Neonatal Immunology

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Learning Outcomes

You should have an understanding of –

- Current hypotheses regarding the association of neonatal experiences with long term respiratory health

- The importance of respiratory infections in early life

- The mechanisms underlying failure of vaccination in neonates

- How B cell, T cell and antigen presenting cell responses differ in neonates

The Influence of Early Life on Respiratory Health: 1. Respiratory Infections

Infectious disease is the major cause in deaths in under 5s

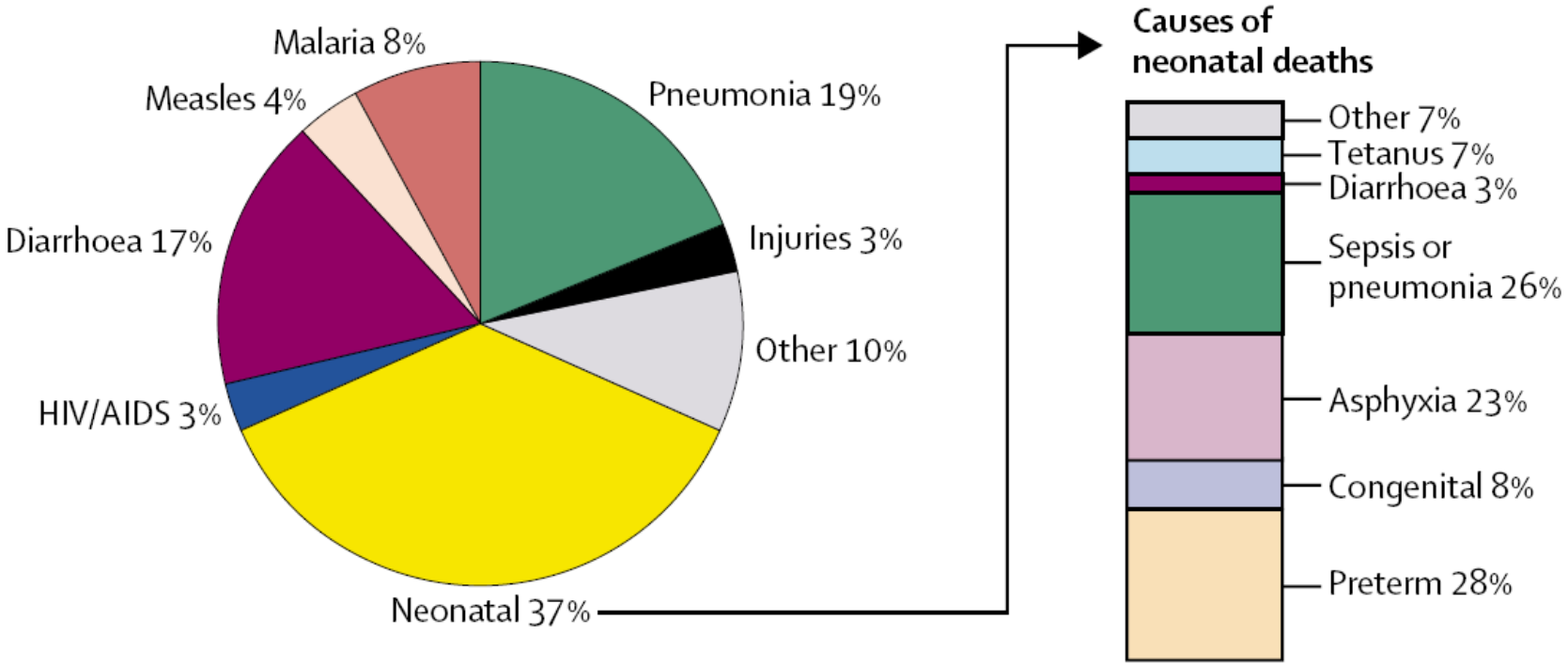


Figure 2: **Major causes of death in children younger than age 5 years and in neonates (yearly average for 2000–03)**

Malnutrition is a major underlying contributor to these deaths.

Bryce *et al*. WHO estimates of the causes of death in children, The Lancet (2005) 365: 1147–52

Major Pathogens in Young Children

Measles Virus

Respiratory Syncytial Virus (RSV)
Bortetella pertussis
Streptococcus pneumoniae
Haemophilus influenzae b (Hib)

Rotavirus
Salmonella

Malaria
HIV

Black et al Where and why are 10 million children dying every year?
Lancet 2003; 361: 2226–34

Acute Respiratory Infections

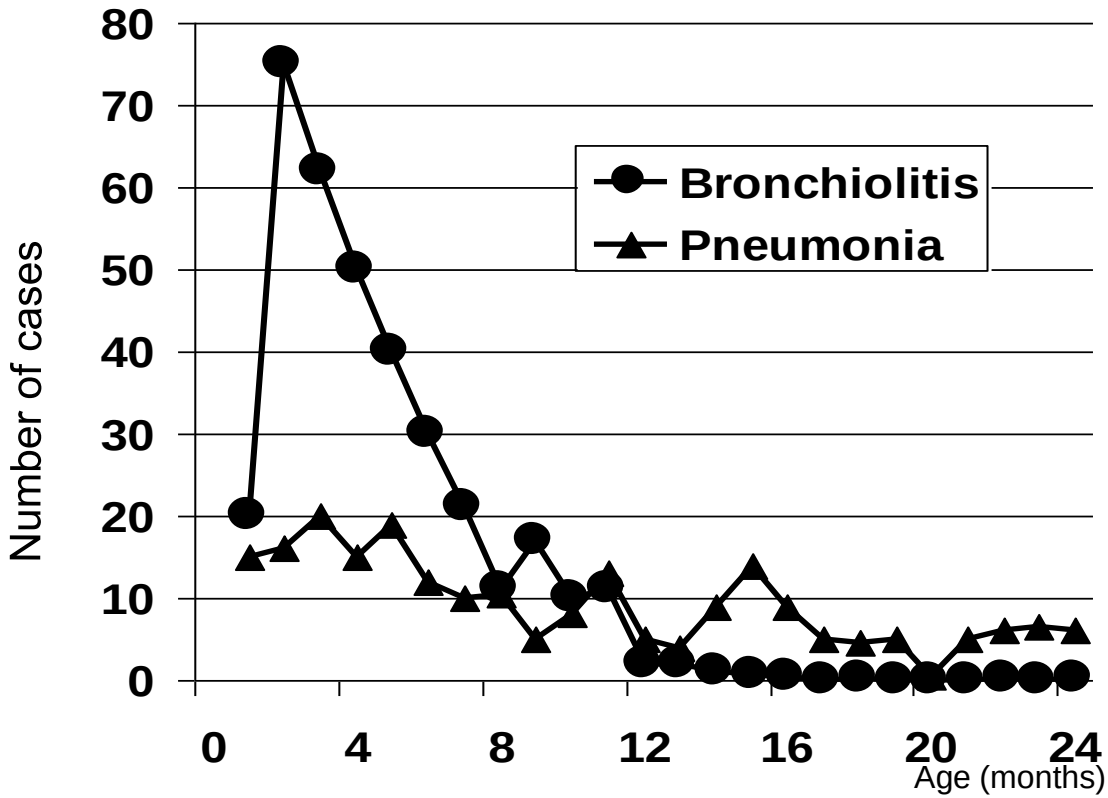
Respiratory syncytial virus (RSV) and parainfluenza virus type 3 are the main causes.

RSV causes 70% of cases of viral bronchiloitis - the most common cause of hospitalisation in the developed world.

