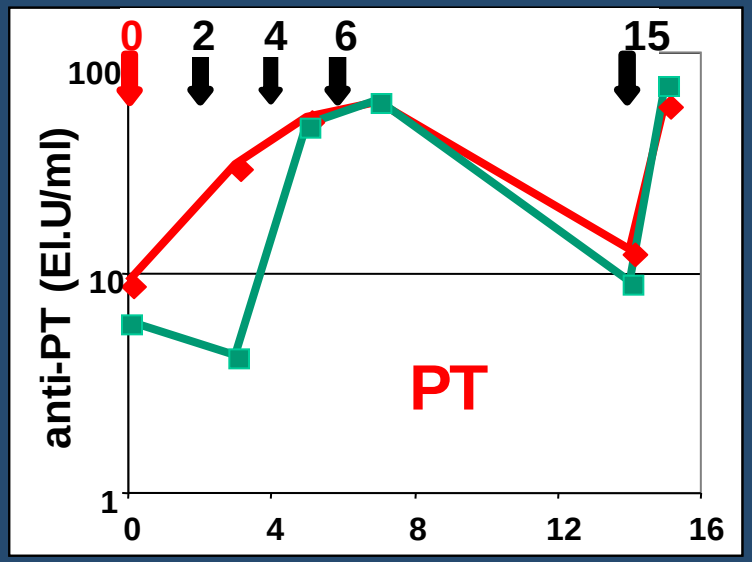
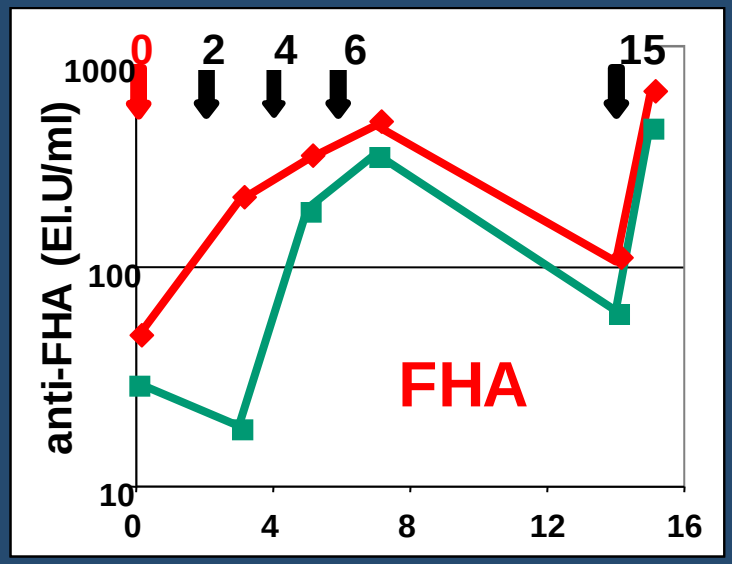


