Tolerance and Immune Deviation

Christopher B. Wilson, M.D.
University of Washington
Department of Immunology and Pediatrics

Neonatal Vaccination - *Primum non Nocere*

Can/could neonatal vaccination

- Induce tolerance/long-term hypo-responsiveness to vaccine antigens?
- Abrogate self-tolerance $\rightarrow$ autoimmune disease or injury in genetically at risk individuals?
- 'Deviate' the immune response to vaccine antigens and bystander environmental antigens $\rightarrow$ allergy in genetically at risk individuals?

What is the Purpose of Tolerance and How is It Maintained?

- Self-tolerance – protection from self-injury by autoreactive T and B cells
- T and B cell receptors for antigen are produced by random V(D)J rearrangement, generating $>10^7$ different receptors – including receptors recognizing self-antigens and harmless environmental antigens (e.g., allergens)
- T and B cells are hugely useful but, unlike the innate immune system lack intuition, must be educated to assure they respond to 'dangerous non-self' but ignore 'self'
- Multiple mechanisms in concert assure self-tolerance
  - Physical or functional deletion of self-reactive T and B cells
  - Some self-antigens are normally sequestered from the immune system 'Immunological Ignorance'
  - Dominant suppression of self-reactive T and B cells
  - <u>These mechanisms are non-redundant</u>

Physical and Functional Deletion of Self-reactive T and B cells

- Central Tolerance - T and B cell precursors with receptors that react strongly with self-antigens are deleted – BUT only if the self-antigens are present in the thymus and bone marrow, respectively
- Peripheral Tolerance - Newly-formed naïve self-reactive T and B cells are permanently inactivated (become anergic) and deleted in 2^{o} lymphoid organs.

  How is anergy induced? Newly formed T or B cells soon encounter self-antigens, providing signal 1, in the absence of an essential signal 2

Activation of Naïve T or B Cells Requires 2 Signals

- T cell Activation Requires Both
  - Signal 1 – T cell receptor-antigenic peptide/HLA on APC
  - Signal 2 – CD28 on T cell-B7 on APC
- B cell Activation Requires Both
  - Signal 1 – B cell receptor-antigen
  - Signal 2 – CD40 on T cell – CD40 ligand on B cell (T cell-dependent response)

Physical and Functional Deletion of Self-reactive T and B cells

- Fallibility of deletional tolerance and anergy
- Not all self-antigens are present in the bone marrow and thymus or in 2^{o} lymphoid organs in sufficient abundance to remove all potentially-self-reactive T cells
- New T and B cells are formed every day –
  - Anergy/deletion must be continuous and completely successful
  - What is not deleted/anergized could be activated in the future if the antigen is presented in sufficient abundance (signal 1) along with an adjuvant or infection that induces/provides signal 2

Implications for Long-Lasting Tolerance to Self-Antigens or Vaccine Antigens

- Since new T and B cells are generated every day, deletion and anergy must be ongoing at all times to maintain tolerance

  AND EITHER

- Antigen(s) must persist, which is the case for self-antigens but not for vaccines (with theoretical exception of some live vaccines)

  OR

- T and/or B cells reactive to specific antigen must either not be generated (or be generated very slowly) after tolerance is induced

  OR

- Tolerance must be dominant and due to long-lived suppressor or regulatory cells that actively inhibit self-reactive T and B cells

Regulatory (Suppressor) T Cells - Treg

- Distinct subset(s) of T Cells
- Best characterized Treg are CD4+CD25+ cells generated in the thymus; regulatory T cells can also be induced in the periphery in response to TGFβ, IL-10
- Inhibit T cell activation and/or function, thereby inhibiting T cell and T cell-dependent B cell responses
- Deficient in IPEX, an X-linked autoimmune disease due to mutations in FOXP3
- Much to be learned about Treg biology, diversity and mechanisms of control
- Dominant suppression = potential for long-term tolerance through induction of memory Treg

Neonatal Tolerance – What is It and What Does It Mean?