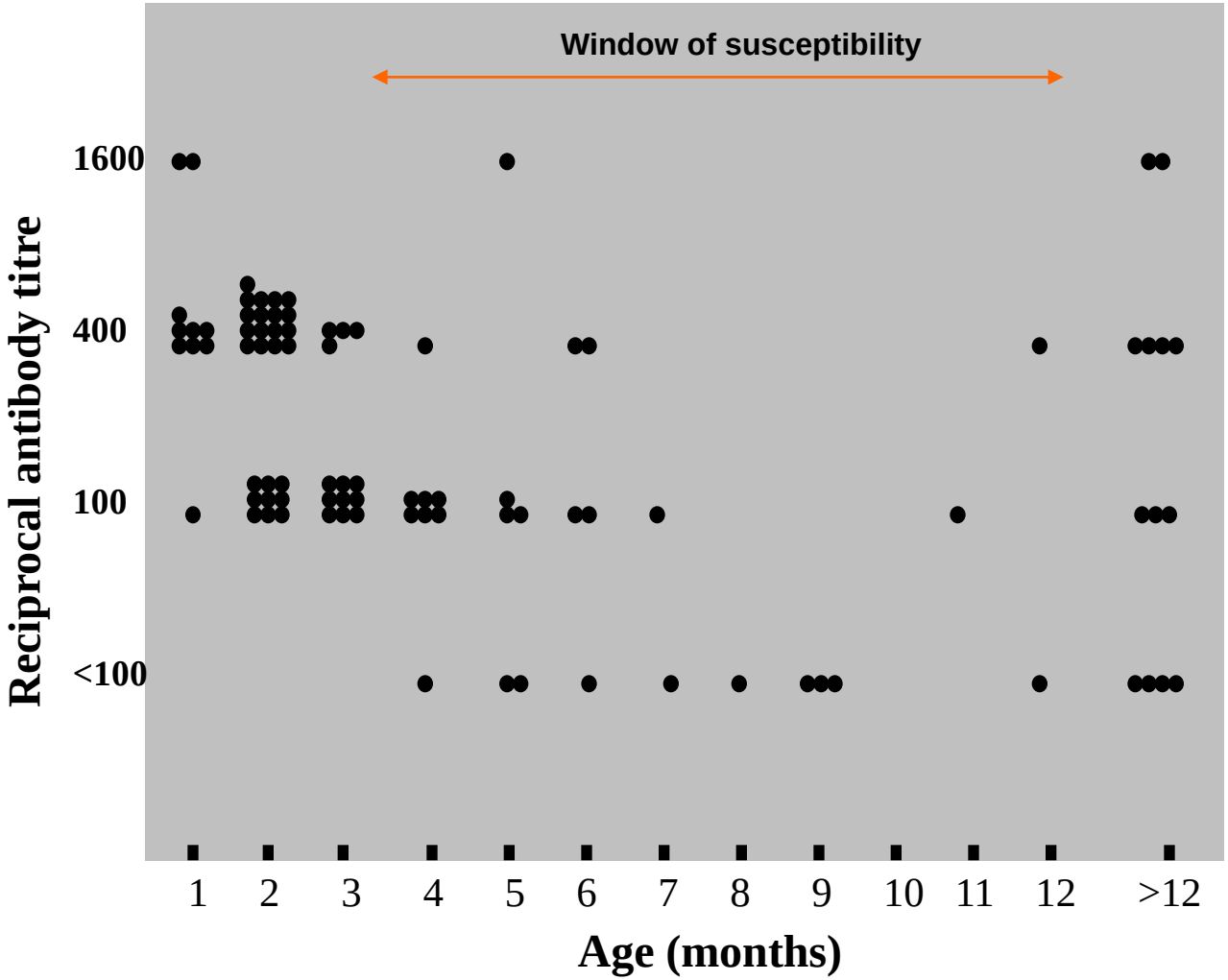
Respiratory Syncytial Virus Causes the common cold in adults.

Age incidence of RSV disease

Data from Parrott et al *Am J Epidemiol* 1973; **98**:289

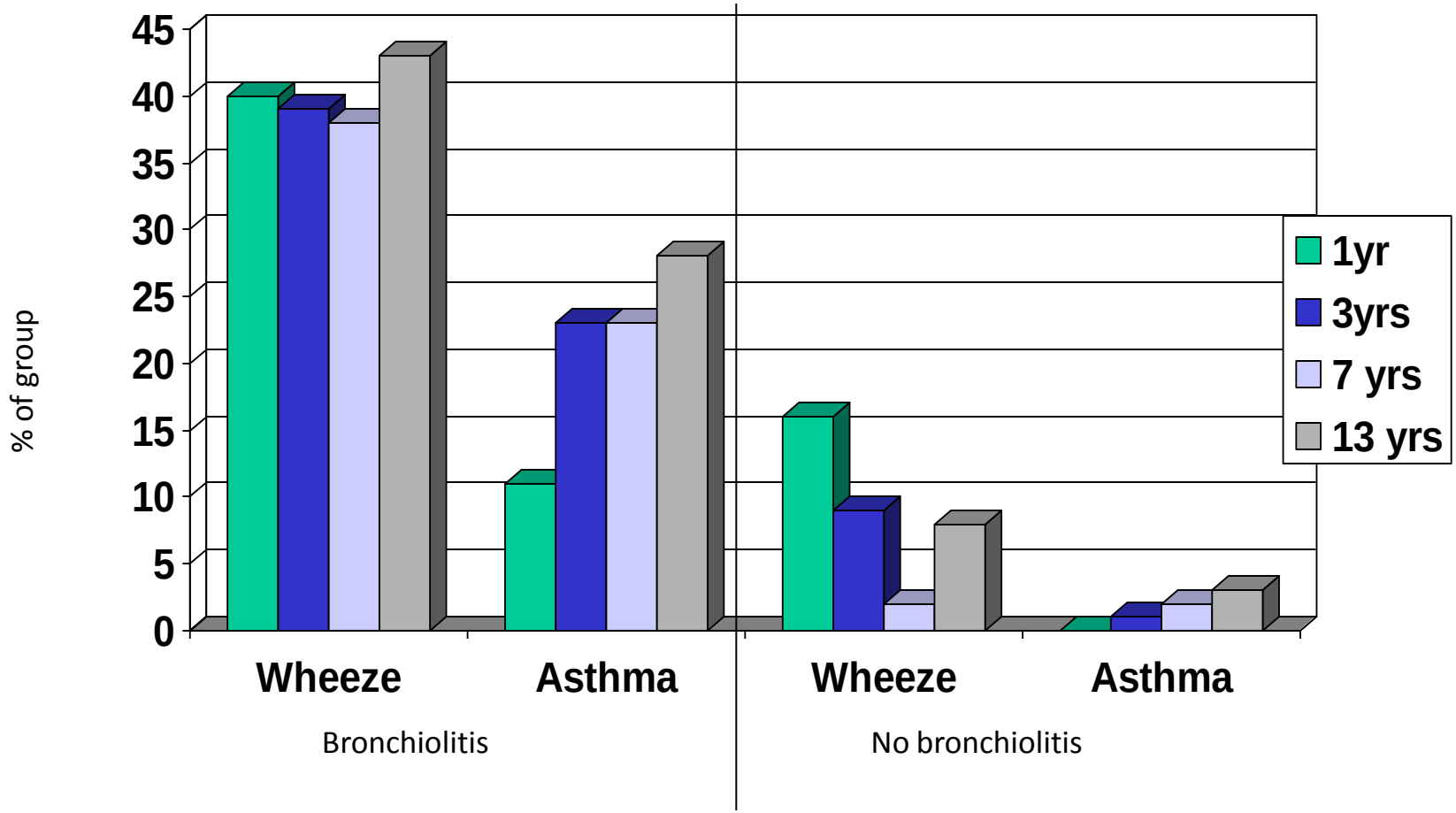


Maternal Antibody:
ELISA antibody titre to RSV at different ages



Data from Richardson et al *Infect Immunol* 1978; **20**:660

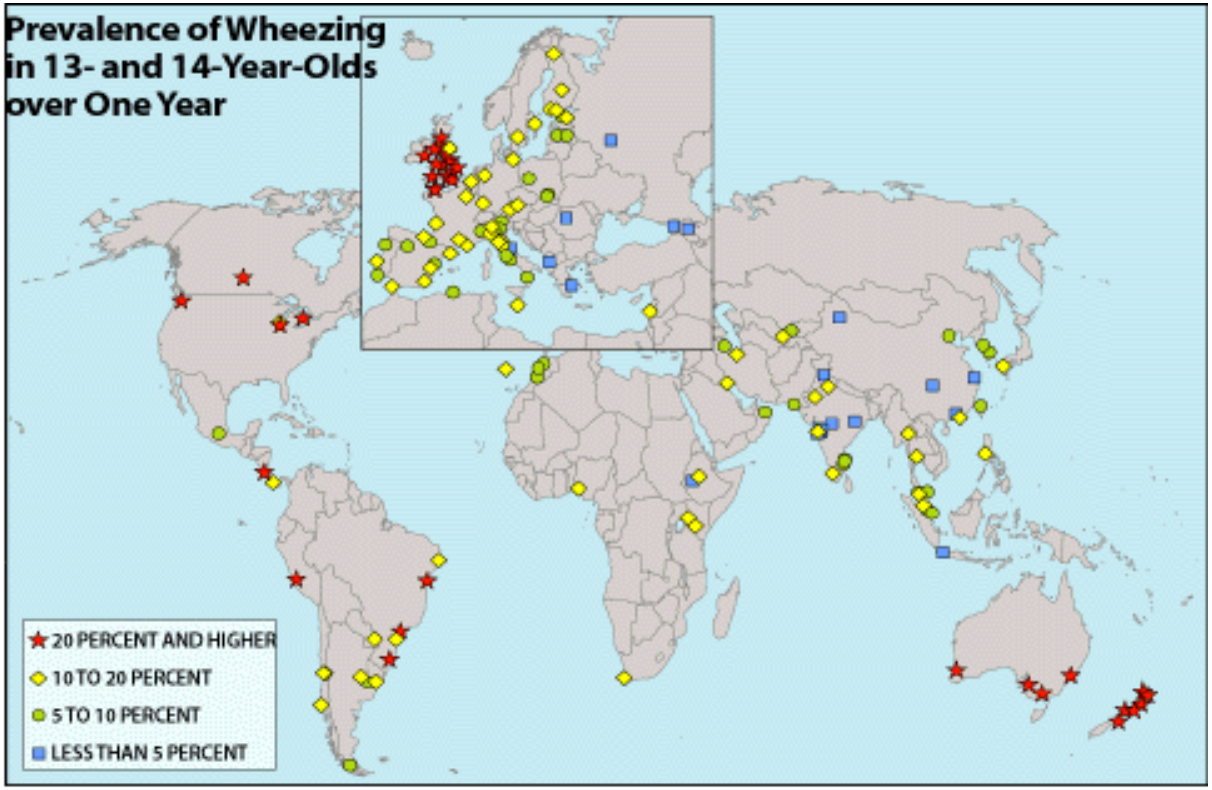
Infections: Wheezing and asthma after RSV hospitalisation



Sigurs N et al. *Pediatr* 1995; 95:500-05., Sigurs N, et al. *Am J Respir Crit Care Med* 2000;161:1501-07. Sigurs N, et al. *Am J Respir Crit Care Med* 2005;171:137-41.

