Immune Deficiency and Dysregulation

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Overview

- Definitions
- Classification of PIDs
- Clinical prediction of PIDs
- Explanation of concepts governing immune regulation and inflammation
- Illustrative cases
Global Causes of Death in Children 2013
Theme of this talk

“There’s no such thing as bad luck”
Definitions

- **Primary Immunodeficiency** – Characterised by absent, reduced or dysfunctional immune cells or antibodies leading to an increased susceptibility to infection.
- **Autoimmunity** – Characterised by organ damage mediated by auto-reactive T cells or auto-antibodies (adaptive immune system).
- **Autoinflammation** – Characterised by chronic inflammation secondary to defects / dysregulation of the innate immune system.
- **Immune Dysregulation** – Characterised by features of autoimmunity, autoinflammation and/or an abnormal response to infection.
“Classic” PIDs – susceptibility to multiple organisms

- Combined T and B cell
  - SCID
- Syndromes with Immunodeficiency
  - Wiscott-Aldrich; DiGeorge; AT
- Predominantly Antibody Deficiency
  - XLA; CVID
- Phagocyte Defects (number or function)
  - Congenital Neutropenia; CGD
- Complement Deficiency
Innate Immune Defects

- Susceptibility to more specific organisms
  - Mycobacterial Susceptibility
    - IFNg pathway defects; GATA2
  - Encapsulated Bacteria & Mycobacteria
    - NEMO; IRAK4
  - Candida
    - STAT1 GOF (CMCC)
  - HSV Encephalitis
    - TLR3
Autoinflammatory Disorders

- Autoinflammatory Syndromes
  - Inflammasome related
    - FMF; CAPS; PAPA
  - Non-Inflammasome related
    - TRAPS, HIGD, Blau Syndrome
  - Receptor Antagonist related
    - DIRA, DITRA
Disorders of Immune Dysregulation

- Familial Hemophagocytic Syndromes
  - with or without abnormal pigmentation
- T Regulatory Cell Abnormalities
  - IPEX; CTLA-4 deficiency
- Syndromes of Autoimmunity with or without lymphoproliferation
  - APECED; ALPS or ALPS-like
- Immune Dysregulation with colitis
  - IL-10 deficiency; IL-10R deficiency
- Type 1 Interferonopathies
  - Aicardi – Goutieres Syndrome (AGS 1-7); STING-associated vasculopathy
JM - 10 Warning Signs of PID

- Based largely on expert opinion and Ig deficiencies in adults
- Require 2 or more to investigate for PID
- Aimed at primary care physicians and the public
- Not Validated

Table 1. Ten warning signs of primary immunodeficiency

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How predictive are the warning signs?

- Basically identify a susceptibility to bacterial infection
- Good at predicting a neutrophil defect
- Poor for B cell, T cell or Complement defects
- Strongest predictor = family history of PID
Diagnosis?
A FEW USEFUL CONCEPTS COMING UP ......
Inflammasomes
T Cell Activation: Co-stimulators
Immune Regulation – Control of inflammatory response

A. B7-CD28 interaction
- Dendritic cell
- T cell
- B7 and CD28
- TCR
- Proliferation, differentiation

B. B7-CTLA-4 interaction
- Dendritic cell
- T cell
- B7 and CTLA-4
- Functional inactivation
- Signaling block
Immune Regulation – ‘Self’ specific Treg cells

Central T cell Tolerance

- Negative selection: deletion
- Development of regulatory T cells

Thymus

- Immature T cells specific for self antigen

Periphery

- Regulatory T cell
Regulatory T Cell (Tregs) express CTLA4 and give APC’s a B7 ‘haircut’
ILLUSTRATIVE CASES
Case 1

- 6yo boy born in Melbourne
- Non-consanguineous Turkish ancestry
- Ex-term well infant
- In Turkey:
  - Age 3: developed petechiae
    - Platelets of 4, improved with IVIg
  - 3.5yo: extensive bruising after bumping head
    - Thrombocytopenia, anaemia, neutropenia
    - Cervical and axillary lymphadenopathy (oncology r/v)
    - Diagnosed with Evan’s Syndrome
- 4yo: Returned to Australia (December 2013)
Case 1

- Recurrent (purulent) ear discharge from 4.5yo
- General Paediatrician referred to Haematologist in early 2014
- Ongoing anaemia and thrombocytopenia managed with oral steroids
- Referred to Immunology June 2014 due to cytopenias and recurrent otitis media ?CVID
Case 1– Initial results

