CDC Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Stakeholder Engagement and Communication (MECFS-SEC) Webinar/Conference Call

September 23, 2020
AGENDA

• Welcome – Christine Pearson
• CDC Program Overview – Dr. Beth Unger
• Guest Speaker – Dr. Maureen Hanson
• Questions and Answers

Federal Relay
Event ID: 4537697
For closed captioning, please visit
ustomerID=321

The findings and conclusions in these presentations are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Immune Dysfunction in ME/CFS

Maureen Hanson
Department of Molecular Biology and Genetics

Cornell Center for Enervating Neuroimmune Disease
The Reality of ME/CFS

Few people with the illness are able to work full-time
At least 25% are housebound or bedbound
The most severely ill victims cannot speak, eat, nor tolerate light and sound
Prognosis is poor; fewer than 5% of adults recover most of their prior function
No FDA-approved drug for treatment
A majority of patients indicate onset after a viral-like illness


https://www.nap.edu/catalog/19012/beyond-myalgic-encephalomyelitis-chronic-fatigue-syndrome-redefining-an-illness
Outbreaks of ME/CFS implicate one or more infectious agents

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elk Grove, California</td>
<td>1990</td>
</tr>
<tr>
<td>Lyndonville, NY (between Rochester and Buffalo)</td>
<td>1985</td>
</tr>
<tr>
<td>Incline Village, Nevada</td>
<td>1984</td>
</tr>
<tr>
<td>Chapel Hill, NC (NC Orchestra)</td>
<td>1984</td>
</tr>
<tr>
<td>West Otago, New Zealand</td>
<td>1982-1984</td>
</tr>
<tr>
<td>Mercy San Juan Hospital, Sacramento, California</td>
<td>1975</td>
</tr>
<tr>
<td>Lackland Air Force Base, Texas</td>
<td>1970</td>
</tr>
<tr>
<td>Royal Free Hospital, England</td>
<td>1955</td>
</tr>
<tr>
<td>Adelaide, Australia</td>
<td>1949-1951</td>
</tr>
<tr>
<td>Frohburg Hospital, St. Gallen, Switzerland</td>
<td>1937</td>
</tr>
<tr>
<td>Los Angeles County Hospital</td>
<td>1934</td>
</tr>
</tbody>
</table>

Not a complete list: see large compilation in *The Clinical and Scientific Basis of Myalgic Encephalomyelitis - Chronic Fatigue Syndrome*, 1992
Byron Hyde et al., ed.
What could cause continued symptoms following an acute infection?

Chronic infection
   either by inciting organism
   or by loss of control of known chronic infections (e.g. EBV) or endogenous retroviruses
Damage from the acute infection
Epigenetic alterations in response to the infection
Autoimmunity
Disrupted microbiomes
The Immune System

Immune System

- Mucous Membranes
- Lymphatic Vessels
- Thymus
- Skin
- Bone Marrow
- Tonsils
- Lymph Nodes
- Spleen

NIH.gov

https://lab-a-porter.com/
Analyzing specific cell types in peripheral blood will reveal features that cannot be detected when mixed cell populations are assayed.
T cells are key elements of the immune system.

Bone Marrow

Thymus

CD4

CD8

Secrete cytokines to induce other immune cells to respond to antigens

Cause death of pathogen-infected cells or cancer cells

Circulation
T cells become activated when they interact with a dendritic cell that informs them of the presence of a foreign antigen.
T cells use various types of energy sources to maintain themselves and to respond to activation signals.
The energetic functioning of T cells can be examined by measures of metabolic pathways and mitochondrial characteristics.

- Agilent Seahorse assays to measure activity of:
  - Oxidative phosphorylation
  - Glycolysis
  - Fatty acid oxidation

- Flow cytometry
- Mitochondrial size, shape, membrane potential
- Round Red Fluorescence Detector
- Tubular Green Fluorescence Detector
- Fluorescence microscopy

Created with Biorender: Jessica Maya
Patient population for T cell study

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>ME/CFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>45</td>
<td>53</td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>22</td>
</tr>
</tbody>
</table>

**Illness duration**

21.7 ± 12 yrs

**Daniel Peterson, M.D.**
**Gunnar Gottschalk**
**Marco Maynard**
**Jineet Patel**
**Incline Village, Nevada**
Dysfunction of CD4+ and CD8+ T Cells in ME/CFS

**CD4+ T cells**
- No significant differences
- Normal mitochondrial mass
- Basal and compensatory glycolysis at rest

**CD8+ T cells**
- Mitochondrial membrane potential
- Basal and compensatory glycolysis at rest and after activation

More in:
- Harvard OMF Symposium
  https://www.youtube.com/watch?v=QAdZNU6D7Gs
- Videos from InvestinME Conference and the April NIH Conference at https://neuroimmune.cornell.edu/news/

Myalgic encephalomyelitis/chronic fatigue syndrome patients exhibit altered T cell metabolism and cytokine associations

Alexandra H. Mandarano, Jessica Maya, Ludovic Giloteaux, Daniel L. Peterson, Marco Maynard, C. Gunnar Gottschalk, and Maureen R. Hanson

Assays of metabolism of immune cells tell about the functioning of the immune system: not necessarily applicable to other tissues and organs in the body.

A mixture of many cell types using different fuels.
Immune cells also communicate through both release and uptake of both plasma cytokines and extracellular vesicles.

All three types of vesicles were isolated together by a plasma precipitation method.
Purification and characterization of EVs from plasma

Nanoparticle Tracking Analysis

Immunoblot

Transmission Electron Microscopy

Size and number

Protein markers

CD9

CD81
Study Population for Extracellular Vesicle Study

Manhattan, NY
Subjects recruited by Susan Levine, M.D.