2. The modern environment

Asthma was rare in 1900, but now it has grown into an epidemic: more than 150 million are affected around the world. Every year it kills 180,000 worldwide.



SOURCE: "Worldwide Variations in the Prevalence of Asthma Symptoms: The International Study of Asthma and Allergies in Childhood (ISAAC)." M. I. Asher et al. in European Respiratory Journal, Vol. 12, pages 315–335; 1998. Data based on surveys of 463,801 children in 155 centers and 56 countries. Fieldwork conducted in 1991–95. Map reprinted with permission from the ISAAC Steering Committee on behalf of the ISAAC Phase One Study Group and with permission from the European Respiratory Journal.

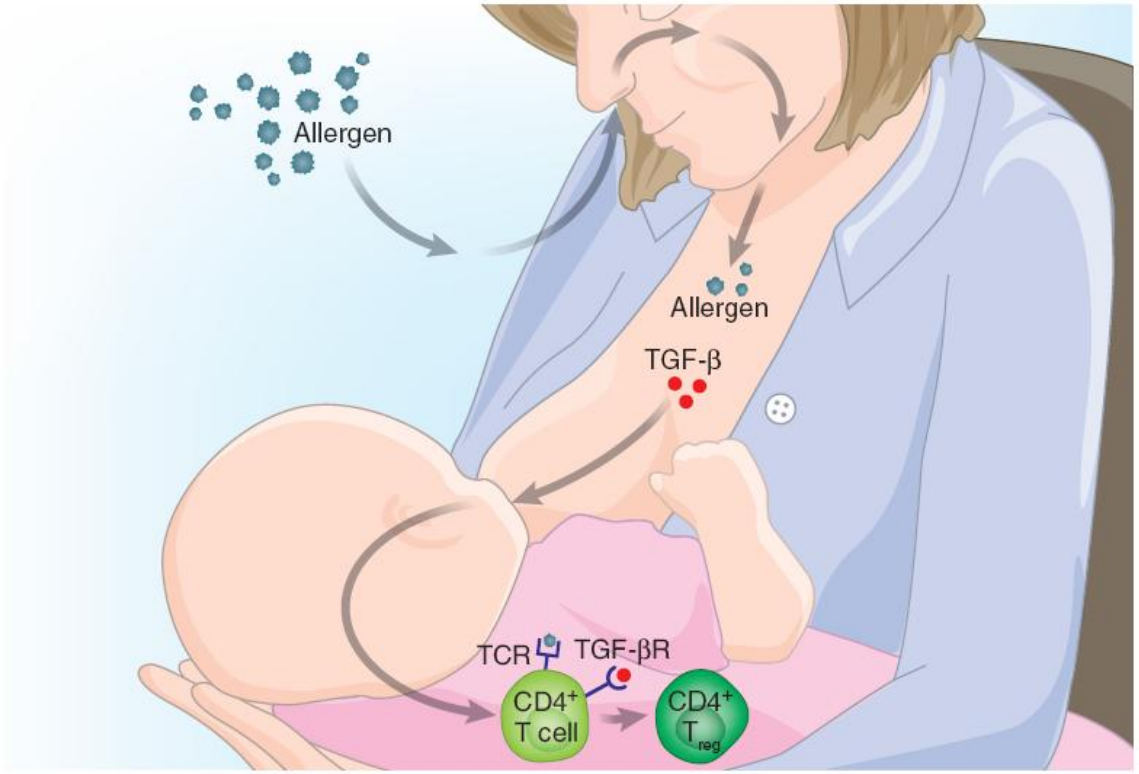
Epidemiology: The hygiene hypothesis

Children from large families or raised on farms are at reduced risk of developing:

- Hay fever
- Eczema
- Allergen sensitisation

Immunological mechanisms?

3. Tolerogenic Effects of Breast Milk



Breast feeding is believed to protect against allergy and wheeze. Mouse studies have demonstrated that regulatory T cells are induced after ingestion of allergen and TGFβ in breast milk, rendering offspring tolerant to the allergen.

Friedman and Zeiger, J Allergy Clin Immunol 2005;115:1238-48;Verhasselt et al Nature Medicine (2008) 14 (2): 170

4. Microflora

Differences in microflora are associated with different diseases *eg* allergy and inflammatory bowel disease (most studies are on gut flora).

There is growing evidence that microflora can influence development of disease.

Microbiota can be sufficient to transfer disease *eg* Tbet-/- RAG2-/- (TRUC) mice transfer colitis to co-housed wild type mice.

Active interaction between host microbiota and immune response

The host innate immune response influences the bacteria...

eg Myd88 − / − mice or Nod1 − / − mice exhibit dramatic changes of the composition of gut microbiota.

The bacteria influence the host...

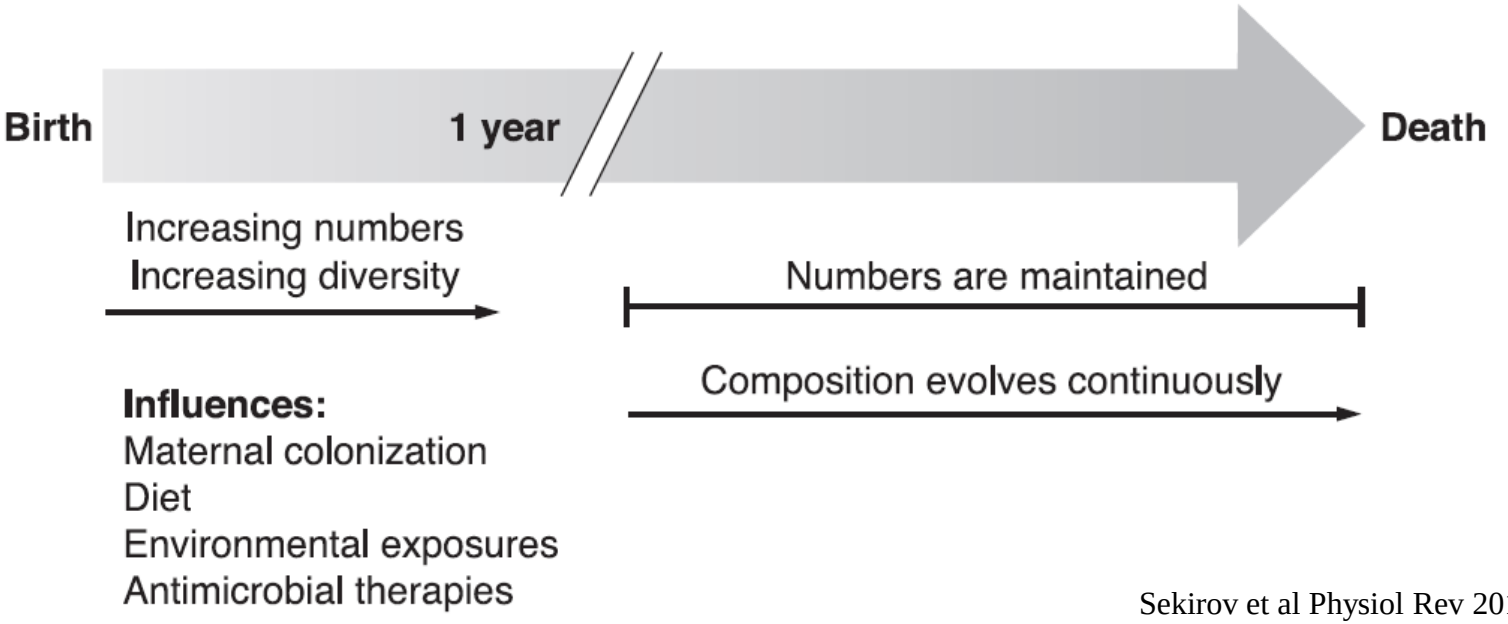
eg Some can promote Tregs.

A symbiosis factor (PSA, polysaccharide A) of *B. fragilis* signals through TLR2 directly on Foxp3+ regulatory T cells to promote immunologic tolerance. *B. fragilis* lacking PSA is unable to restrain T helper 17 cell responses and is defective in niche-specific mucosal colonization.

PSA is a “symbiont-associated molecular patterns (SAMPs)”
Round *et al* Science May 2011

Acquisition of the microbiota

Microbiome is acquired from the mother during birth and from random environmental encounters. Stabilises at about 1 year old.



Sekirov et al Physiol Rev 2010

This may be influenced by delivery route, breast feeding, diet, antibiotics and cleanliness of the environment (controversial).

According to Hill and Artis, bacteria in the gut undergo two main phases of colonisation – during breast feeding and upon weaning. In mice the immune response to colonisation of the gut peaks within a week of birth and stabilises over the first year of life.

Microbiome of the Lung

Bronchial tree is not sterile but contains a mean of 2,000 bacterial genomes per cm^2 surface

A rich microbial environment in infancy protects against asthma.

Caesarian section, early life antibiotic use are associated with asthma.

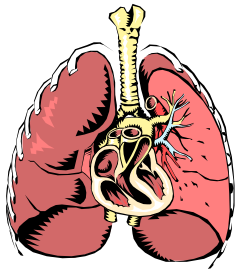
The microbiome is altered in COPD and asthma (Hilty, Mowat, Cookson 2010).

