May 20th, 1:15 PM - 2:45 PM

Activated Innate Immunity in Childhood: A Novel Treatment Target

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Activated Innate Immunity in Childhood: A Novel Treatment Target

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University of Massachusetts Medical School
Division of Pediatric Endocrinology and Diabetes
May 20, 2011
DISCLOSURE

I have no actual or potential conflict of interest in relation to this program or presentation.
An Ecological Framework: Multiple Influences on Physical Activity and Eating Behaviors

- Individual Factors (personal)
  - Cognitions
  - Affective
  - Skills
  - Demographic
  - Biological
  - Genetic
  - Improving the understanding of deleterious effects of high fat food on innate immunity

- Social Environment (networks)
  - Role modeling
  - Social support
  - Social norms
  - Friends
  - Peers
  - Family
  - Co-workers

- Macro-level Environments (sectors)
  - Legislative, regulatory, or policy actions
  - Societal and cultural norms and values
  - Industry
  - Marketing and media
  - Food production & distribution systems
  - Food assistance programs
  - Land use and transportation, zoning
  - Health care systems
  - Government & political structures and policies

- Physical Environments (settings)
  - Availability
  - Access
  - Barriers
  - Opportunities
  - Home
  - Worksite
  - School, Afterschool
  - Child-care
  - Neighborhoods & Communities
Childhood obesity is an epidemic


Based on current trends 86% of U.S. adults will be overweight by 2030.

Obesity is a risk factor for numerous medical conditions

- Pulmonary disease
  - abnormal function
  - obstructive sleep apnea
  - hypoventilation syndrome

- Idiopathic intracranial hypertension
- Stroke
- Cataracts

- Nonalcoholic fatty liver disease
  - steatosis
  - steatohepatitis
  - cirrhosis

- Gall bladder disease

- Gynecologic abnormalities
  - abnormal menses
  - infertility
  - polycystic ovarian syndrome

- Coronary heart disease
  - Diabetes
  - Dyslipidemia
  - Hypertension

- Severe pancreatitis

- Cancer
  - breast, uterus, cervix
  - colon, esophagus, pancreas
  - kidney, prostate

- Osteoarthritis

- Skin

- Gout

- Phlebitis
  - venous stasis
However … not all obese individuals develop complications.

51% of overweight adults

31% of obese adults

Metabolically healthy

Increased visceral fat is associated with a high risk of diabetes

Gesta et al. Cell 2007
Monocytes may be a modifiable source of proinflammatory cytokines

Mononuclear cells from adults with T1DM and T2DM have increased expression of TLR2, TLR4, CCL2 and increased secretion of IL6 and Tnfα.

Mononuclear cells from obese adults have increased NFκB binding and increased expression of IL6 and Tnfα.

Mice lacking TLR2 or TLR4 are protected from high fat diet induced insulin resistance.

Objectives

Adolescents
- Overweight with metabolic syndrome (Overwt-MetSyn)
- Overweight without metabolic syndrome (Overwt-Healthy)
- Lean

1. Assess inflammatory state
   Gene expression
   - Toll-like receptors (TLR2, TLR4)
   - Cytokines (Tnfα, IL6)

2. Correlate monocyte inflammation with anthropometric measurements and serum markers
   - BMI, Waist circumference
   - Glucose, Insulin, Lipid profile
   - Tnfα, IL6

Hypotheses:
1. Monocytes from Overwt-MetSyn subjects will have increased gene expression of TLRs and cytokines when compared to Overwt-Healthy and Lean subjects
2. TLR and cytokine expression will show a positive correlation with anthropometric and serum markers of metabolic disease
Twenty four adolescents were recruited to participate in this pilot study

