White Cell Enzymes – what are they?

Jean Kirk
RHSC Edinburgh
White cells are an easily obtainable source of Lysosomes

- Intracellular organelles
- contain hydrolytic enzymes at acid pH
- contain no DNA
- most enzymes targeted by mannose-6-phosphate recognition signal
Lysosomes

Are the bulky molecule recycling and disposal centre for the cell

Rubbish (macromolecules) is engulfed whole by the lysosome

Is sorted and broken down according to chemical structure by a series of lysosomal enzymes. The resulting reusable small molecules are transported out of the lysosome by specific carriers
Major Pathways Catalysed

Stepwise degradation of
- Glycosaminoglycans (mucopolysaccharides)
- Glycolipids
- Glycoproteins

Each pathway is catalysed by a series of lysosomal enzymes.
- Deficiency causes a specific disorder
- 50-60 in total. Some diagnosed by lysosomal enzyme profile
Glycosaminoglycans  
(previously known as mucopolysaccharides)

Repeating sequence in hyaluronan, a simple GAG

Repeating disaccharide

Chondroitin sulphate  Normal
Dermatan sulphate  Hurler, Hunter, Maroteaux Lamy
Heparan sulphate  Sanfilippo
Keratan sulphate  Morquio
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<td>sulphatides</td>
<td>Metachromatic Leucodystrophy</td>
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Glycoproteins
important constituents of connective tissue
tend to present with coarse features

- oligosaccharide side chains
  - a and b mannosidosis
  - fucosidosis
  - sialidosis
  - aspartylglycosaminuria
Lysosomal storage disorders

The substrate of the specific enzyme accumulates
LYSOSOMAL STORAGE

Secondary changes in Neurons

Accumulation of toxic proteins
Extra lysosomal storage

Impairment of Autophagy

Dysfunction of cellular systems

- Induction of ectopic dendrites, megaleneurites and axonal spheroids
- Altered axonal transport and Retroendocytic trafficking
- Neuroinflammation

- Mitochondrial dysfunction and accumulation

- Altered ER and Golgi functions

- Perturbation of lipid rafts
- Altered Calcium storage
- Altered Iron storage
- Altered cellular homeostasis

Cellular damage / Oxidative distress

Inflammatory response

Cell death
Clinical Presentation

Lysosomal enzyme deficiencies result in

- lysosomal engorgement with unmetabolised substrate
- excretion of unmetabolised substrates

Presentation depends on

- which tissue is most active in metabolising that specific substrate (liver, spleen, brain)
- or on specific toxicity of the stored material
Specific Features

- startle response
- cherry red spot
- vacuolated lymphocytes or storage cells

characteristic dysmorphic features
- organomegaly
- developmental regression
- dysmorphic features

May lead straight to diagnostic enzyme test
But findings may not be specific of a single disorder (e.g., trivial names for disorders Hurler, pseudo Hurler)
Non specific Presentation – Investigation Strategy

Same clinical syndrome may result from different enzyme deficiencies (eg Tay Sachs & Sandhoff Disease, MPSIII San Filippo Types A, B C and D)
Same enzyme deficiency may present with a spectrum of clinical severity and age of onset (see Tables on next 2 slides)

Lysosomal storage disorders are individually rare
Few clinicians will have extensive experience of them
Patients usually first present to community or non specialist services
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<th>Optic atrophy or ophthalmoplegia</th>
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Strategy for Investigation

Specific features may suggest a specific disorder
– request specific enzyme eg
  - Pompe (cardiomyopathy),
  - Fabry (male with painful extremities, unexplained renal failure, angiokeratomas)

Otherwise
Urine Mucopolysaccharide screening test
Blood lysosomal enzyme profile

Evidence of storage???
– but ask any haematologist – vacuolated wbcs on peripheral films seen in much commoner disorders than lysosomal storage diseases.
Other enzymes selectively (Battens, NPC etc)
Mucopolysaccharides (Glycosaminoglycans)

14 deficient enzymes

1st line screen urine quantitative dye binding test for excess MPS
Good for
MPS1 (Hurler/Scheie),
MPS II (Hunter),
MPS III (San Filippo)

Won’t detect Mucolipidoses, and other lysosomal storage disorders, that may present with similar clinical presentation

Not reliable in babies <3 months (high excretion due to rapid growth & tissue turnover) or dilute urine samples (creatinine <1.0 mmol/L – additive errors in calculating ratios. How reliable are non enzymatic methods in urine at this concentration?)
DMB MPS all data
860 blue diamonds = reported as within ref range,
121 orange triangles = eph done and NAD, 23 pink squares = abnormal eph & MPS
diagnosis confirmed