Neonates colonized in the hypopharyngeal region with *S. pneumoniae, H. influenzae,* or *M. catarrhalis*, or with a combination of these organisms, are at increased risk for recurrent wheeze and asthma early in life. (Bisgaard *et al* NEJM, 2007).

Differences in the composition of the gut flora between infants who will and infants who will not develop allergy are demonstrable before the development of any clinical manifestations of atopy. (Björkstén B *et al* , JACI, 2001)

Taken together this suggests that bacterial colonisation of the airways may influence respiratory health.

The Neonatal Respiratory Tract



- Respiratory infections are the major cause of infant mortality and hospitalisation.

- Long-term respiratory health may be strongly influenced by neonatal experiences:

- Breast feeding, microflora, exposure to environmental factors and microbial components may be important for preventing allergy and asthma.

- Some infections (RSV) may predispose to the development of asthma.

Why study neonatal immunology?

- To understand how the immune system develops

- To understand why the young are more susceptible to infection and the development of allergy.

- To understand how external influences can shape our developing immune system.

- To manipulate the immune system to promote protection against infectious disease (vaccination) and allergy.

Studying Neonatal Immunology

Murine Models

One week old pups are considered to be similar to new born humans in terms of their immunological maturity. Neonate <7d old.

Human Studies

Ethically difficult to get hold of blood or tissues from newborns. Many studies carried out on cord blood.

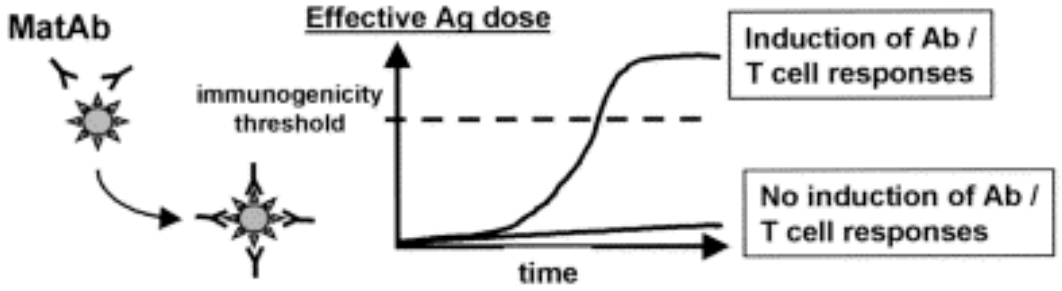
Neonate <28d old.

Neonatal Vaccination

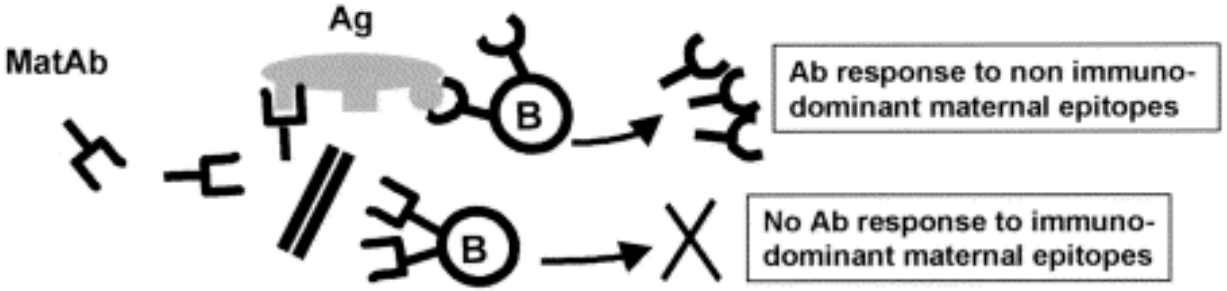
Many commonly used vaccines are ineffective if administered during infancy - they do not protect and generate **weak**, **short-lived** or **inappropriate** immune responses.

Neonatal Vaccination: Influence of maternal antibodies on induction of vaccine responses

1. Neutralisation of live vaccines, depending on MatAb / Ag load ratio



2. Epitope-specific B cell masking, preventing binding by epitope specific infant B cells



3. APC uptake of immune complexes, processing and Ag presentation

Siegrist CA *Vaccine* (2001) **19** : 3331-46

Neonatal Vaccination: MEASLES

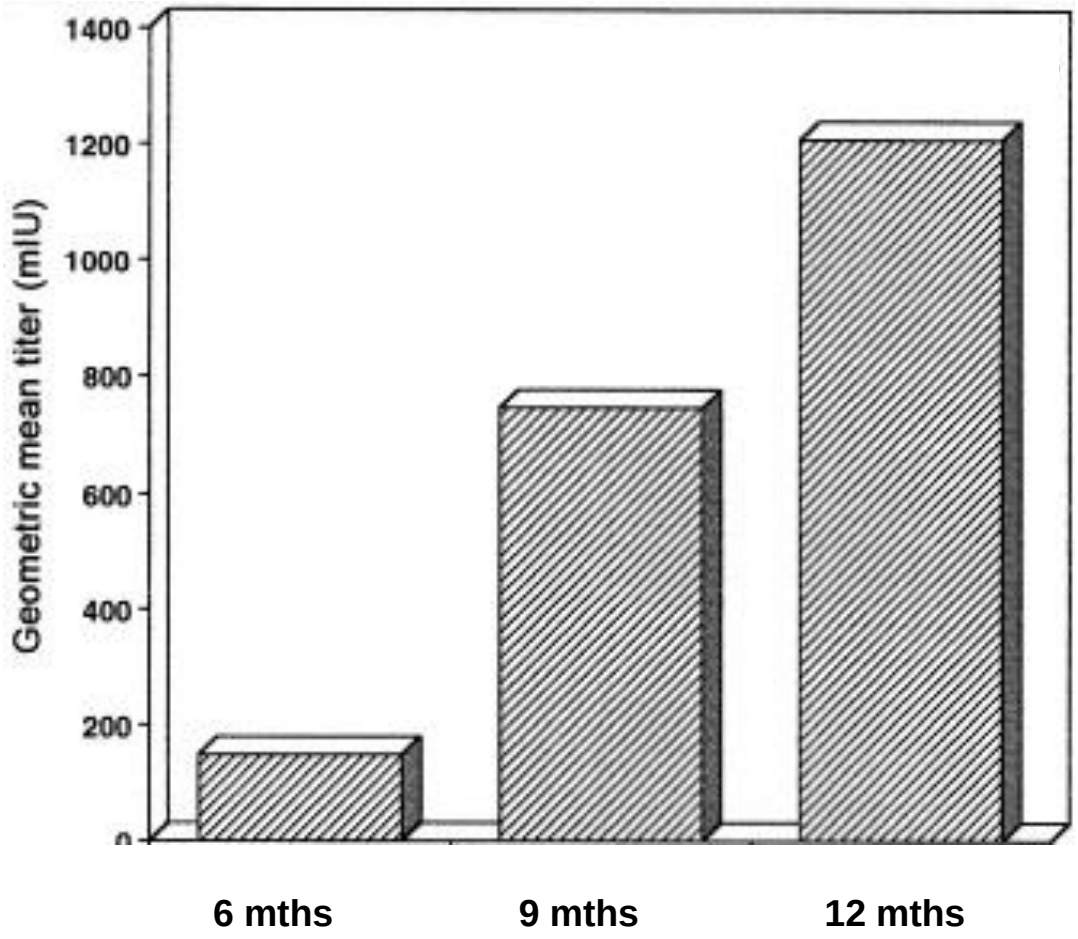
Despite the availability of a vaccine, measles still ranks as one of the leading causes of childhood mortality worldwide.

- 42 million cases of measles annually.
- 1 million annual fatalities from measles and its complications.

•Infants become susceptible to infection as soon as maternal measles antibodies have disappeared (from 6mths)

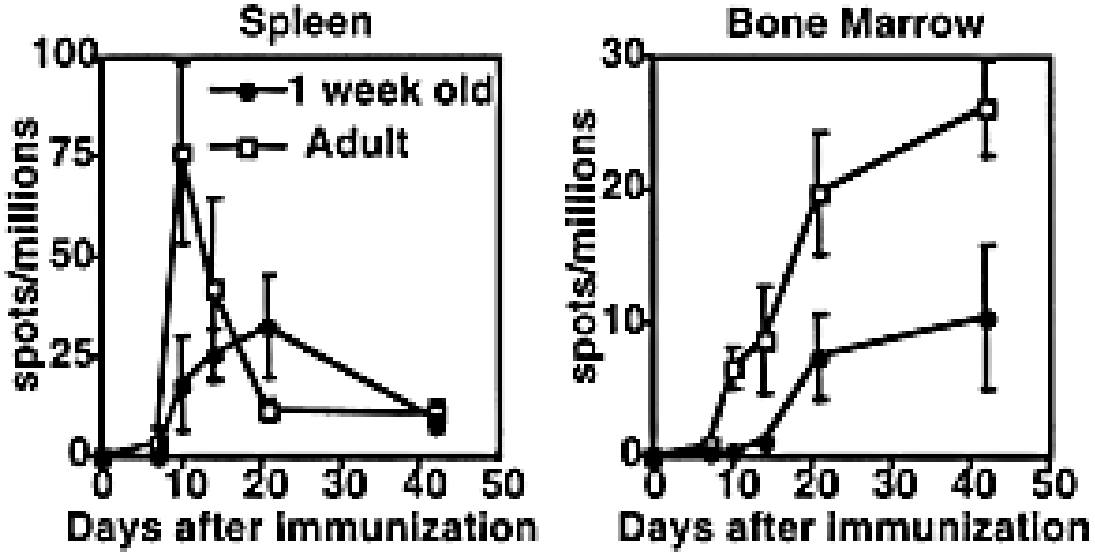
- **Vaccine is usually administered at 12-15 mths old.**