UMMS clinics
- Pediatric endocrinology
- Adolescent medicine
- Nutrition

Dorchester Academy – inner city high school

- Lean – BMI < 85% age and gender
- Overweight – BMI > 85% age and gender
- Metabolic syndrome – 3 of the 5 components
  * Waist circumference > 75% age, gender, ethnicity
  * Triglycerides > 100
  * HDL < 50 (girls) and < 40 (boys)
  * Systolic blood pressure > 95% age, gender
  * Fasting glucose >100 mg/dl
## 3 groups are similar in age, gender, ethnicity

<table>
<thead>
<tr>
<th></th>
<th>Overwt MetSyn (n=6)</th>
<th>Overwt Healthy (n=9)</th>
<th>Lean (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female, No. (%)</strong></td>
<td>6 (100)</td>
<td>6 (67)</td>
<td>6 (67)</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>16.5 (15.4-17.2)</td>
<td>16.8 (15.9-19.8)</td>
<td>16.6 (15.6-18)</td>
</tr>
<tr>
<td><strong>Ethnic group, No. (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>4 (67)</td>
<td>6 (67)</td>
<td>7 (78)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0</td>
<td>2 (22)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (33)</td>
<td>1 (11)</td>
<td>1 (11)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>39 (30-52)*</td>
<td>32 (26-40)*</td>
<td>21 (18-25)^</td>
</tr>
<tr>
<td><strong>BMI %</strong></td>
<td>98 (96-99)*</td>
<td>94 (85-99)*</td>
<td>50 (36-84)^</td>
</tr>
<tr>
<td><strong>Waist circumference</strong></td>
<td>117 (98-142)**</td>
<td>95 (80-112)*</td>
<td>74 (61-83)^</td>
</tr>
<tr>
<td><strong>Systolic BP (mm Hg)</strong></td>
<td>122 (102-139)</td>
<td>117 (108-141)</td>
<td>114 (104-129)</td>
</tr>
<tr>
<td><strong>Diastolic BP (mm Hg)</strong></td>
<td>75 (61-84)</td>
<td>71 (53-85)</td>
<td>73 (63-81)</td>
</tr>
<tr>
<td><strong>White blood cell counts (k/uL)</strong></td>
<td>8 (5-12)*</td>
<td>6 (4-9)</td>
<td>6 (4-7)</td>
</tr>
<tr>
<td><strong>Monocytes (%)</strong></td>
<td>8 (4-12)</td>
<td>9 (6-14)</td>
<td>8 (5-11)</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dL)</strong></td>
<td>119 (72-162)</td>
<td>113 (83-155)</td>
<td>109 (73-145)</td>
</tr>
<tr>
<td><strong>Triglyceride (mg/dL)</strong></td>
<td>61 (23-125)</td>
<td>48 (24-97)</td>
<td>51 (34-90)</td>
</tr>
<tr>
<td><strong>HDL (mg/dL)</strong></td>
<td>40 (32-47)</td>
<td>41 (24-56)</td>
<td>42 (29-56)</td>
</tr>
<tr>
<td><strong>LDL (mg/dL)</strong></td>
<td>67 (27-90)</td>
<td>62 (49-100)</td>
<td>57 (32-81)</td>
</tr>
<tr>
<td><strong>CRP (pg/mL)</strong></td>
<td>3.5 (1-11)</td>
<td>3.1 (1-16)</td>
<td>1 (1-1)</td>
</tr>
<tr>
<td><strong>Fasting glucose (mg/dL)</strong></td>
<td>98 (82-119)</td>
<td>89 (66-109)</td>
<td>95 (84-110)</td>
</tr>
<tr>
<td><strong>Fasting insulin (uIU/mL)</strong></td>
<td>13 (2-31)*</td>
<td>6 (2-14)</td>
<td>3 (2-6)^</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>2.9 (0.8-7.1)*</td>
<td>1.3 (0.3-3.3)</td>
<td>0.7 (0.4-1.4)^</td>
</tr>
<tr>
<td><strong>TNFα (pg/mL)</strong></td>
<td>2.14 (0.75-4.66)**</td>
<td>0.86 (0-2.5)</td>
<td>0.95 (0.3-1.84)</td>
</tr>
<tr>
<td><strong>IL6 (pg/mL)</strong></td>
<td>2.75 (0.49-3.89)**</td>
<td>1.46 (0-2.74)*</td>
<td>0.61 (0-2.2)^</td>
</tr>
</tbody>
</table>

