Overview of the Neonatal Immune System

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Innate vs. adaptive immunity

- **Innate immunity**
  - Evolutionarily ancient, no need for prior exposure for action
  - Effective first line of defense
  - Present in all individuals at all times
  - Immediate response (0 – 4 hours) but non-specific
  - Does not generate lasting protective immunity

- **Adaptive (acquired) immunity**
  - Kicks in later (> 96 hours)
  - Initiated if innate immune response is not adequate
  - Antigen specific immunity
  - Lasting protective immunity (Abs, memory T/B cells)
  - Humoral and cell-mediated components

Innate Immune System

- **Physical barriers**
  - Epithelial cells, mucus, tears

- **Receptors**
  - Pattern recognition type (PRR) - Toll-like, Nod-like, Mannose receptor (MR)
  - Scavenger receptors, C lectin like receptors

- **Antigen presenting cells**
  - Dendritic cells and macrophages
  - Neutrophils and monocytes
  - NK cells

- **Soluble factors**
  - Complement (see later – opsinises, direct killing – MAC, recruits cells)
  - Fibronectin (binds pathogen / activates T cells)
  - Mannose Binding lectin (binds to CHO moieties, opsinises, activates complement)
Pattern recognition receptors - major player in Innate Immune Response

- Majority of innate immune response mediated by PRRs
  - PRRs present on the surface of cells
  - Recognizes pathogen associated molecular patterns (glycoproteins, phospholipids, polysaccharides)
  - Recognises self from non self
  - Recognises live vs dying cells
- Two broad groups
  - Endocytic – binding, phagocytosis and intracellular destruction of pathogens without signalling (MR)
  - Signalling - PRR binding induces intracellular signalling that primes adaptive immunity (eg Toll / Nod)

Neonates first line of defence has a few holes

- Epithelium - Intact
- Receptors
  - Not fully clarified, some equivalent (TLR2, MBL) others lower (TLR1), can be up or down regulated.
- APC
  - Monocytes
    - impaired phagocytosis of bacteria, impaired response to some viral agents, cytokine responses less well regulated
  - Neutrophils
    - impaired adhesion and migration, reduced opsonisation and phagocytosis, altered superoxide production
  - Dendritic cells
    - reduced response to pathogens, altered cytokine production, impaired phagocytosis and cell killing
- Soluble Factors
  - Complement – variable, less than adults, more severe reduction in prems
  - Fibronectin – reduced, normal by 1 year
  - Mannose Binding Lectin – “adult levels” in term infants, low in prems

Adaptive Immune Response

- Complex interactions and feedback loops
- Includes humoral and cellular components
- Most start with pathogen ingestion by macrophages / dendritic cells
- Antigens of pathogen are processed and presented to T cells (both CD4 and CD8)
- CTL (CD8) generated – direct killing
- CD4 secrete cytokines and interact with B cells
  - Promotes B cell maturation to plasma cells
  - Immunoglobulin production occurs early if previous Ag exposure (memory B cells) delayed if new Ag

Humoral components - Immunoglobulins

- Immunoglobulins / antibodies
  - Present in plasma, body fluids and on B cell surface
  - Recognise and bind to specific antigens
  - 5 classes - IgG, A, M, E, D but IgG >75% of all antibodies
  - Actions
    - Neutralization
      - Ab bind and neutralizes bacterial toxins, bacteria and virus particles – preventing interaction with host cells, promotes ingestion by macrophages
    - Opsonization
      - Ab assists recognition by phagocytes or NK cells (ADCC) for ingestion / killing
    - Complement activation
      - Ingestion by phagocytes, upregulates T cell response, cytokines

IgG deficiency associated with significant infection risk
Production of antibodies

- Pathogen (virus or bacteria) binds to B cell
- Peptides from the pathogen are presented (MHC II) to the T cell resulting in the activation of the B cell
- B cell proliferation
- B cells differentiate into antibody-secreting plasma cells
- Produce antibodies against pathogen

Immunoglobulin in fetus / neonate

- Synthesis regulated by developmental and genetic mechanisms
- Intrinsically linked to B cell number, maturation and function
- But - production of specific IgG relies on exposure to antigens (infections)
- In utero environment such that neonates relatively protected from exposure to infection / antigens
- RESULT = Limited or no intrinsically produced specific IgG or IgM antibodies at birth

There is a back up plan!!