Neutralizing antibody responses of infants after measles immunization in the absence of maternal Ab



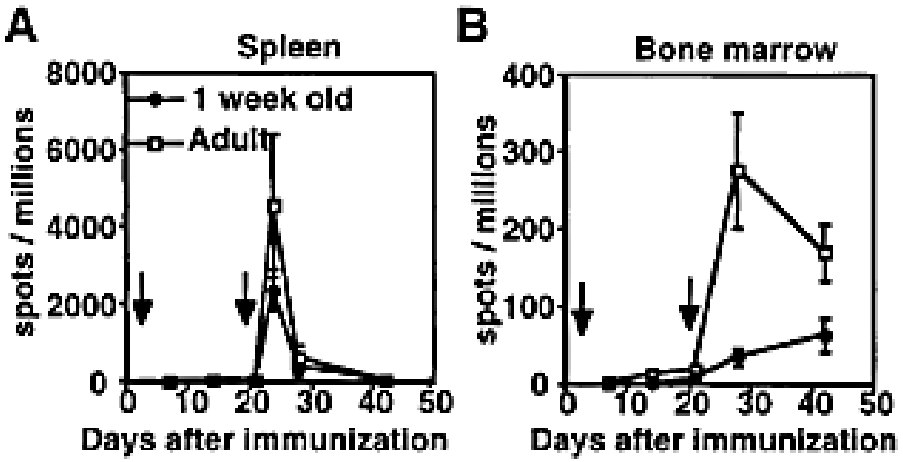
Gans et al, *JAMA. 1998;280:527-532*

Neonatal B-cell response is delayed and deficient



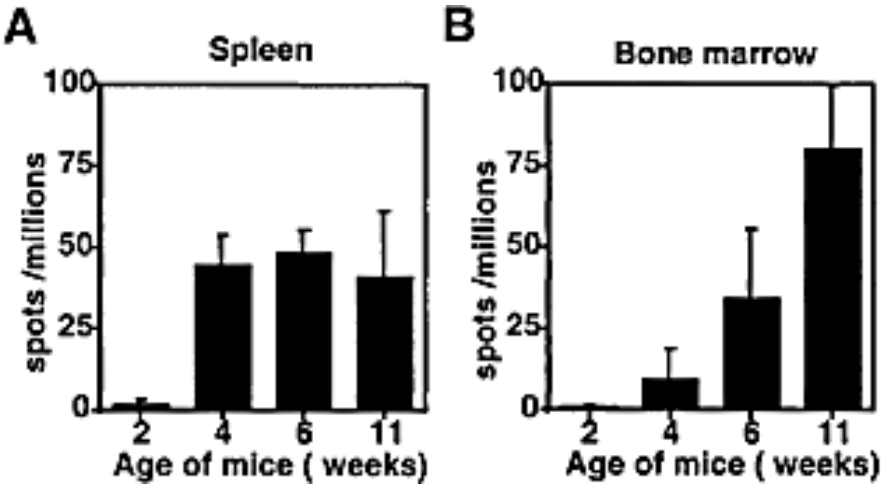
Antibody secreting cell frequency in the spleen and bone marrow following immunisation of 1 week old mice with TT and Al $(OH)_2$

Mice immunised as neonates respond poorly to boosting



Boosting Antibody secreting cells

Secondary immunization fails to correct limitation of bone marrow antibody secreting cells in early life.

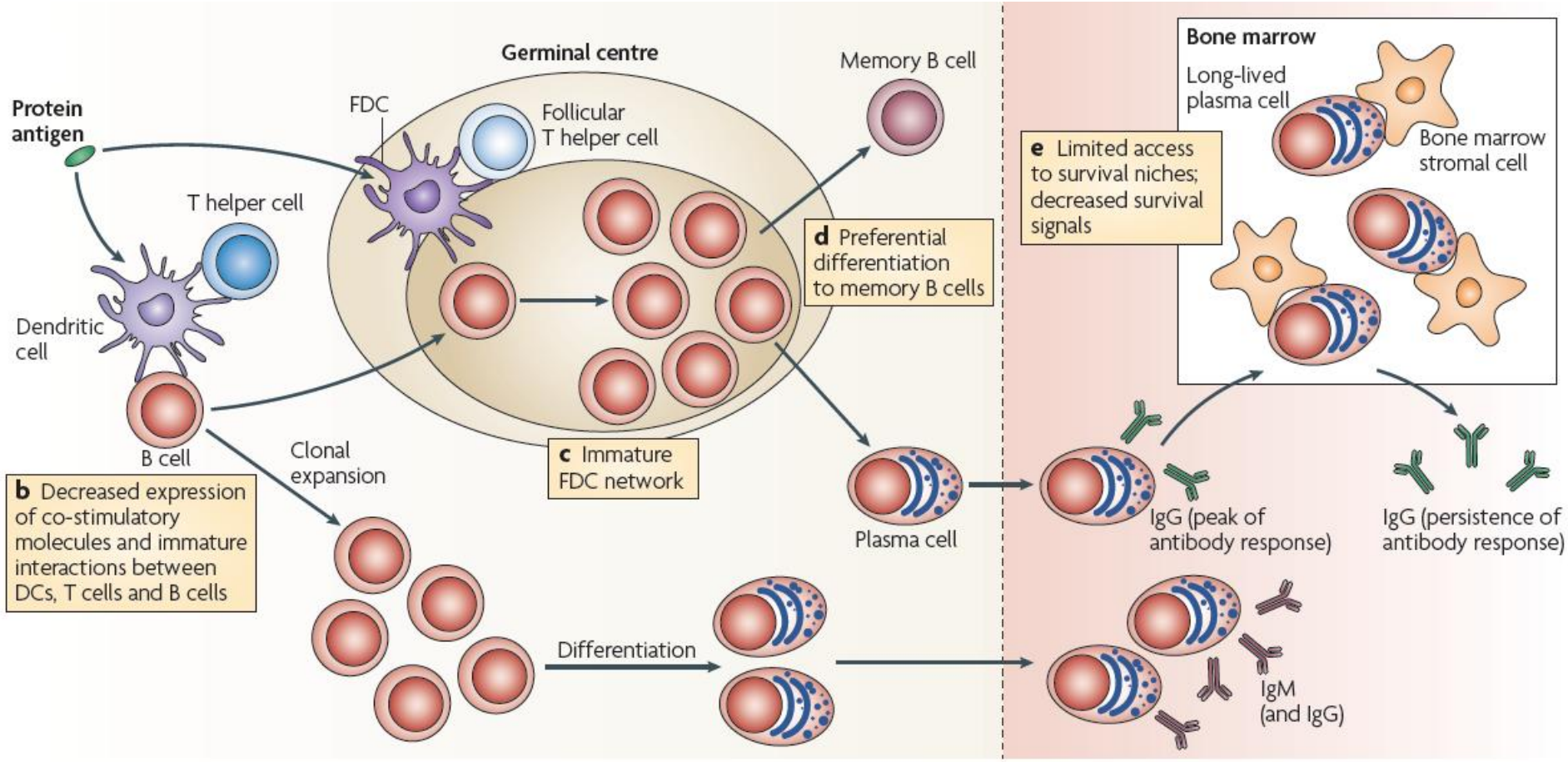


Total IgG secreting cells

Neonates lack B-cell follicles in the spleen and bone marrow

Pihlgren *et al*, *European Journal of Immunology* 2001. **31**: 939

Early-life Limitations of B-cell responses

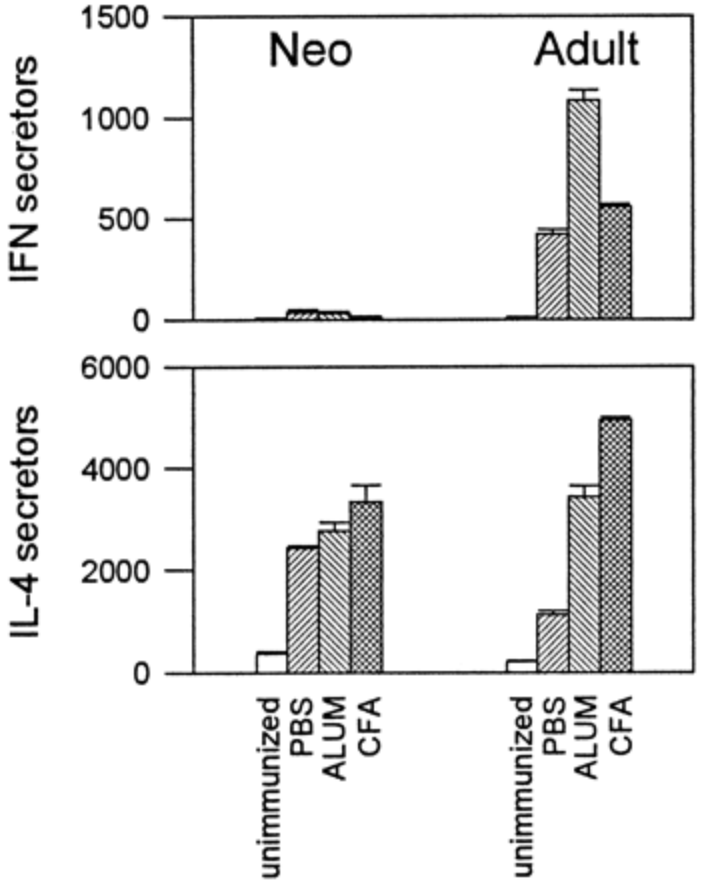


Siegrist and Aspinall, Nature Reviews Immunology, 2009

Summary: Neonatal Antibody Response

- Delayed and weaker IgG response
- Poor affinity maturation
- Rapid decline of Ab production
- Preferential memory cell production, but short lived
- Lower responses to polysaccharides
- Maternal antibody may interfere