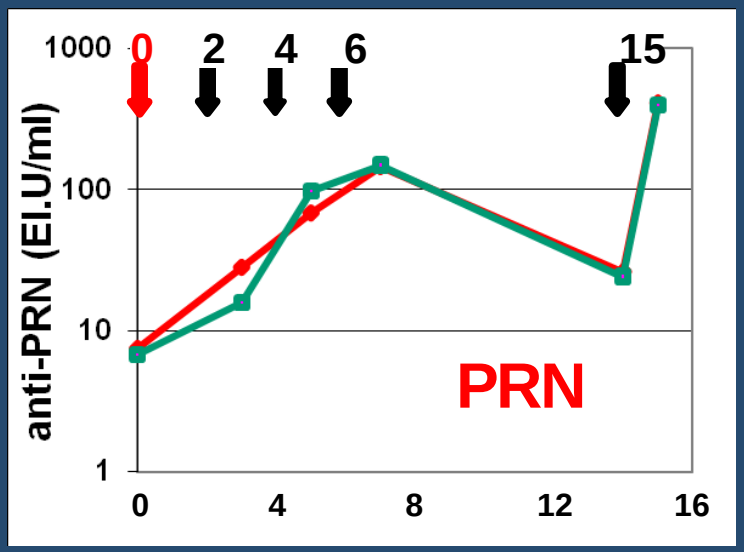

**Normal or ↑ infant T cell responses !**

*Siegrist CA, Vaccine 2003, 21:3406-3412*

# Infant responses to acellular pertussis may be primed at birth !



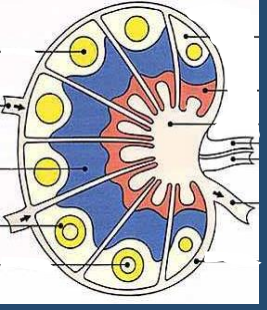




Pa at birth
HBV at birth

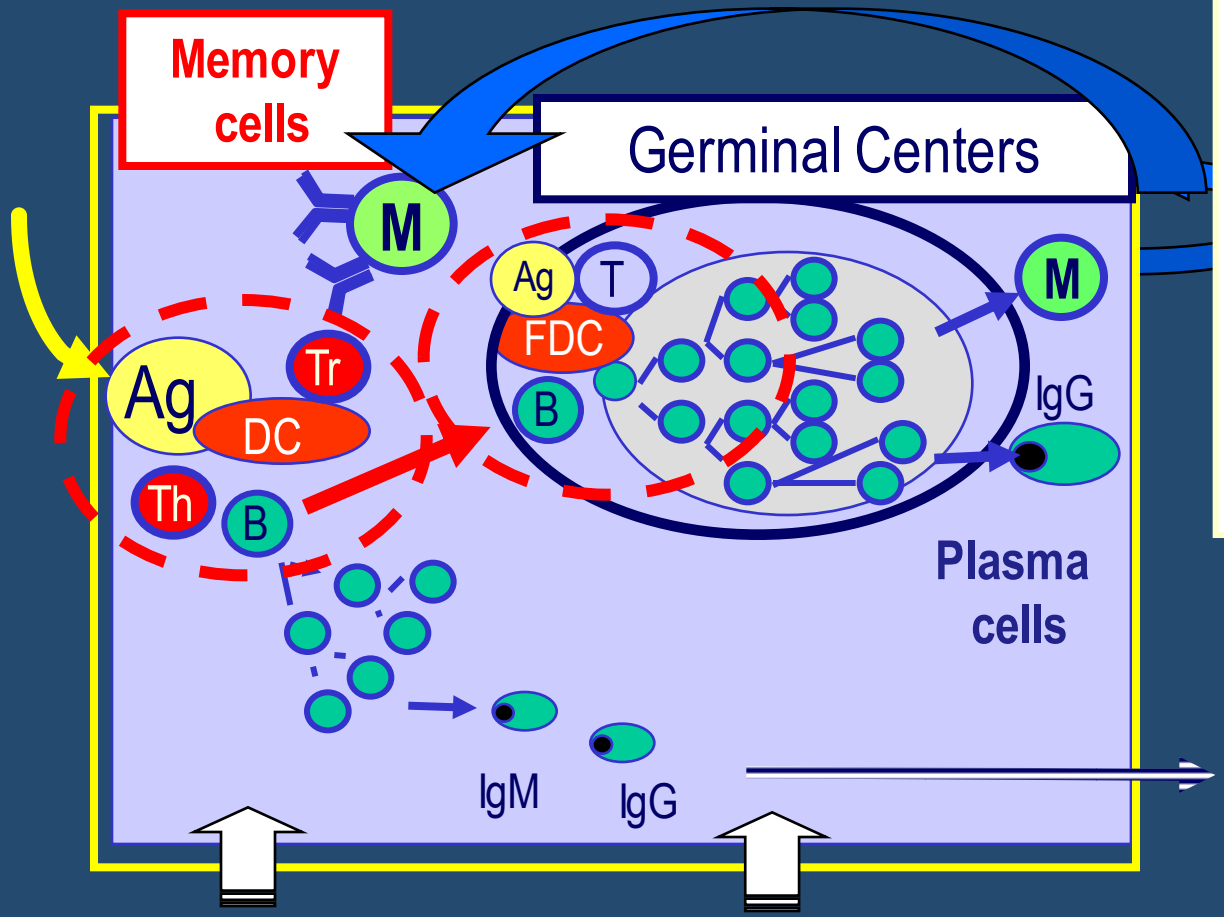
… but only with a **monovalent aP vaccine** *(DTaP: interference…)* !