- Freemartin calves - non-identical dizygotic calf twins sharing a single placenta - are permanently tolerant of grafts from each other (Owen 1945)
- Newborn rodents given genetically disparate skin grafts do not reject future grafts of that genotype
- Infection or immunization in newborn rodents often induces long-term inability to generate protective immunity
- This 'neonatal tolerance' is dominant and persistent – mechanism?
  - 'Immune deviation' from Th1/CTL to Th2 response >> complete unresponsiveness mediated by Treg
- Relevance to humans?

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- Can human fetal or neonatal infection or vaccination induce tolerance / persistent hypo-responsiveness to vaccine antigens?

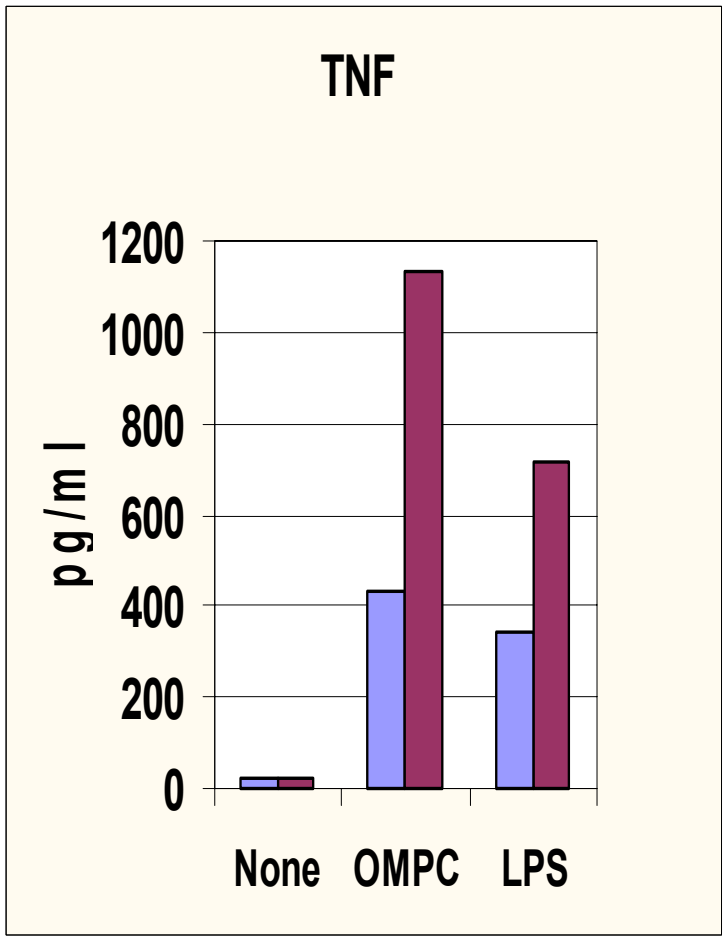
Tolerance/Unresponsiveness to Congenital or Perinatal Infection in Human Neonates