Spleen T-cell responses are Th2 - biased in neonates

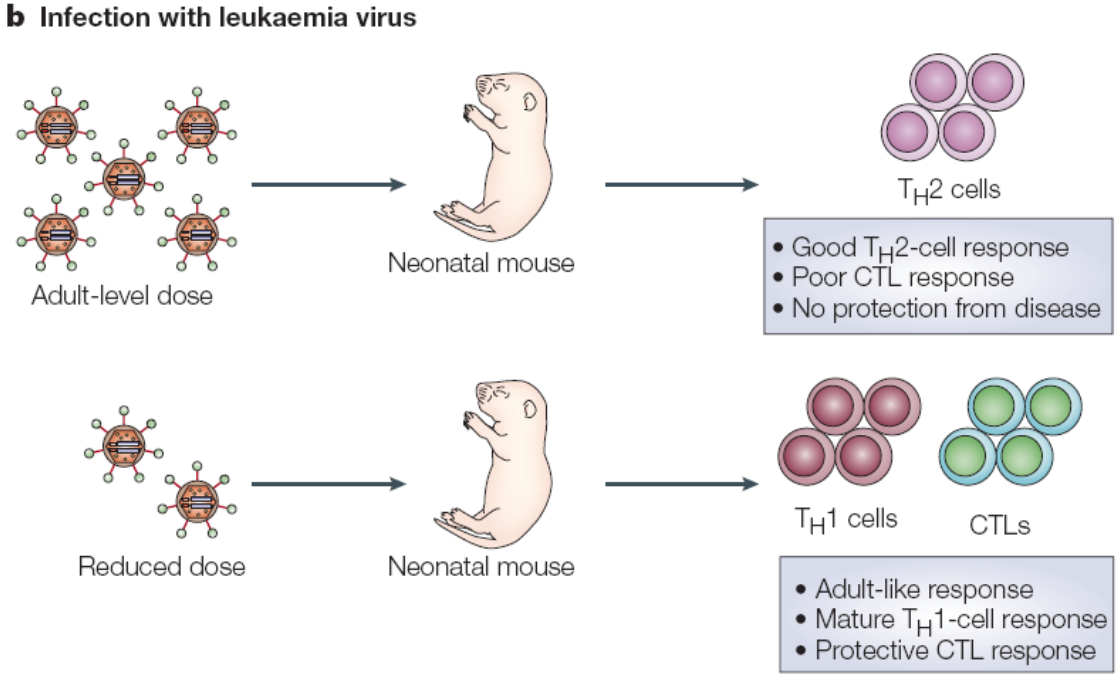


ELISPOT analysis in spleens following immunization with Ag in adjuvants.

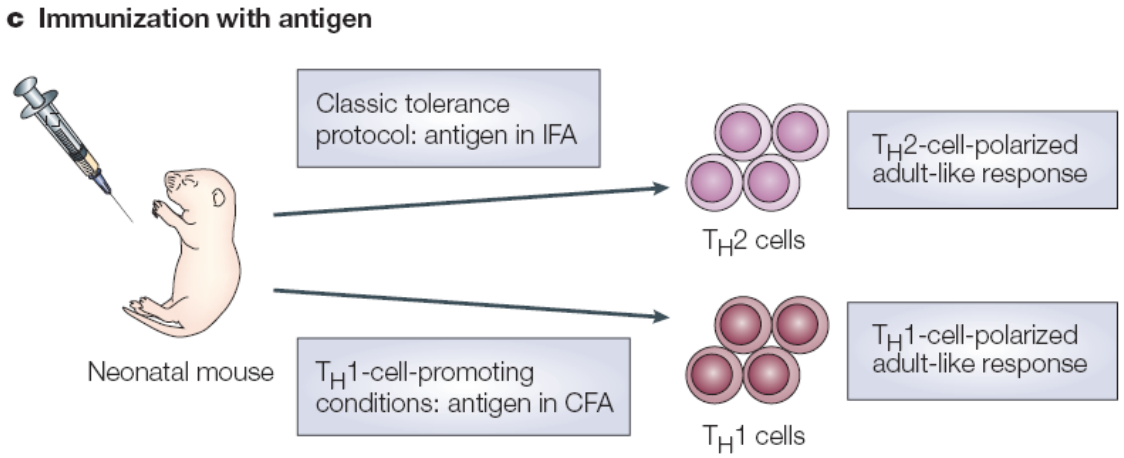
Mice were immunized with KLH in PBS, CFA, or ALUM.

Adkins B *et al*, *J. Immunol.* (2000) 164:2347.

Under certain circumstances neonates can produce adult-like responses



Dose



Adjuvant

BCG vaccination works in newborns

Adkins *et al* Nature Reviews Immunology (2004) VOLUME 4: **553**

Is ‘defect’ at the level of the T-cell or its environment?

Environment:

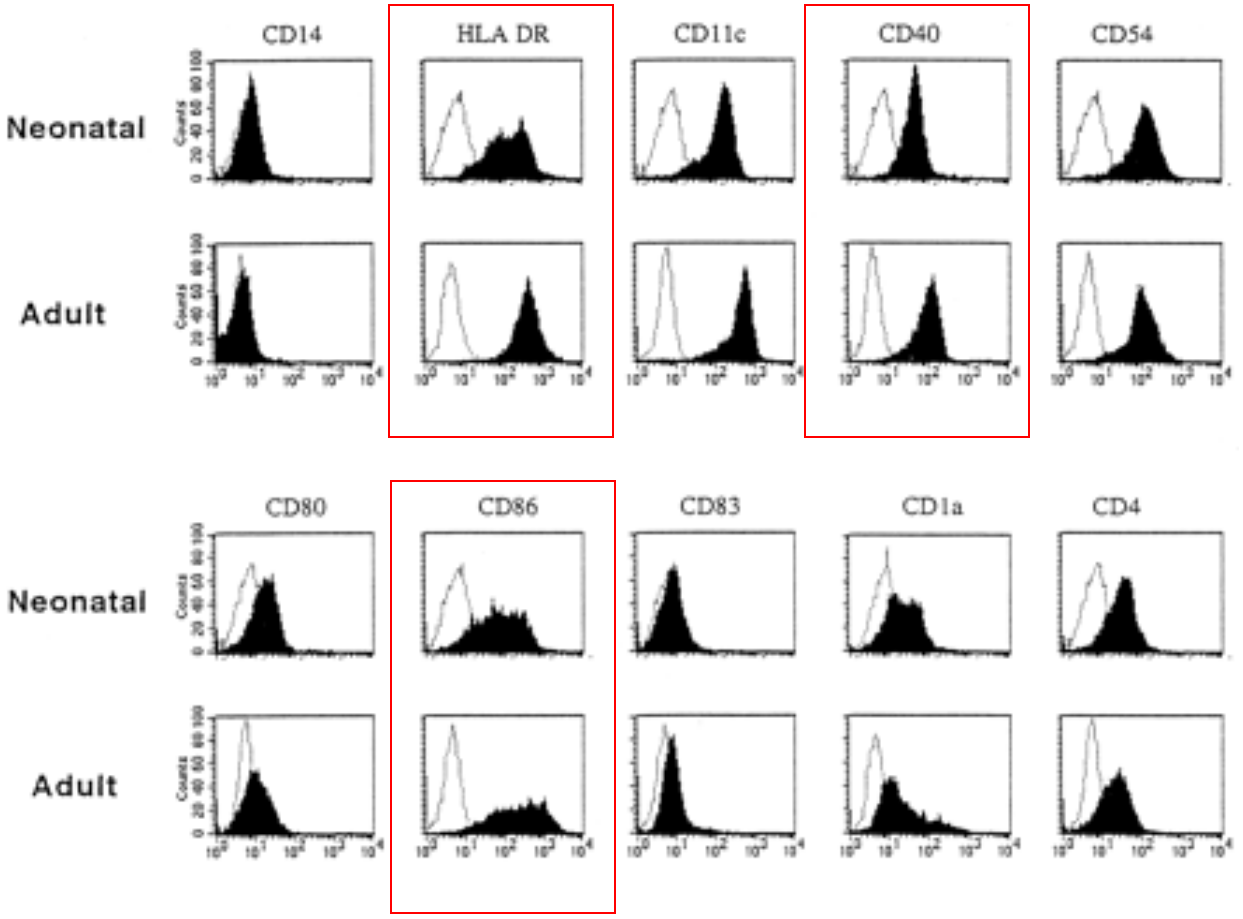
Neonatal T-cells are able to resolve *P. carinii* infection when transferred into adults, whereas neonates are not, suggesting the neonatal environment is preventing T-cell function (Qureshi and Garvy, 2001).