- Maternal IgG actively transported across the placenta
  - "passive" immunity (but only to antigens to which mother is immune)
  - protects against more common viral and bacterial infections
  - degraded over time; decline in maternal IgG titres to nadir @ 3 – 6 months postnatally
- Amount of foetal / neonatal IgG is dependent on gestational age
  - most transport is in 3rd trimester
  - total IgG in normal term neonate are higher than adult levels
- IgM, IgA, IgE and IgD do not cross placenta

So when will "the force" be with me?

- IgG
  - Increasing amounts after birth, ~ 25% of adult at 1 year
  - Response to protein antigens are good
  - Blunted response to polysaccharide antigens till 18 – 24 months
- IgM
  - Rises rapidly after birth, ~60% of adult levels @ 1 year
  - Important role in neonatal immune defence (complement interaction)
- IgA
  - Levels ~ 20% of adult in first year of life
  - Breast milk provides enteral IgA
- IgE
  - Levels related to atopy / role in primary Ag recognition
- IgD
  - Less well understood - role in primary Ag recognition
Complement System – another loop

- Consists of large group of plasma and cell surface proteins
- Induces bacterial cytolysis
- Solubilises immune complexes
- Releases of anaphylatoxins and cytokines
- Induces B cell proliferation/differentiation
- Activates T cells
- Activated by classical, alternative and lectin pathways

Complement in foetus/neonate

- Complement components are detectable from 5-6 wks gestation
- Levels increase with gestation
- > 28 weeks levels are 50 - 75% of normal adult range
- No placental transfer
- Functional aspects of complement pathway in premature and term neonates are not extensively studied
- Appears that there are limitations/reduction in activity
  - probably related to reduced levels of components
  - activation via alternate pathway or MBL
  - limited ability to activate via classical pathway (little IgM)

Cellular components of the Adaptive Immune System

- Monocytes, dendritic cells
  - Ingest and kill pathogens
  - Break these down into peptides
  - Package peptides with MHC
  - Present antigen to T cells
  - Migrate to lymphoid tissue
  - Produce cytokines
  - Stimulate T cell migration and recruitment
Monocytes, macrophages and dendritic cells

- **Monocytes**
  - Present from early gestation, but few in blood until 5 months gestation
  - Relative monocytosis at birth (term)
  - Functional differences in pathogen response, phagocytosis, killing and chemotaxis cf adult

- **Dendritic cells**
  - Normally DC have highly developed APC function
  - Detectable early in gestation
  - But fetal / neonatal DC have
    - Poor APC functionally
    - Impaired cytokine release
    - Reduced co-stimulation of T and B cells

- Developmental reasons for reduced function
  - Related to need to allow tissue senescence and remodelling without inflammation

**T cells**

- Activation largely via
  - Peptide / protein fragments of foreign pathogen that sit in the MHC binding cleft
  - TCR interacts with MHC 1 or 2 on APC
    - MHC I - CTLs / CD8
    - MHC II - TH 1 or 2 / CD4
  - Cytokine release
    - Activation of other cells
      - TH 1 or TH 2 dependent
    - Proliferation of T cells
    - Direct cell death

**Antigen recognition by T-cells**

- T<sub>1</sub> cells (CD4) recognize antigen presented by MHC II
  - IF gamma mediated activation of macrophages and of B cells (opsonising Ab)
  - Cell mediated Imm
  - Humoral Immunity

- T<sub>2</sub> cells (CD4) recognize antigen presented by MHC II
  - IL4 mediated activation of B cells (neutralising Ab)

**Neonatal Lymphocytes**

- T, B, NK cells produced from early gestation
- Vary with gestational age
- Number and functions differ from older child
- T cell functional differences
  - Immature T cells, predominantly naive (normalises by 2 yrs) – no memory T cells
  - Altered helper/suppressor ratios (more CD4, less CD8)
  - Impaired proliferation in response to Ag
  - Impaired Th1 response to antigen (not so good at stimulating macrophage / DC / PMNs)
  - Diminished delayed type hypersensitivity
  - Cytotoxic T cells less efficient in inducing antigen independent cellular lysis / apoptosis
Neonatal Lymphocytes

- **B Lymphocytes**
  - Can produce IgM, IgG and IgA
  - Response is qualitatively and quantitatively different to adult
  - Restricted repertoire
    - Respond to some antigens but not others
    - Low affinity Abs, mostly IgM
    - Result of intrinsic responsiveness of B cells
    - Effects of T cell functional differences or altered T suppressor ratios

- **NK cells**
  - Numbers adequate
  - But reduced lytic activity

Neutrophils

- Haematology “people” are pretty familiar with these!