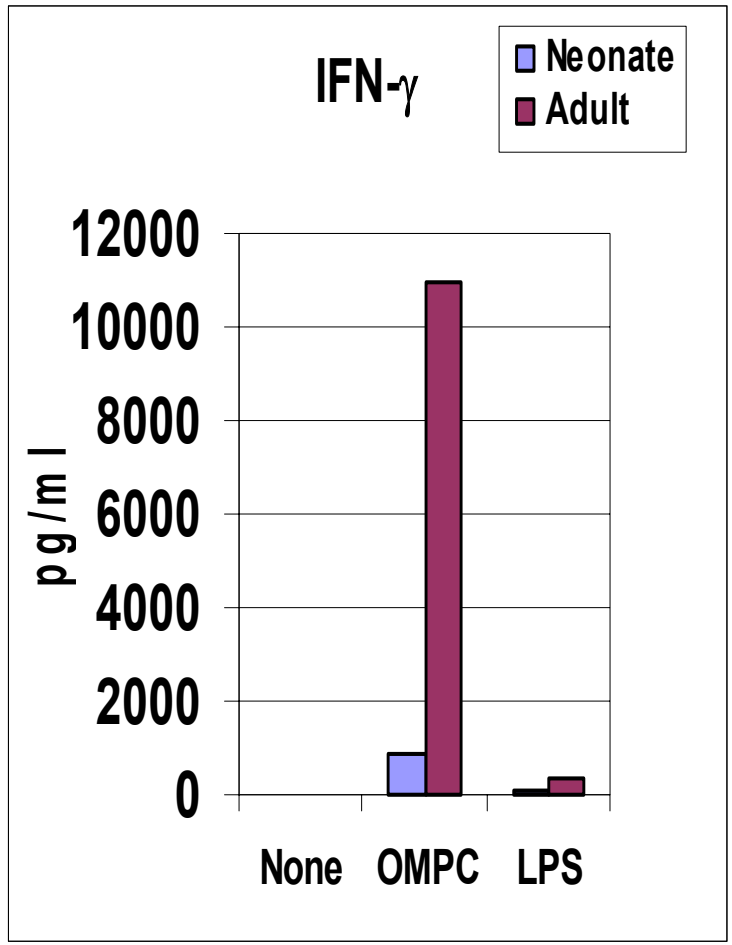
- Many (but not all) infants with congenital CMV, rubella, and Toxoplasma infection do not have detectable antigen-specific CD4 T cell responses (and occasionally Ab responses) until months or more after birth
- Infants with perinatal HSV (Sullender, Burchett) and perinatal/early life CMV (Marchant, Tu) infection commonly have delayed development of antigen-specific CD4 but not CD8 T cell responses
- CD4 T cell unresponsiveness is associated with active viral shedding and resolves after shedding ceases
- Mechanism unknown – anergy, suppression by Treg and/or sequestration at sites of viral replication plausible

Tolerance to Vaccines in Human Neonates?

- Conflicting evidence for persistent (> 9 mo) B cell/antibody hyporesponsiveness to whole cell pertussis given at birth
  - Profound - but no parallel controls (Provenzano,1965)
  - Slight – many other studies – per Peter McIntyre
  - Slight, limited to PT, and only apparent in subset of neonates lacking maternal Ab (Baraff,1984)
  - DTaP - slightly lower PT, slightly higher FHA, similar PRN (Belloni,2003)
- PRP-OMPC at birth $\rightarrow$ B cell/antibody hyporesponsiveness in infancy(Ward, Keyserling), but respond normally at 18 mo to HbOC (Ward)
- Mechanism? - What's special about OMPC?

OMPC Contains Microbial PAMPs that Activate Innate Immunity, APC and B cells via TLR2 > TLR4





Perez Melgosa et al, 2001

Mechanisms of Hyporesponsiveness to PRP-OMPC in Neonates?

- Counterintuitive that PRP-OMPC, containing a microbial adjuvant, is more immunogenic in 2 mo olds but induces hyporesponsiveness at birth
- PRP-OMPC, like PRP-T, induces a cognate T cell-dependent B cell response (proved in mice, Perez-Melgosa)
- Unlike PRP-T, PRP-OMPC provides both signal 1 and signal 2 for B cells, perhaps inducing T-independent B cell activation before T-dependent B cell activation can fully develop
- Are T cells hyporesponsive to OMPC or is defect limited to B cells, specific for Hibid2 B cells?
- Also described for Mening C polysaccharide (Gold, 1977)

Neonatal Vaccination - *Primum non Nocere*

- Abrogation of self-tolerance $\rightarrow$ autoimmune disease. What is the relative risk in neonates?