T-cells:

T-cells are still Th1 deficient when transferred into adults (Adkins *et al* . 2002).

The IFNγ promoter is differentially methylated, suggesting different gene regulation in neonatal T-cells (White et al, 2002)

Human Neonatal Cord Blood-derived Dendritic Cells are deficient in Co-receptor expression

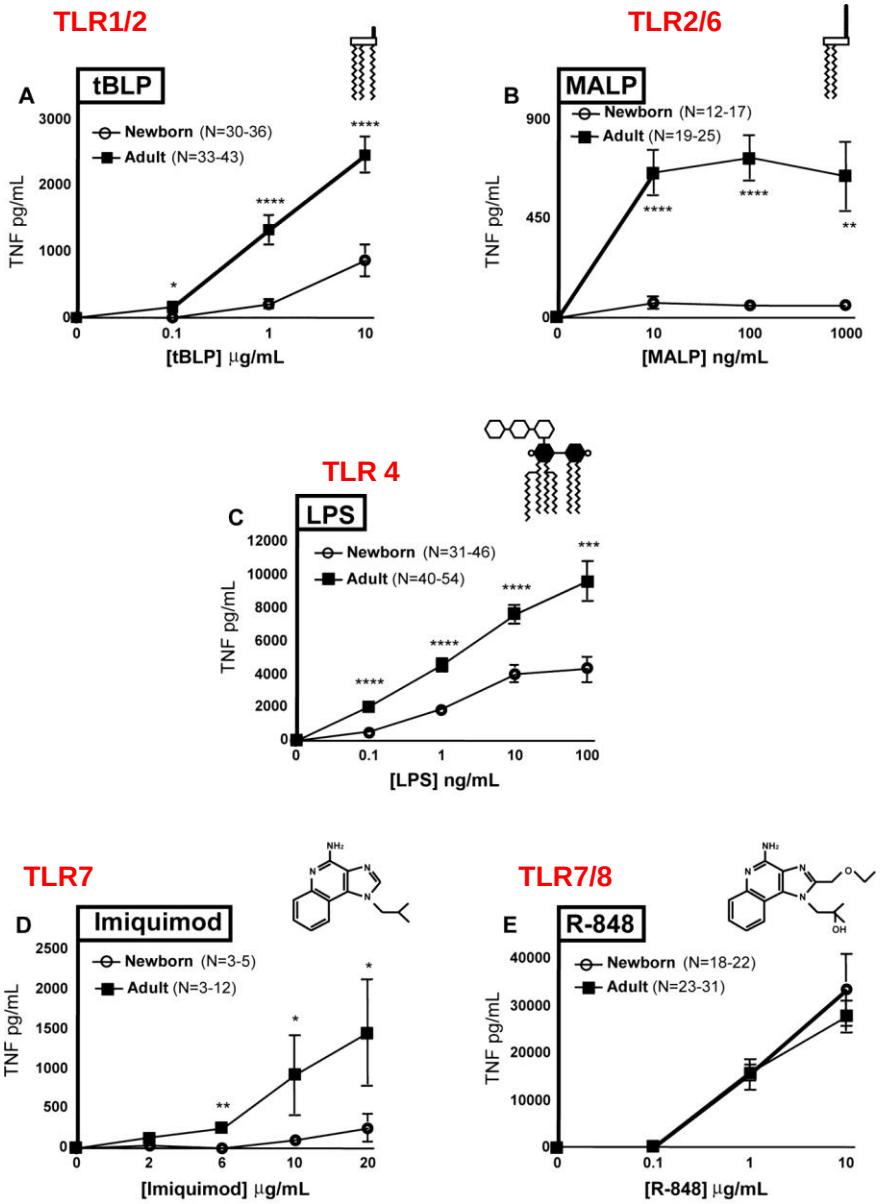


Goriely et al *The Journal of Immunology*, 2001, 166: 2141-2146.

Toll like Receptors

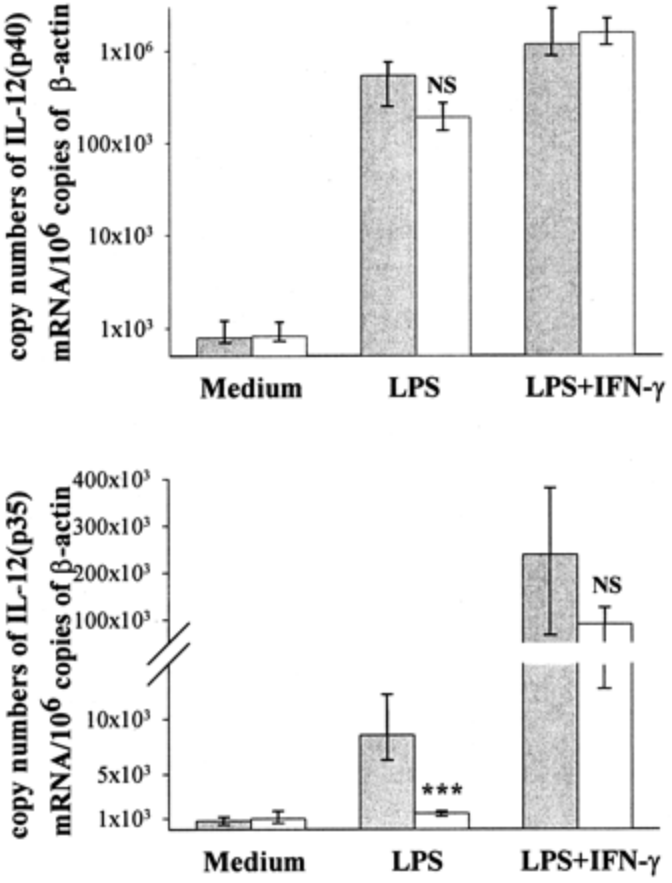
TLR responses in neonates are less than adults

Human neonatal cord blood monocytes



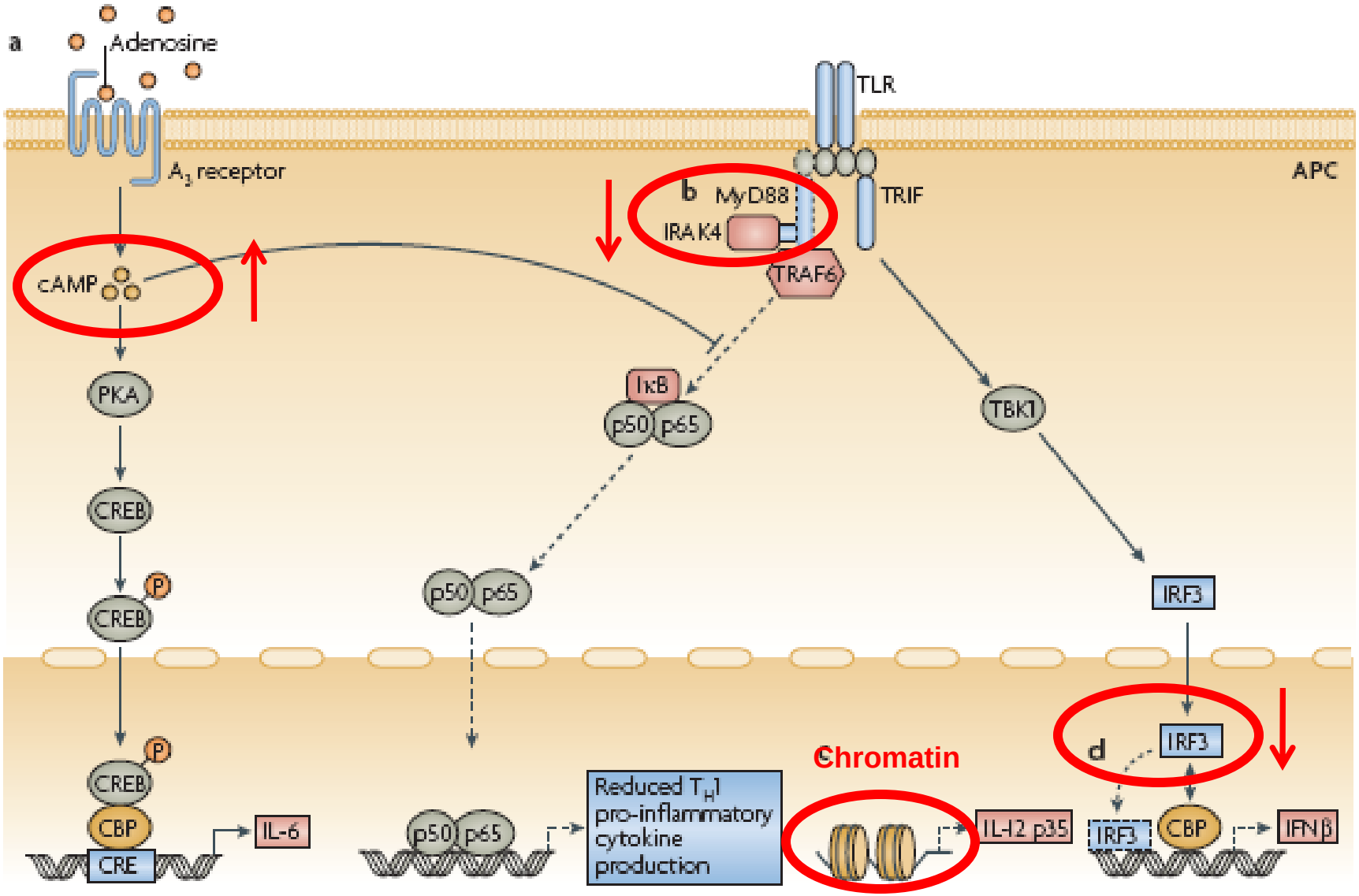
From Levy *et al* JI 04

Neonatal DCs are deficient in IL-12 p35 production in response to LPS, CD40 ligation, or poly(I:C)



Goriely ey al *The Journal of Immunology*, 2001, 166: 2141-2146.

