Summer Research Report

Presenter: Shane Greene
Mentor: Dhananjay Gupta, PhD, UVM Endocrinology
Type 1 Diabetes Mellitus

- Auto-immune destruction of the insulin-producing β-cells of the islets of Langerhans
- Most patients depend on exogenous insulin to prevent serious metabolic consequences
- Estimated annual cost of disease in the US: $14.4 billion
Current Research in T1DM

• Long Term Goal: a ‘curative’ treatment for T1DM
  • ? pancreas transplant
  • ? β-cell transplant
  • ? in vivo regrowth of β-cells

• To do so, it is necessary to explicate the mechanisms that regulate β-cell growth and longevity
The Vagus Nerve and the Pancreas

• The autonomic nervous system via the vagus nerve is responsible for curtailing innate immune system over activity

• “The cholinergic anti-inflammatory pathway”

• The central cellular component of this system is the α7-nicotinic acetylcholine receptor, which is expressed on the surface of pancreatic β cells
Proposed Inflammatory and Anti-Inflammatory Pathways in β-cells
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SMALL SIGNALING MOLECULES
IL-1, IL-6, TNFα
Proposed Inflammatory and Anti-Inflammatory Pathways in β-cells

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A transcription factor that moves to the cell nucleus in response to cell stress
Proposed Inflammatory and Anti-Inflammatory Pathways in β-cells

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The promoter to which NFκB binds and whose downstream gene produces NO
Proposed Inflammatory and Anti-Inflammatory Pathways in β-cells

A receptor whose activation results in the phosphorylation of STAT3

A transcription factor that moves to the cell nucleus in response to cell stress

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SMALL SIGNALING MOLECULES
IL-1, IL-6, TNFα

A transcription factor that, when phosphorylated, decreases the activation of NFκB

A receptor whose activation results in the phosphorylation of STAT3

Cytokines

STAT3

NFκB

iNOS

Cellular damage
Hypothesis

- Reducing the amount of STAT3 present in pancreatic β-cells will increase the activation of NFκB and iNOS despite concurrent activation of α7AChR via an exogenous agonist
Proposed Inflammatory and Anti-Inflammatory Pathways in β-cells

**Small Signaling Molecules**
- IL-1, IL-6, TNFα

**A transcription factor** that moves to the cell nucleus in response to cell stress

**A receptor** whose activation results in the phosphorylation of STAT3

**A transcription factor** that, when phosphorylated, decreases the activation of NFκB

**The promoter** to which NFκB binds and whose gene produces NO
Greta
Experimental Theory

Drosha

Dicer cleaves to about 22 nucleotides

RNA-Induced Silencing Complex

extensive match

less extensive match

mRNA

 "SLICING"

AAA

5′ RISC

AAA

AAA

ATP

ADP

RISC released

AAA

TRANSLATION REDUCED transfer of mRNA into P-bodies and eventual degradation

Figure 7-112 Molecular Biology of the Cell 5/e © Garland Science 2008
Experimental Approach

1. Purification of STAT3 shRNA plasmid

2. Stable transfection of INS-1 cells with STAT3 shRNA cassette

3. Evaluation of the cytokine (TNF-alpha, IFN-gamma, IL-1beta) mediated inflammatory response in STAT3 knock down in INS-1 cells
Stable Transfection of INS-1 cells with STAT3 shRNA cassette
Evaluation of Cytokine Mediated Inflammatory Response in STAT3 Knock Down in INS-1 Cells

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- iNOS
- P-65 NFkB
- Beta-Actin

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- P-Stat3
- Total-Stat3
- Beta-Actin

The University of Vermont
Reasons to do a Summer Research Project

- Learn a lot of cool science!
- Work with really smart people!
- Produce tangible results!
- Contribute to worthwhile research!
Questions?
Implications of Depression and Obesity on Poor Asthma Control

Sonam Kapadia
Mentor: Dr. Anne E. Dixon, MD
Pulmonary Disease & Critical Care Medicine
Asthma Background

Galli & Tsai, Nature 2012
Asthma Background

Pathogenesis of asthma

antigen

naive T-lymphocyte

Th-0

IL-12

Th-1 response

(dendritic cell

IL-12)

Cell mediated immunity and Neutrophilic inflammation

Th-2 response

IL-4, IL-13

IL-9

IL-3

IL-3, IL-5

GM-CSF

IgE

Mast cells

Basophils

Eosinophils

Mediators of inflammation (eg. histamine, prostaglandins, leukotrienes, enzymes)

Asthma symptoms

Bronchial hyperresponsiveness

Airway obstruction

Medscape Asthma 2013
Background

- Obesity is associated with poor asthma control
- Asthmatics are more likely to suffer from anxiety & depression
- Depression is associated with poor asthma control
- Obesity & depression share a positive, bidirectional association
Hypothesis

- A significant interaction between obesity and depression contributes to worse asthma control among obese asthmatics compared to lean asthmatics.

Beuther et al, 2006 – Obesity & Asthma
Research methods

- Retrospective analysis of ALA TAPE clinical trial

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Asthma and lower airway disease

**Randomized trial of the effect of drug presentation on asthma outcomes: The American Lung Association Asthma Clinical Research Centers**

Robert A. Wise, MD, a,b Susan J. Bartlett, PhD, c Ellen D. Brown, MS, b Mario Castro, MD, d Rubin Cohen, MD, e Janet T. Holbrook, PhD, b Charles G. Irvin, PhD, f Cynthia S. Rand, PhD, g Marianna M. Sockrider, MD, g and Elizabeth A. Sugar, PhD, b for the American Lung Association Asthma Clinical Research Centers* Baltimore, Md, Montreal, Quebec, Canada, St Louis, Mo, New Hyde Park, NY, Burlington, Vt, and Houston, Tex

Background: Information that enhances expectations about drug effectiveness improves the response to placebos for pain. Although asthma symptoms often improve with placebo, it is not known whether the response to placebo or active treatment can be augmented by increasing expectation of benefit.