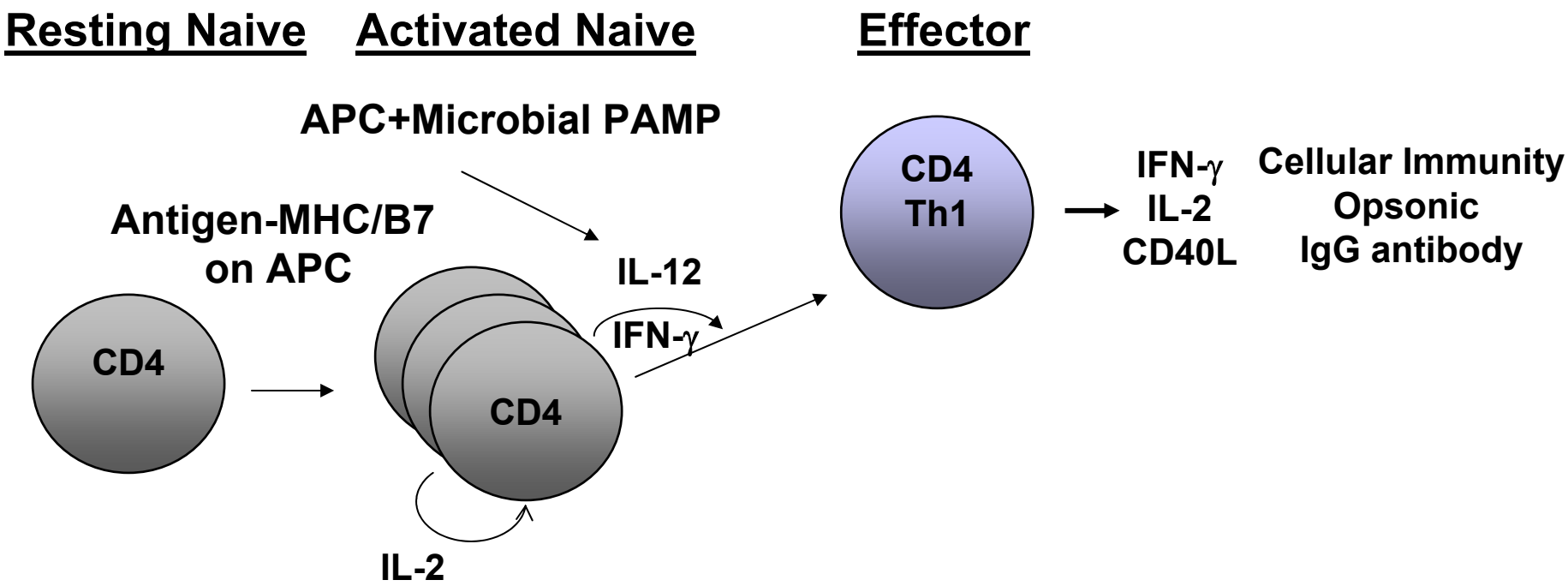
Self-tolerance in Human Neonates?

- Neonatal responses to microbial PAMPs is reduced; prediction $\rightarrow$ less risk for loss of self-tolerance via vaccine acting as adjuvant to break self-tolerance
- Tolerance to alloantigens can be more readily induced *in vitro* with neonatal T-cells than adult T cells (Broxmeyer) - mechanism?
- Unlike rodents, in which CD4+CD25+ putative Treg are not present at birth, human neonates have normal numbers of CD4+CD25+ Treg in thymus and blood
- Regulatory T cells can be induced from cord blood T cells by TGFβ, IL-10
- But there is little functional information about neonatal Treg, or how their responses are modulated by the post-natal environment

Neonatal Vaccination, Immune Deviation and the Hygiene Hypothesis

- Might neonatal vaccination induce a qualitative ‘deviation’ of the immune response not only to vaccine antigens but also to third-party environmental antigens $\rightarrow \uparrow$ risk for allergy in genetically at risk individuals?

Cytokines From Innate Immune Cells/APC Instruct T cells Whether and How To Respond – the Th1/Th2 Choice