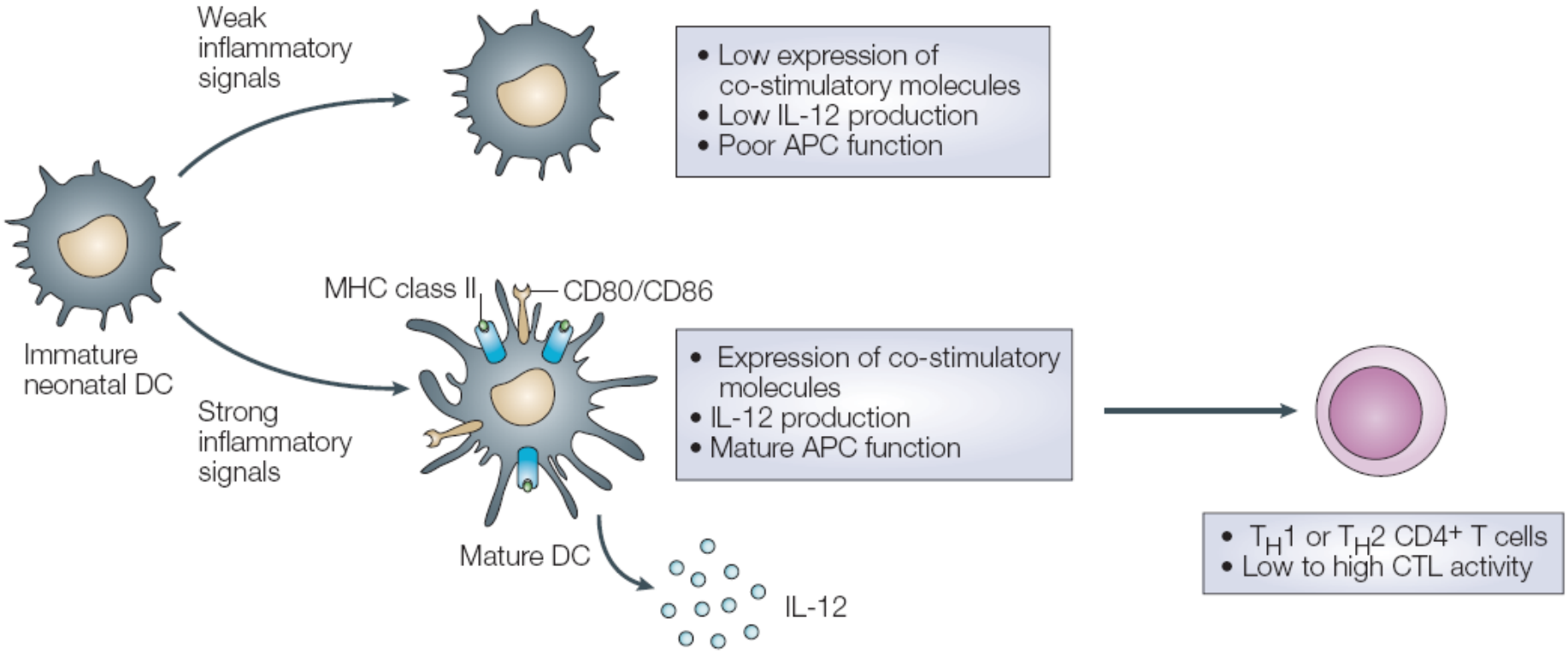
Differences in Neonatal APC



Levy. Nature Reviews Immunology VOLUME 7 | MAY 2007 | 379

Neonatal Dendritic Cells

Low numbers
Poor responses to TLR ligands



Adkins *et al* Nature Reviews Immunology (2004) VOLUME 4: **553**

Why are neonates different?

- The fetus is an allograft onto the mother and is at risk of rejection, so there is a strong T helper 2 (T_H2)-cell bias to fetal innate immune responses, a tendency also initially manifest in the newborn.

Advantages -

- At birth the neonate leaves a sterile environment and moves to a microbe rich one. Neonates must establish tolerance to environmental antigens, including microflora. Avoids over-exuberant and potentially harmful reactions.

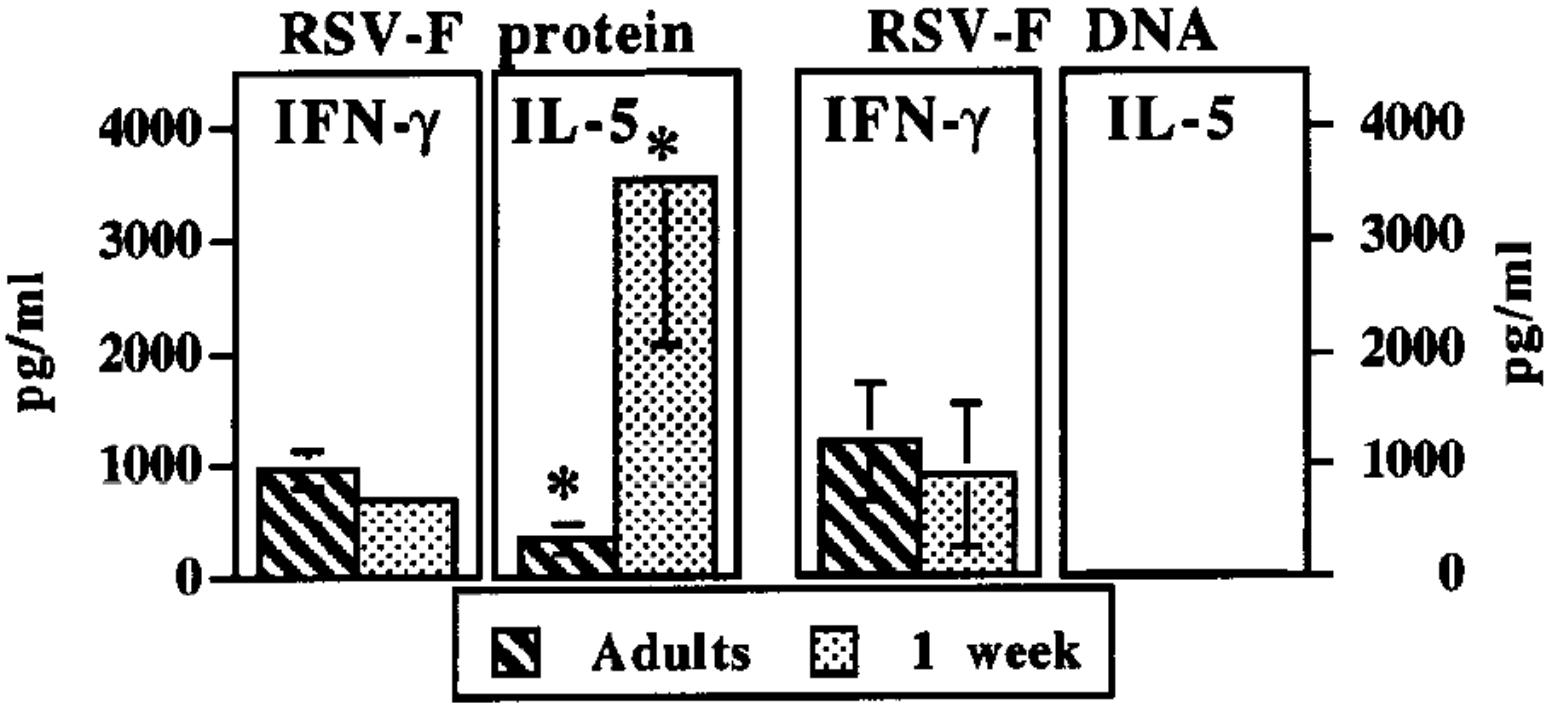
- Inflammatory responses can be inherently dangerous to postnatally developing tissues, such as the lungs.

Adkins et al (2004) *Nature Reviews Immunology* **4**: 553
Levy (2007) *Nature Reviews Immunology* **7**, 379-390

New Vaccine Strategies

The challenge is to develop novel vaccine strategies that overcome maternal antibody and weak neonatal responses.

DNA Vaccination



Further Reading

Adkins *et al* (2004) *Nature Reviews Immunology* **4**: 553

Black *et al* (2003) *The Lancet* 361: 2226

Bryce *et al (2005) The Lancet* **365**: 1147–52

Levy (2007) *Nature Reviews Immunology* **7**, 379-390

Siegrist and Aspinall (2009) *Nature Reviews Immunol.* 9: 185 -193

and references cited in the slides.