Controls vs. ME/CFS:
- Controls: 28
- ME/CFS: 7

Age:
- Controls: 47.8 ± 13.0
- ME/CFS: 50.6 ± 13.7

SF-36

Physical functioning
Absence of pain
Role limitation due to physical health
Energy/Fatigue
General health
Concentration of smallest particles (exosomes) is higher in ME/CFS subjects
Samples from 38 subjects were analyzed for cytokines EVs and whole plasma

19 ME/CFS and 19 CTRLS in both EVs and whole plasma

45 cytokines

- G-CSF
- GM-CSF
- Granzyme B
- IFN-alpha
- IFN-beta
- IFN-gamma
- IL-1 alpha
- IL-1 beta
- IL-1ra/IL-1F3
- IL-2
- IL-3
- IL-4
- IL-5
- IL-6
- IL-7
- IL-8/CXCL8
- IL-10
- IL-12 p70
- IL-13
- IL-15
- IL-17A
- IL-17E/IL-25
- IL-33
- PD-L1/B7-H1
- PDGF-AA
- PDGF-AB/BB
- TGF-alpha
- TNF-alpha
- TRAIL
- VEGF
Principal component analysis does not separate cytokines present in patients vs. controls.

Plasma

- PC 1 (48.2%)
- PC 2 (11%)
- H < 0.5

CTRL CFS

Extracellular Vesicles

- PC 1 (26.5%)
- PC 2 (20.3%)
- H < 0.5

CTRL CFS
Principal component analysis does separate cytokines present in plasma vs extracellular vesicles.
Are cells communicating normally through cytokines?

When a particular cytokine’s level is high, is another cytokine’s level also high?

Or when a particular cytokine’s level is high, is another cytokine’s level low?
Dysregulation of cytokine-cytokine interactions in plasma

- Positive correlations:
  - 483 (+)
  - 385 (+)

- Negative correlations:
  - 0 (-)
  - 13 (-)

IP-10 involved in numerous ME/CFS negative correlations
Dysregulation of cytokine-cytokine interactions in EVs

Many fewer positive correlations between cytokines in ME/CFS
Conclusions of pilot extracellular vesicle study

- No difference in EV size between ME/CFS and controls
- Significant increase in the concentration of 30-130 nm particles in ME/CFS
- No significant differences in the cytokine levels in plasma and EVs between groups
- Dysregulation of intercytokine associations in both plasma and EVs

Cytokine profiling of extracellular vesicles isolated from plasma in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: a pilot study
Ludovic Giloteaux, Adam O’Neal, Jesús Castro-Marrero, Susan M. Levine and Maureen R. Hanson
Under Review
Our current studies use samples before and after a provocation.

Healthy:
- Good before exercise
- Bad after exercise

ME/CFS:
- Bad before exercise
- Post-exertional malaise after exercise
How does the cargo carried by extracellular vesicles change before and after exercise?
Preliminary cytokine data from exercise subjects

22 ME/CFS
17 Controls
Exercise affects EV intercytokine cargo correlations

Controls preDay1

ME/CFS preDay1

Controls postDay1

ME/CFS postDay1

Controls preDay2

ME/CFS preDay2
Other types of protein cargo are being analyzed by mass spectrometry.

90 samples

15 ME/CFS  preDay1, postDay1, preDay2 = 45
15 controls  preDay1, postDay1, preDay2 = 45
194 proteins total detected in EVs

139 in common
33 in controls only
22 in ME/CFS only
Differential protein content in control vs ME/CFS EVs increases with exercise.

ME/CFS
- 34 proteins lower than in controls
- 57 proteins lower than in controls
- 73 proteins lower than in controls
- 1 higher

Control
- 57 proteins lower than in controls
- 1 higher
Plasma metabolite comparisons may reveal differences in functioning of tissues and organs.
Plasma metabolites analyzed by Metabolon

30 Sedentary controls
45 ME/CFS subjects

LA
Ithaca
NYC
Exercise increases the number of metabolites significantly different between controls and patients.
The majority of metabolites are lower in ME/CFS vs. Controls

- **D1PRE**: n=7
- **D1POST**: n=24
- **D2PRE**: n=30
- **D2POST**: n=56

$q < 0.05$

- **ME/CFS > Controls**
- **ME/CFS < Controls**
Number of lipids and fatty acids in ME/CFS that are higher than in controls greatly increases after the second CPET.

Lipid Species

Fatty acids

100% ME/CFS > Controls
Work in Progress

Pathway Analysis

Integration of physiological measures and clinical information
Acknowledgments

The Hanson Lab Biomedical Group

Carl Franconi
Jessica Maya
Ludovic Giloteaux
Arnaud Germain
Adam O’Neal
Alex Mandarano
Ivan Falstyn
Madeline McCanne
Vivian Huang
Jesús Castro-Marrero

Metabolon, Inc.
Cornell NIH Center
Cornell proteomics: Sheng Zhang

Cornell University
Sloan Foundation
Private donors
Simmaron Research

Daniel Peterson
Gunnar Gottschalk
Marco Maynard
Ivan Falstyn
Jineet Patel

Cornell NIH Center
Betsy Keller
Geoffrey Moore
Susan Levine
John Chia
Staci Stevens
Jared Stevens
Dikoma Shungu
Xiangling Mao
The Cambridge Dictionary defines "Enervating" as:

adjective

causing you to feel weak and lacking in energy

Foremost among cryptic neuroimmune diseases is one variously known as Myalgic Encephalomyelitis or Chronic Fatigue Syndrome or Systemic Exertion Intolerance Disease. The Center’s mission is to promote research to identify its cause(s), biomarkers, and pathophysiology in order to lead to prevention and effective treatments.

Patient-focused webinars available under News tab
Questions and Answers

If you have additional questions following the call, please email CDC at MECFSSEC@cdc.gov.