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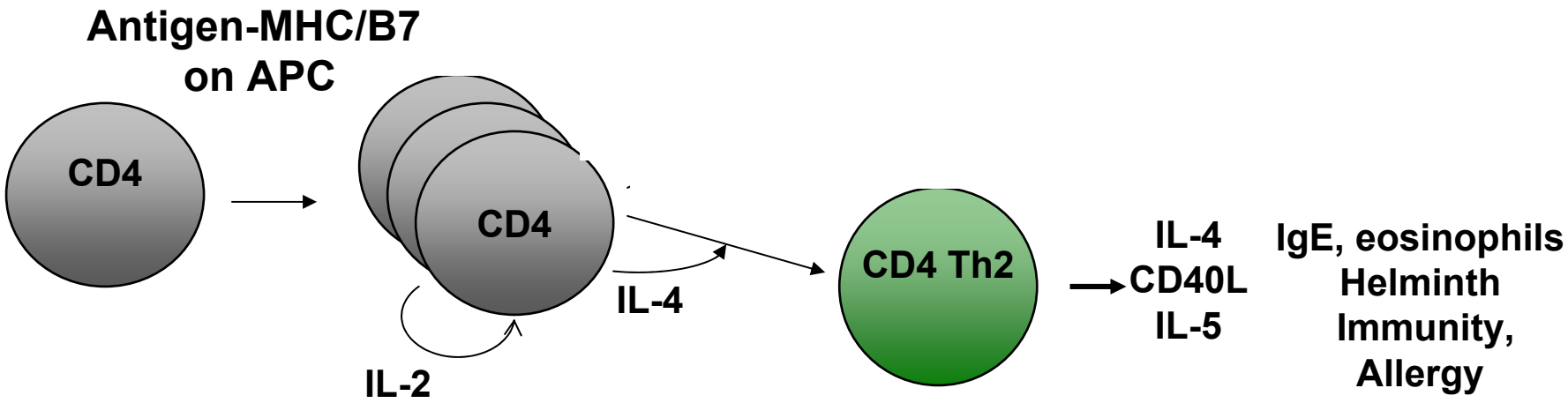
Resting Naive **Activated Naive** **Effector**



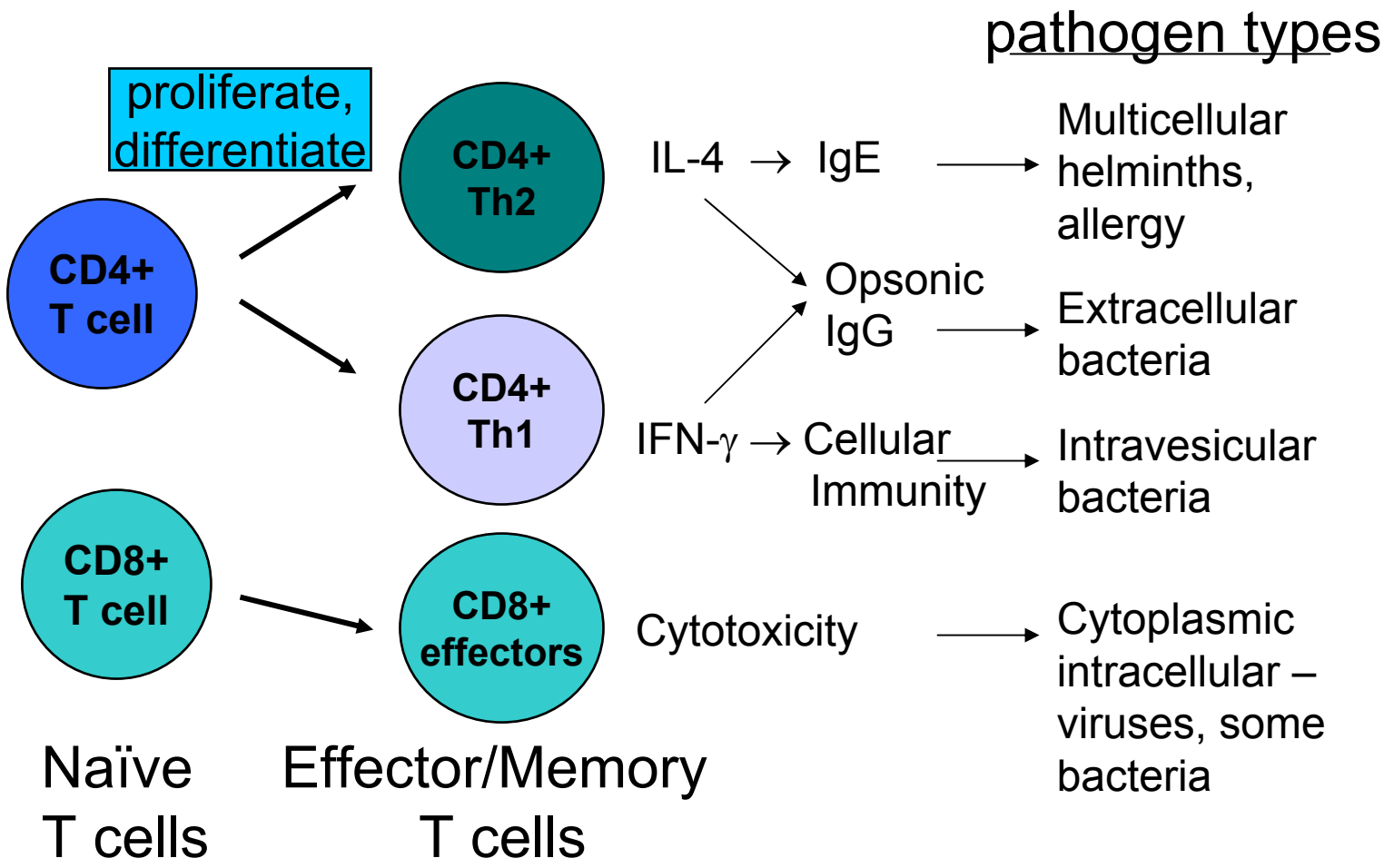
Immune Deviation
Shifting the Response From Th1 to Th2