Age (Years)
Lysosomal enzyme profile (11 enzymes)

Prepare Leucocyte pellet
- 5mL EDTA blood is the MINIMUM
- Differential lysis of red cells
- Requires several incubations & centrifugations – 45 min minimum
- Yield of mixed leucocytes depends on age of sample, patient’s white cell count

ENORMOUS improvement in pellet quality by preparing leucocytes locally and transporting pellets on dry ice, compared to overnight (at best) transport of whole blood to specialist lab before leucocyte isolation.
Leucocyte layer

Prepared leucocyte pellet
Stored −70°C pending analysis
Cells and lysosomes are disrupted by carefully controlled sonication.
Substrates for measuring lysosomal enzymes

- Fluorimetric tag & artificial substrate – 4MU
  4-Methylumbelliferyl β-D-glucopyranoside
  4-Methylumbelliferyl β-D-galactopyranoside
  4-Methylumbelliferyl α-D-galactopyranoside

- Colorimetric tag & artificial substrate – p-nitrophenol
  Despite looking nothing like the natural substrate below, the “aryl” sulphatase enzyme is fooled

- Radioactive tag – natural substrate with $^3$H or $^{14}$C
Pipette sonicate dilutions into tubes. 7 patients = >200 individual tubes. Start enzyme reaction by timed addition of appropriate substrate. Incubation times depend on enzyme. Stop reactions at same timed intervals by addition of an inhibitor.
Fluorimetric and colorimetric enzyme reactions are read directly
Radioactive assays require further manual steps (phase separation or precipitation) to separate product from unreacted substrate.

Separated radioactive product is then measured overnight on a scintillation counter.
Protein is measured in all samples, and the enzyme activity reported /mg protein
Obtain white cell concentrate from Blood Transfusion Service
Bulk prepare white cell pellets
Same method as for patient samples
Store -70°C
Analyse 1 in each batch

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EQA

- ERNDIM lysosomal enzyme scheme
- ~ 80 participants worldwide
- Few teething problems
- To obtain enough material to ship to all participants 2011 scheme used transformed lymphocytes from diagnosed patients and controls.
- Enzyme activity in lymphocytes may be very different from mixed leucocytes eg α-iduronidase ~10%.
- Report results as % mean normals
Reference ranges

- Lysosomal enzyme activities are not usually changed by intercurrent illnesses
- Possible to use patient data from those not diagnosed to construct reference range
- In-house methods – many different suppliers of substrates
- Recheck stability of reference range at regular intervals or following known changes of supplier or instrumentation.
ASA data 2002-6 used to check RefR (0.5 – 1.7)
1 exclusion: 0.06 – diagnosis of Metachromatic Leucodystrophy

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Carrier Detection

Mean enzyme activity of carriers 50% mean of non carrier population – but can’t reliably identify carriers by enzyme analysis.

successful screening programmes in selected communities

DNA mutation studies used within families
- eg for prenatal diagnosis, alone or in combination with enzyme
- Phenotype/genotype correlations for some disorders

In this family prenatal diagnosis was carried out by mutation studies.
Pseudodeficiency

- First described for arylsulphatase A
- Healthy relatives of MLD patients with deficient enzyme activity
- Many have common mutation in MLD gene
- Population estimates 1:7-14 carriers
- 1:50-200 homozygous for pseudodeficiency
- MLD incidence 1:40,000
Chart shows results on 240 children assayed by the same method, and not diagnosed Metachromatic Leucodystrophy.
Two patients with results <0.2 were shown to be homozygous for the pseudodeficiency allele.
Not included above are:
Diagnosed MLD patients: 0.12, 0.07, 0.14, 0.1, 0.22, <0.01
Presumed heterozygotes (parents): 0.36, 0.32, 0.60, 0.41, 0.53, 0.64, 0.75, 0.55
Other disorders - not single enzyme

- targeting of enzyme to lysosomes
  - absent mannose 6 phosphate recognition signal
  - I-cell (mucolipidosis II)
  - Measure very high levels of lysosomal enzymes in plasma
- transport of small molecules out of lysosome
  - Salla disease, infantile sialic acid storage disease
  - Cystinosis
  - Measure storage material in relevant tissue
- multiple sulphatase deficiency
  - features of MLD, Hunter, Morquio
  - Measure more than one sulphatase enzyme
Summary

- Lysosomal enzyme profile is a useful panel of tests to investigate a number of storage disorders that may clinically present similarly.
- Usually in early childhood with developmental regression and/or hepatosplenomegaly.
- It does not rule out all storage disorders, let alone all inherited metabolic diseases.
- Deficient enzyme activity is not diagnostic unless backed up by other evidence.