- Dysgammaglobulinaemia
  - IgG 5.6 – 8 g/L, low IgA and low IgM
  - B cell lymphopenia (subsets otherwise normal)
- Poor vaccine responses
- 8% double negative T cells (4% gamma/delta) - ?ALPS
- NK cell function – normal degran, reduced lysis
- Normal SAP / XIAP expression
- ‘Report’ from Turkey that “ALPS genes all normal”
- Normal PHA
- Normal red cell ADA
October 2014: Admission for severe OM
  • Polyps removed from ears – neg micro studies
November 2014: Worsening diarrhoea (?Augmentin)
November 13 2014: Re-vaccinated
November 19 2014: Worsening anaemia
  • Required ICU admission for severe haemolytic anaemia (Coombs +ve) – Hb nadir 47 g/L
November 28 2014: Screening CT Chest – Multiple nodules
December 16 2014: Lung biopsy – non-contributary
March 2015: cervical lymphadenopathy and dyspnoea
April 2015: Endoscopy – c/w EoE, enteritis and colitis, post op rectal bleed
May/June 2015: Admitted with febrile neutropenia
September 2015: Worsening diarrhoea, weight loss, anaemia, thrombocytopenia
Case 1 - Progress

- Steadily worsening splenomegally from November 2014
- FAS, CTLA4, STAT3 (GOF) normal
- Confirmatory email from Turkey that ALPS genotype normal
- Lymph node biopsy October 2015 – Reactive
- Treatment Options October 2015
  - IVIG (since Dec 2014)
  - Prophylactic Bactrim
  - Ongoing ‘complementary’ therapies from family including Kombucha and Bovine colostrum
  - ?? BMT
Cytopenia

ICU

Febrile Neutropenia
PET Scan Sept 2015
CT Chest

- Progressive lymphadenopathy:
  - Massive splenomegaly & lung nodules
What is this?

- **Symptoms**
  - Thrombocytopenia
  - Haemolytic anaemia
  - Neutropenia
  - Lymphadenopathy / Splenomegally
  - Lung nodules
  - Chronic Diarrhoea and FTT

- **But**
  - **Infections**
    - Recurrent otitis media and 1 salmonella only
    - No OI or systemic viruses
  - IgG normal; IgA/M low
  - Lymphocyte subsets essentially normal
  - PHA normal
  - Genetics unhelpful
  - Biopsies unhelpful
Case 1

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<th>✗</th>
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Whole Genome Sequence

- Compound heterozygous truncating mutations in the LRBA gene
- Frameshift deletion + premature stop codon
LRBA: what does it do?

- LRBA ‘controls’ CTLA4 surface expression
- CTLA4 on the cell membrane removes the CTLA4-ligand (CD80/86)
- CTLA4 highly expressed on Treg cells
- Its presence limits the degree of immune activation and autoimmunity
LRBA: clinical features

- Immune Dysregulation with autoimmunity
- Cytopenia: ITP, Evan’s Syndrome, neutropenia
- Inflammatory bowel disease
- Hypogammaglobulinaemia
- Poor antibody responses
- Recurrent infections
- Lymphadenopathy
LRBA Deficiency - Decreased T reg cells in periphery

- Percentage of CD4+FOXP3+ Tcells in periphery of patient vs control
- Open circles – control n = 8
- Solid circles – Patient n = 6
LRBA Deficient- impaired Treg function

In control - large proliferation of T cells in response to stimulation with anti alpha CD2/CD3/CD28
- Addition of Treg cells causes decrease in proliferation

In patient - large proliferation of T cells in response to stimulation
- Addition of Treg cells almost no change in proliferation
Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy

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Abatacept

A Human Immunoglobulin Receptor Fusion Protein

CTLA4

External domain
External
Cell membrane
Internal

Abatacept (CTLA4Ig)

IgG1

Heavy-chain constant region
Abatacept

Abatacept Selectively Modulates T Cell Activation

Without Abatacept

With Abatacept

DC

CD80/86

CD28

Activated T cell

T

DC

Abatacept

DC = dendritic cell.
LRBA: treatment

- 9 patients from 8 unrelated families with known LRBA deficiency
- Treated them with Abatacept (Orencia)
- CT scans, pulmonary function tests, overall inflammation decreased rapidly & dramatically
- Minimal side effects
LRBA: Abatacept treatment
Lessons Learned

• Autoimmunity major presenting feature of PID
• Subtle clues from ‘routine’ tests eg. lgs
• Affected T cell subset (Treg) undetectable on ‘routine’ tests
• High index of suspicion necessary
• Benefit of genetic diagnosis
• Availability of specific therapies
Case 2

