Central Hypothesis for ME/CFS

There are one or more Metabolic Traps

These are the existence of two stable metabolic states:
  a healthy state and a Disease state

ME/CFS Patients rarely if ever become healthy
However, the very few that do become healthy are totally normal
What is blocking the return to a healthy state?
Metabolic Trap by Substrate inhibition

There are 80 enzymes that show substrate inhibition

The kynurenic pathway shows substrate inhibition and could form a metabolic trap

The kynurenic pathway is important for immune regulation
IDO1&2 catalyzes the first step of the kynurenic pathway
IDO1 is substrate inhibited.

**Biochemistry**
Yamamoto JBC 1967

**Nonlinear Systems theory**
Robert Phair

IDO Flux

<table>
<thead>
<tr>
<th>Tryptophan (uM)</th>
<th>IDO Flux</th>
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<tbody>
<tr>
<td></td>
<td>IDO1</td>
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<tr>
<td></td>
<td>IDO2</td>
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<td>Total flux</td>
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IDO2 Deficient
Genetic

IDO2 Sequencing

• All 20 severe patients have (1.7) non-functional mutations in IDO2

• 46 additional ME/CFS patients
  All have non-functional mutations

About 75% of population have IDO2 non-functional mutations
Race Between TRP import and TRP Degradation

TRP

TRP Transport

TRP

IDO2 ? IDO1

L-KYNURENINE

Robert Phair
Can Cells be Traped

- Substrate inhibition only demonstrated *In Vitro*

- Can yeast cells be trapped using Human IDO1?
  No Kynurenine to NAD = No Growth

- Can Human Immune cells be trapped?
  No depletion of TRP and No Kynurenine
BH4 may be involved in ME/CFS.
Tecan Grower Systems for YEAST

96 well multiwell plate shaking incubators with timed OD measurements
Yeast Model for Metabolic Trap

• Yeast: Added Human IDO1 Gene under yeast control. Removed all other genes that make NAD and all other genes that consume TRP. Yeast growth now dependent upon IDO1 function (NAD needed to make ATP).

• Modified Yeast grow normally at low TRP but **stop** growing at high TRP. They are **trapped**

• This shows that the trap can function in a cell

• Can now screen for a drug that reactivates IDO1 that would consume the TRP and get them out of the **trap**
Yeast in Metabolic Trap

TRP

+ Kynurenine

Independent of IDO1

Dependent on IDO1

GROWTH

Angela Chu
Race Between TRP import and TRP Degradation

TRP Import

TRP

L-KYNURENINE

? TRP Transport

IDO1

Low Fe

TRP

IDO2 ?

L-KYNURENINE

Robert Phair
Preliminary Results

• It appears Human Immune Cells can be trapped
• In High TRP Human cells stop metabolizing TRP to produce Kynurenine.
• Our first experiment shows IDO2 does not block the trap. But much more needs to be done
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