**DTaP-X : @ 6 wks**

*Knuf et al J Ped 2008; J Ped 2010*

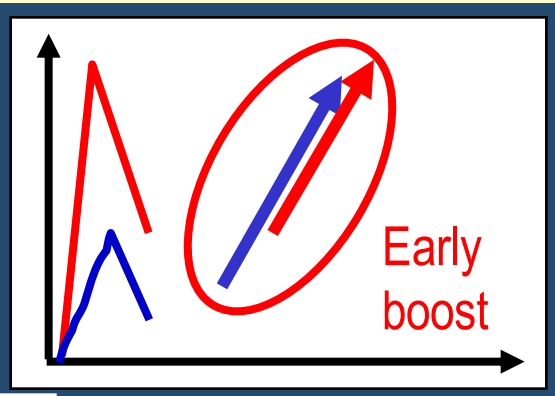


# Early life B cell responses are polarized towards the induction of memory cells !




**Efficient neonatal priming in humans:**

- Oral polio vaccine
- Hepatitis B
- Hib (PRP-T)
- Pertussis (Pa)
- PCV




**B cell activation (DC, T cells) ↓↓↓**

**Germinal center responses ↓↓↓**
**Early memory B cell induction !**

*M. Knuf, J. Pediatrics 2008*
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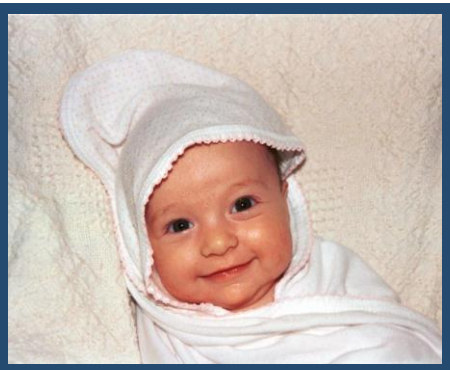
# Early life B cell responses

***Infant B cell responses (measles, TT, DT, Pw/Pa, HBsAg, pneumo, HIV, etc…)***



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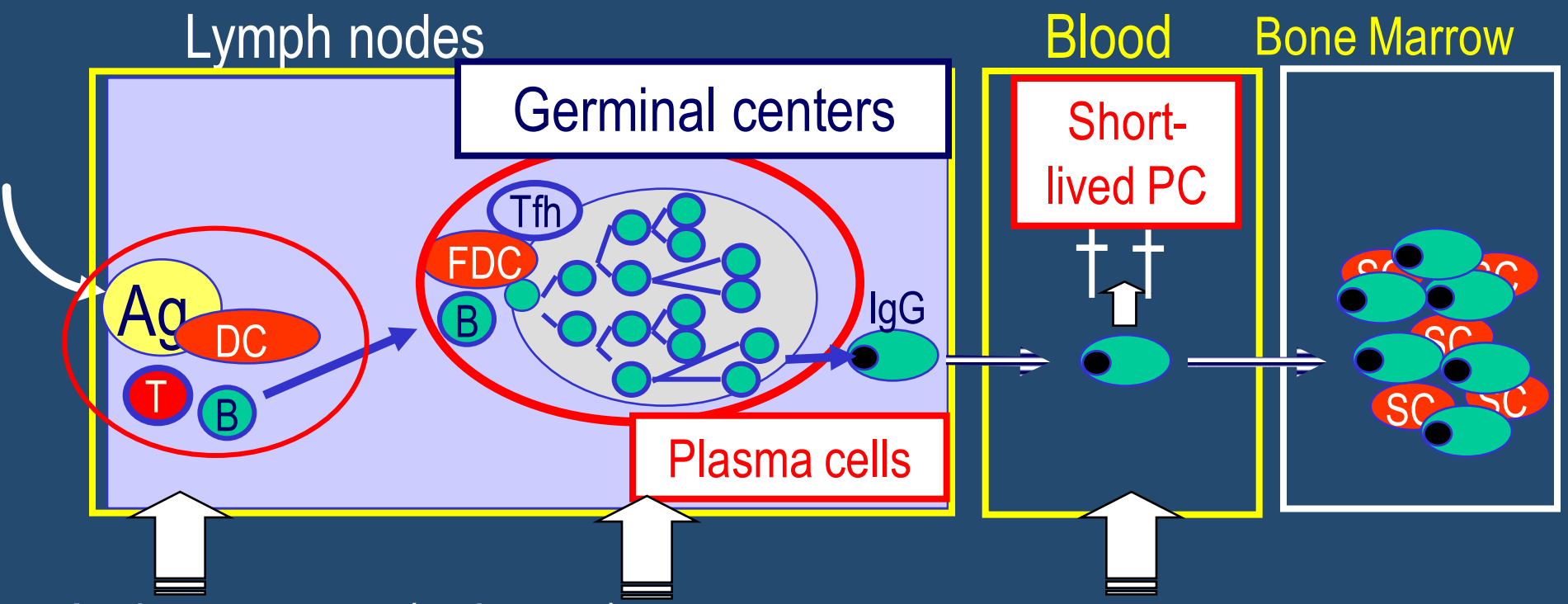
**Conserved patterns ↔ immune regulation**

# Age-specific patterns of infection in early life

| Neonates (28 d): | Infants (1-12 mo): | Toddlers: |
|---|---|---|
| •Low Th1 / CD8 | • ↑ Th1 / CD8 | •Adult-like Th1 / CD8 |
| •↑ Th2/Th17 | • ↓ Th2/Th17 | •Adult-like Th2/Th17 |
| •B cell priming | •B cell priming | •B cell priming |
| | •Short lived PCs | •Long lived PCs |

**Conserved patterns ↔ immune regulation**

# Determinants of early life B cell responses




- Antigen nature (epitopes)
- Antigen dose
- B / T cell repertoire
- APCs: activation status
- Tfh cells ↔ GC responses

**Vaccine antigens**

**Genetics**

- Innate immunity ← adjuvants
- ← nutrition (feeding, vitamins)
- ← environment (*microbiome?*)

# *Vaccine responses may be elicited early – including in very low birth weight preterm infants*

< 32 wks or 1500g :
↑ risks of brady-apnea in hospitalized preterm infants
➡ monitoring for 48h !