PMNs in fetus and neonate – functional issues

- Present from early gestation
- Many functional differences from adults
  - Signal transduction, cell surface protein expression, cytoskeletal rigidity, oxygen metabolism, intracellular antioxidant mechanisms
  - WITH ADDED IMPAIRMENT
    - reduced levels of growth factors and inflammatory mediators
  - RESULT
    - reduced chemotaxis/migration
    - reduced adherence
    - reduced ability to recognise opsinised bacteria = reduced phagocytosis
    - impaired intracellular killing - esp Group B strep, staph aureus, pseudomonas
  - AND
    - Other illnesses eg RDS, compound defects

Not only functional problems

- Altered Neutrophil kinetics
  - reduced storage pool; more severe in prems
  - exaggerated release of available neutrophil pool in response to stimulus
  - neutrophil proliferation is maximal at baseline
  - results in a reduced ability to increase production
  - RESULT
    - less function and reserve
    - initial burst, unsustained
    - “dump and burn”
Summary – neonatal immune response

- Neonate have a “naïve” immune system
- Some aspects function better than others, eg innate functions
- Multi layered defects of proteins, cell numbers and functions
- Creates a relative “immunodeficiency” compared to older child/adult

END RESULT

- Neonates have an intrinsic susceptibility to bacterial, viral and fungal infections
- Presence of additional acquired defects greatly increase the risk

So what?

- Most babies don’t develop sepsis
- Protected in utero
- Effect of relative isolation and lack of exposure
- Protective role of breast milk

BUT!!

Case BB

- Term infant, normal pregnancy and delivery
- Noted to have tachypnoea within 2 hours of birth
- Oxygen / FBE and CXR
- Fluffy opacities in lungs
- WCC 29,000, mostly PMNs, increased band forms
- Antibiotics commenced
- Deteriorates soon after these are commenced
- Tachycardic and shut down with increasing respiratory distress
- Ventilated. Sick++, progressively deteriorates, DIC, renal failure
- Death
- Gp B Strep grown blood and surface swabs

When it goes bad, it can be really bad!

- Infected infants often deteriorate quickly
- Particularly with bacterial infections
- Diagnosis often delayed by lack of typical symptoms and signs
- Need an awareness of risk
- High index of suspicion
- Early intervention with appropriate treatment can salvage these infants
Can the haematology lab help?
Yes, a bit

- FBE – WCC, bands, IM / IT ratios
- Film
  - bands ? Bacteria esp GBS
  - * odd mononuclear cells ? Listeria
  - * blastoid reactive lymphs ? Viral ?? CMV
- Vacuolation
  - ? NEC, ??Staph Epi
  - if prominent ? Candida
- Eosinophilia
  - ? Gram negatives
- + Red cell changes (Fragmentation / haemolysis)
  - ?Gram Negative ? NEC

Interventions – what and when?

- Immunoglobulin - IVIg
  - Controversial, unclear if beneficial in treatment of sepsis
  - ~3 – 4 % reduction in sepsis without reduced morbidity / mortality
  - ? Selection bias ?? Adequate patient numbers.
  - Possibly more useful in premature, ? Prophylaxis more useful
- Granulocyte transfusion
  - Limited data Benefit, who, how often, when???
  - Difficult to get
  - Irradiation NB (GVHD); CMV risk ? Other viral infections
- GCSF
  - Safe, long term effects not known
  - May have a role if neutropenic ? Thresholds, ? Dose
  - ? role for recombinant purified component (complement), ? FFP
- ? gamma interferon
- ? MBL concentrate

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Summing Up

- Be aware of the relative deficiencies of immune function in neonates
  - “Susceptibility to infection” is intrinsic
- Exposure and other acquired defects confound these issues
- Early suspicion, diagnosis and treatment are particularly important
- Broad antinfective cover may be necessary
- Unclear role of immunomodulation
  - May be beneficial in some situations???
- So hopefully a happy ending!!
**TH 1 vs Th2 – what do they do?**

- Still evolving understanding of responses
- Roles in different types of infections
  - Th1 – intracellular pathogens
  - Th2 – extracellular pathogens
- Different cytokines secreted, different cellular activation patterns
  - Th1 ("Cell mediated")
    - Promotes opsinising Ab (IgG1)
    - Induces cellular cytotoxicity
    - Activates macrophages
  - Th2 ("Humoral")
    - IL-4 upregulation
    - Promotes neutralising antibodies (IgG4 and IgE)
    - Stimulates eosinophils development