- Male 2nd child
  - Non-consanguineous Indonesian parents
- Presented 12/7 old
  - URTI → ALTE → ICU
- Ulcer at 3/52 old
  - Biopsy - > viral inclusions
  - CMV positive on IHC
- 60,000 copies/ml of CMV in serum
- Negative Guthrie CMV
Immunological Phenotype

- Normal other than
  - Mildly low B-cells \((0.57 \times 10^9/L, 11\%)\)
  - Ferritin \(500 \rightarrow 1000\) with fever onset at 4/52
  - Reduced NK cell function with degranulation defect
Diagnosis: Familial HLH

- Centogene gene panel for FHL demonstrated:
  - Both mutations predicted to be pathogenic by:
    - Mutation in exon 27: previously reported as causing HLH
    - Mutation in exon 14:
      - Only reported as causative for HLH in digenic cases
      - Homozygous frequency predicted to be 1:15,000 in Asians

Could we confidently assume these mutations were causative?
Infections

- **CMV**
  - Treated with ganciclovir
  - Rapid decrease in CMV viral load, defervescence
  - Discharged home on valganciclovir

- **Bocavirus pneumonitis**
  - Readmitted to ICU with marked respiratory distress
  - Bocavirus present on NPA & BAL
  - Inhaled budesonide \(\rightarrow\) marked clinical improvement

- Evidence of inability to clear virus & need for BMT
CMV Viral Load & Ferritin
Case 2 : BMT

- Decision made to proceed to BMT
  - Genetic diagnosis
  - Inability to clear virus
  - Response to treatment with antivirals and budesonide

- At the time of diagnosis of FHL (HLH) he did not have HLH !!
Post-BMT (day + 63)
NK Cell Function

<table>
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<th>Effector: target ratio</th>
<th>% of NK Cells Expressing CD107a</th>
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<tbody>
<tr>
<td>20:1</td>
<td>10:1</td>
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<tr>
<td>Patient</td>
<td>7.7</td>
</tr>
<tr>
<td>Control</td>
<td>6.1</td>
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<tr>
<td></td>
<td>11.5</td>
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What is HLH?

- Haemophagocytic Lymphohistiocytosis
- Hyper-inflammatory state
- Immune dysregulation
- Excessive, yet ineffective, immune response

Immune system can ‘see’ a virus but is unable to kill it → cytokine storm → T cell and macrophage activation +++
What is HLH?

- Activated macrophages ‘eat’ cells (haemophagocytosis)
- Increased number of tissue macrophages (histiocytosis)
HLH Pathophysiology

- CTL & NK cells kill target cells via the release of cytotoxic granules

- This requires:
  - Organisation of the granules
  - Transport of granules to the cell surface
  - Perforation of the target cell membrane
  - Release of cytotoxic granules into the target cell

- Defects at any stage lead to impaired cytotoxicity
The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled:

1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)

- Initial diagnostic criteria (to be evaluated in all patients with HLH)
  - Fever
  - Splenomegaly
  - Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood):
    - Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L)
    - Platelets <100 × 10⁹/L
    - Neutrophils <1.0 × 10⁹/L
  - Hypertriglyceridemia and/or hypofibrinogenemia:
    - Fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dl)
    - Fibrinogen ≤1.5 g/L
  - Hemophagocytosis in bone marrow or spleen or lymph nodes
  - No evidence of malignancy

- New diagnostic criteria
  - Low or absent NK-cell activity (according to local laboratory reference)
  - Ferritin ≥500 μg/L
  - Soluble CD25 (i.e., soluble IL-2 receptor) ≥2,400 U/ml
HLH Spectrum

Cetica et al. JACI 2016;137:188-96
Age Distribution of FHL

Cetica et al. JACI 2016;137:188-96
HLH Treatment

- **FHL**
  - Dexamethasone / Etoposide
  - Fatal if untreated & high risk of recurrence
  - BMT

- **Sporadic HLH**
  - Dexamethasone / Etoposide
  - Treat underlying cause if possible
  - Risk of recurrence low
Case 2 – Lessons Learned

- PID presentation may be very subtle
- Suspect PID if unusual case of a common pathogen
- Diagnosis of FHL made before HLH even occurred
- Definitive BMT treatment life saving
Take Home Messages

- PID is an evolving field
- More non-classical PIDs being identified
- Immune over activation (autoimmunity / autoinflammation) can occur in a defective immune system
- Unusually severe or persistent infections with a single common organism should arouse suspicion
Thank You !!