- **Lower primary responses < 32 wks gestational age**
- **Rapid post-natal immune maturation** *(← microbiome ?):*
  - **Early priming (birth)**
  - **Lower PC / Ab responses**

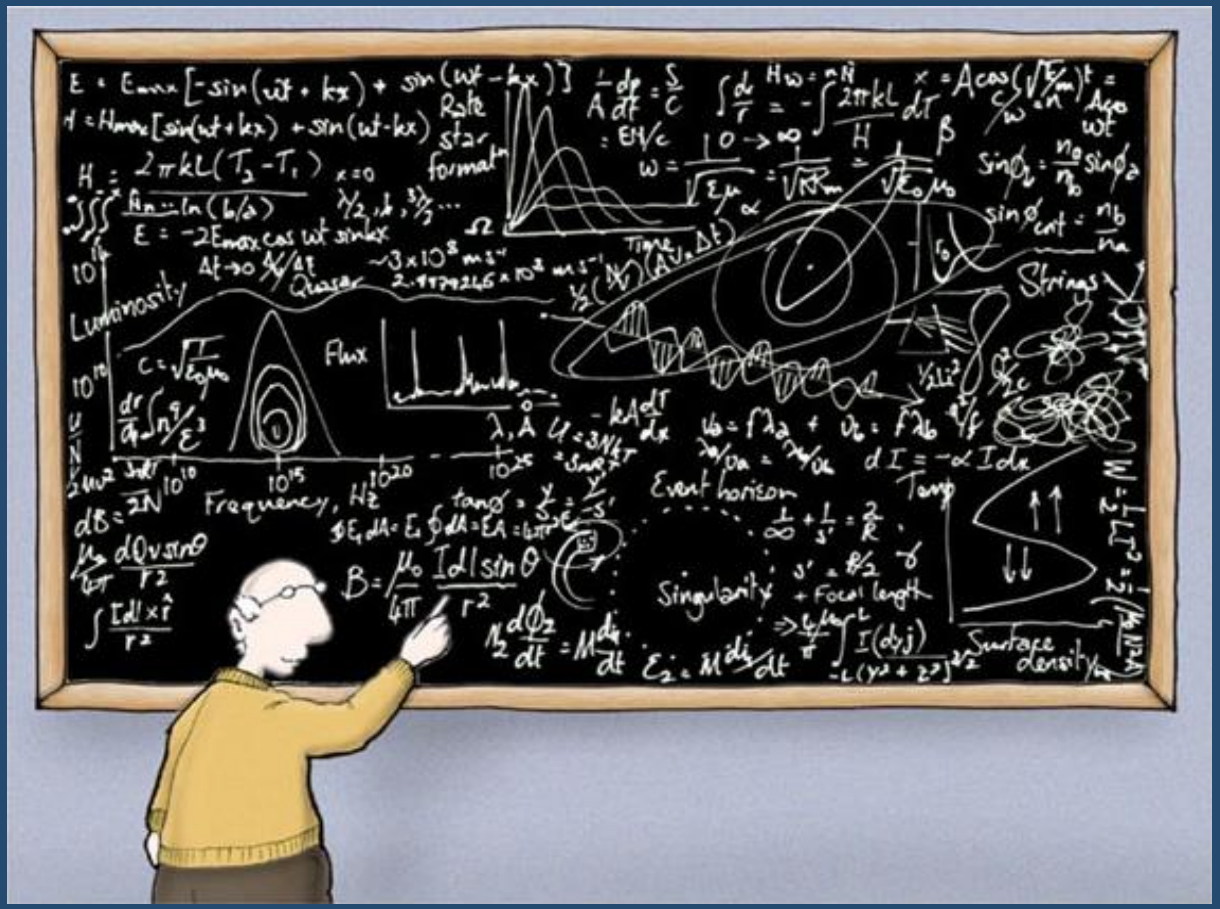
  **↔ 3 primary doses needed**

  ➡ **Immunize @ 6-10-14 wks regardless of gestional age!**
- **Boosting always required !**

*Immune maturation is a fitness-oriented process which progressively builds up defence mechanisms*

***Training should be initiated as early as possible in life … and never interrupted !!!***

# Understanding early life human immunity




**Age-specific requirements!**

**Age-specific patterns !**

**Age-specific competences!**

**Age-specific vaccine strategies !**