- Hypothetical Model
- Limited environmental exposure to microbes, diminished ability of neonatal APC/innate immune system to produce IL-12, IFN-γ and Th1 polarizing cytokines $\rightarrow$ Th2 tendency in neonates
- Vaccines given in alum, which lack microbial PAMPs, could in principle further 'deviate' the neonates response to Th2 $\rightarrow$ $\downarrow$ Th1 CD4 T cell responses, alter Ab isotype to vaccine
- **Could** this also 'deviate' bystander response to environmental antigens $\rightarrow$ allergy in genetically at risk individuals?

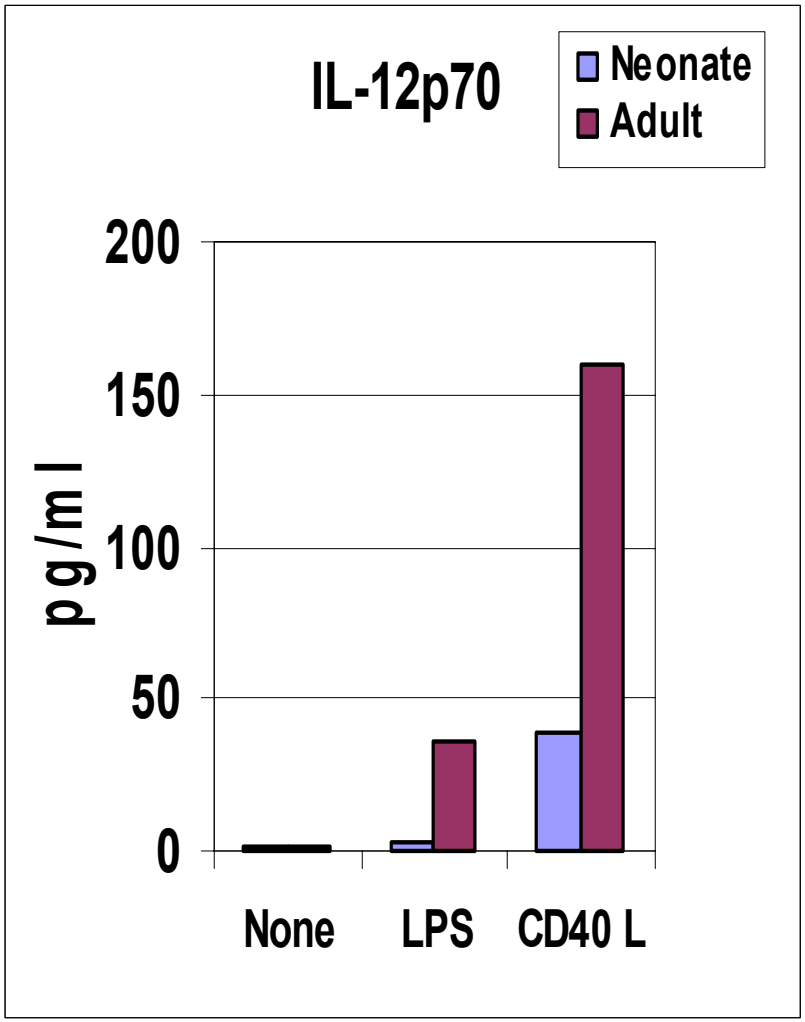
Neonatal Vaccination - *Primum non Nocere*