Data presented as mean (range)

* P < 0.05 compared with lean
** P < 0.05 compared with overweight healthy
^ P < 0.05 compared with all overweight (Overweight healthy and Metabolic syndrome)
Monocytes from Overwt-MetSyn subjects display increased expression of inflammatory genes.
TLR expression in monocytes correlates with circulating cytokines

\[ R^2 = 0.46 \]
\[ p < 0.001 \]

<table>
<thead>
<tr>
<th>TLR4 expression</th>
<th>( R^2 )</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tnf( \alpha ) serum</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>IL6 serum</td>
<td>0.2</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Cytokine expression in monocytes correlates with BMI and central obesity

\[ R^2 = 0.19 \]
\[ p < 0.03 \]

<table>
<thead>
<tr>
<th>Tnfα expression</th>
<th>R²</th>
<th>p -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IL6 expression</th>
<th>R²</th>
<th>p -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>WBC count</td>
<td>0.2</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Conclusions

Assess inflammatory state

Gene expression
- TLR2, TLR4, Tnfα, IL6

Correlate monocyte gene expression with anthropometric measurements and serum markers
- BMI, Waist circumference, Tnfα, IL6

Adolescents
- Overwt-MetSyn, Overwt-Healthy, Lean

1. Monocytes from Overwt-MetSyn subjects display increased gene expression of TLRs and cytokines
2. TLR expression shows a positive correlation with circulating cytokines; cytokine expression correlates with BMI and waist circumference

Ongoing research
1. Recruit more subjects
2. Assess TLR protein expression, surface markers
3. Measure secreted cytokines (Tnfα, IL6) from cultured monocytes at baseline and in response to TLR ligands and dietary lipids
Innate immunity plays a key role in metabolic disease and may be a useful biomarker.

Obese adolescent

↑Tnfα, IL6 serum levels

↑TLR2, TLR4 gene expression in MetSyn

↑Tnfα, IL6 gene expression (trend) in MetSyn

Obese adult

Type 2 diabetes mellitus

Questions
1. Impact of dietary changes on monocyte inflammation?
2. Improvement in monocyte inflammation with weight loss and/or exercise?
3. Reversal of monocyte inflammation with pharmacotherapy or nutritional supplements?

“It is unreasonable to expect that people will change their behavior so easily when so many forces in the social, cultural, and physical environment conspire against change.”

Institute of Medicine, 2000

Acknowledgments

• Mentorship Team
  • Jason Kim, PhD
  • Michael P. Czech, PhD
  • Mary Lee, MD
  • Katherine Luzuriaga, MD

• Collaborators
  • Laura Hayman, PhD (UMass Boston)
  • Jean Wiecha, PhD (UMass Boston)
  • Albert Kim (UMass Boston)

• Study Nurse
  • Carol Ciccarelli, Rn

• Czech Lab Members

• Funding Support
  • CTSA K12 training grant
  • CTSA Life Sciences Moment Fund
  • Diabetes and Endocrinology Research Center Pilot and Feasibility grant
Monocyt es were isolated from whole blood with indirect magnetic labelling

- 25 ml whole blood collected in the fasting state

Indirect magnetic labeling of non-monocytes using a cocktail of biotinylated antibodies and Anti-Biotin Microbeads

Non-monocytes are retained in a MACS Column placed in a MACS Separator. Monocytes pass through the column and are collected as the enriched, unlabeled cell fraction

Topics to be covered in this presentation

Obesity and metabolic syndrome (MetSyn)

Immunologic pathways that may contribute to MetSyn

Potential impact on progression of metabolic disease