- Induction of hypo-responsiveness to subsequent immunization? Apparently so, but risk MAY BE restricted to vaccines able to induce T-independent B cell activation, or theoretically live viral vaccines that result in persistent viral replication. Hyporesponsiveness not permanent but duration may include vulnerable period
- Abrogation of self-tolerance $\rightarrow$ autoimmune disease or injury in genetically at risk individuals?
- Are vaccines a plausible factor in deviating the response to environmental antigens to induce allergy in genetically at risk individuals? Would neonatal vaccination make this more likely?

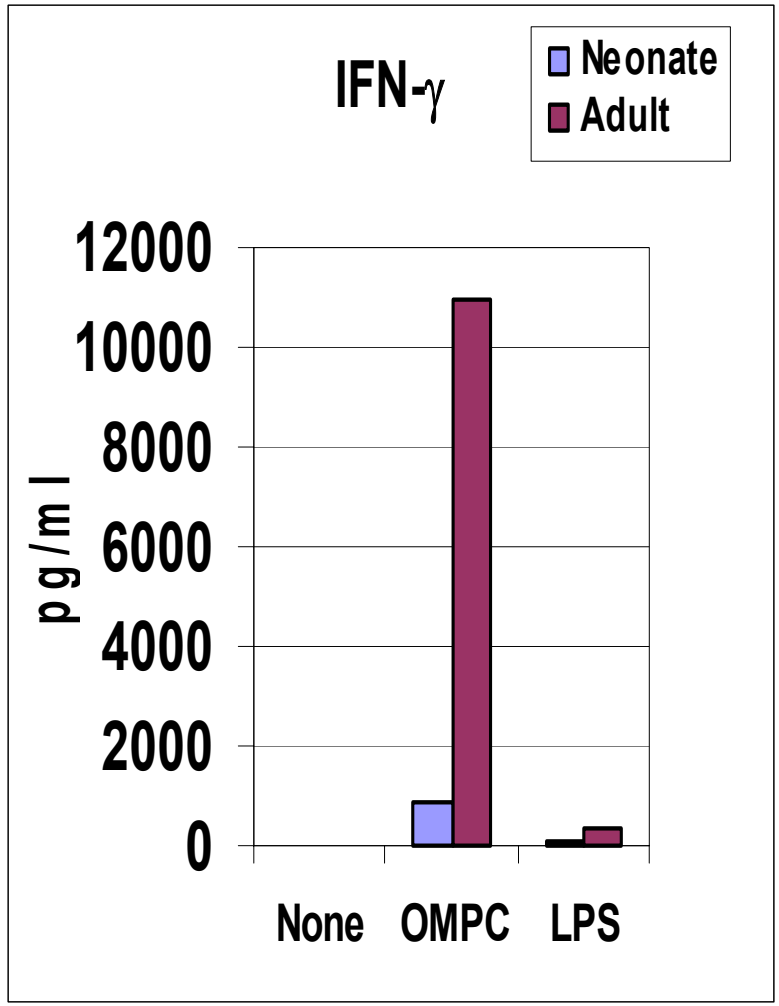
Effector T Cell Subsets – Specialized for Defense Against Different Pathogens



Diminished Production of IL-12 and IFN-γ in response to microbial stimuli and CD40L by neonatal APC/NK cells



Goriely et al, 2001



Perez Melgosa et al, 2001

Microbial Ligands Prime APC Inducing Their Maturation

- T cells are highly specific but not intuitive – they must be educated by APC – APC are the gatekeepers that determine whether naïve T cells become activated or not
- APC receive their instructions from microbes (and microbe containing adjuvants)

Th2 Immune Deviation in Neonates

- Neonatal mice are prone to develop Th2 rather than Th1 CD4 T cell responses to immunization or genetically disparate grafts
- Graft ‘tolerance’ in neonatal mice is due primarily to immune deviation towards a Th2 response rather than clonal deletion or anergy, and is not associated with B cell ‘tolerance’
- Human neonates are also Th2-biased, but the bias may be less dense than in mice