Objective: The study objective was to determine whether response to placebo or a leukotriene antagonist (montelukast) can be augmented by messages that increase expectation of benefit.

Methods: A randomized 20-center controlled trial enrolled 601 asthmatic patients with poor symptom control who were assigned to one of 5 study groups. Participants were randomly assigned to one of 4 treatment groups in a factorial design (ie, placebo with enhanced messages, placebo with neutral messages, montelukast with enhanced messages, or montelukast with neutral messages) or to usual care. Assignment to study drug was double masked, assignment to message content was single masked, and usual care was not masked. The enhanced message aimed to increase expectation of benefit from the drug. The primary outcome was mean change in daily peak flow over 4 weeks. Secondary outcomes included lung function and asthma symptom control.

Results: Peak flow and other lung function measures were not improved in participants assigned to the enhanced message groups versus the neutral messages groups for either montelukast or placebo; no differences were noted between the neutral placebo and usual care groups. Placebo-treated
QUESTIONNAIRES

The University of Vermont
Results

Odds Ratio of Poor Asthma Control

- Obesity\(^1\): 1.91
- Depression\(^1\): 2.17
- Obesity\(^2\): 1.76
- Depression\(^2\): 2.15
- Interaction: 1.22
Results

Beta coefficient of BMI & CES-D effects on asthma control and quality of life

Asthma Control Questionnaire Score

Asthma Quality of Life Questionnaire Score

Unit Increase in BMI or CES-D Score

CES-D, p<0.001
BMI, p = 0.015
BMI, p = 0.007
CES-D, p<0.001
Conclusions

• Obesity and depression are independently associated with poor asthma control and worse asthma related quality of life.
• Mechanisms linking depression and asthma are the same in lean patients as they are in obese patients.
• Factors other than depression also contribute to poor asthma control in obesity.
Questions?
Summer Research 2013

Russell Landry
Why Summer Research?

- It is fun
- It is hard but not impossible to do
- Summer Stipend ($$$)
- Something to put on the Residency Application (Yay - Bonus)
- Vermont is beautiful in the Summer

*DO NOT DO RESEARCH ALL THE TIME*

Some days are long (~8-10 hours)
Some days are short (~2 hours)
You make the schedule
Long incubation times are great for doing something fun outside
Weekends are always “Free Weekend”
Take days off during the week
Fun

- Different from medical
- School new things to learn
- There are no exams

Tips:
1. Find a lab that is a good fit
2. When you walk in, you want to see lab supplies everywhere
3. Find a lab researching a topic you are interested in (does not have to be Residency field specific*)
4. Talk to the PI, and discuss a project to work on before starting
5. Make sure the view is nice
Not that hard (Trust Me)…
Lab’s Research

- Studying Cellular FLIP (c-FLIP) in the regulation of cellular metabolism during coxsackievirus B3 (CVB3) infection
Importance

- CVB3 infection is one of the most common etiologic agents of viral myocarditis (inflammation of the heart muscle)
- Current treatment for myocarditis patients is exclusively supportive treatment (we need drug therapies)
Background

- Increased production of IFN after CVB3 infection
- IFN decreases myocarditis following CVB3 infection
- IFN Pathway:
  RIG-I + MDA5 $\rightarrow$ recognize viral RNA $\rightarrow$ both interact with MAVS $\rightarrow$ increase IFN production
- RIG-I Pathway:
  Regulated by Caspase-8 and c-FLIP
- c-FLIP: Two isoforms – long (c-FLIP<sub>L</sub>) and short (c-FLIP<sub>S</sub>); only c-FLIP<sub>L</sub> activates Caspase-8
  c-FLIP<sub>L</sub> = increase IFN; c-FLIP<sub>S</sub> = decrease IFN
- IFN-induced antiviral response requires energy (ATP)
- ATP produced in mitochondria (electron transport chain)
Hypothesis

- Based on the observation that both forms of c-FLIP differentially regulate IFN secretion and certain viruses usurped the short form of FLIP from the host genome, we undertook studies to investigate how c-FLIP_L, c-FLIP_S, and viral FLIP (v-FLIP) influence metabolic events to enable an antiviral response.
Methods

- Mouse embryonic fibroblasts (MEF) as model
- MEFs transfected to overexpress FLIP variants (c-FLIP<sub>L</sub>, c-FLIP<sub>S</sub>, or v-FLIP (MCV))
- Controls: MEF c-FLIP wild type (c-FLIP WT – has both long and short c-FLIP) and MEF c-FLIP knockout (c-FLIP KO)
1. Cell lines expanded then plated in quadruplets at 30,000 cells per well
2. Infected with CVB3 or not infected (mock); multiplicity of infection of 10 (10 virions per cell)
3. Incubated for 3 or 24 hours
4. Run on Seahorse
5. Count cells in plate to normalize oxygen consumption readings

Figure 1. The Seahorse XF24 measures mitochondrial respiration, and thus the major energy producing pathway of the cell. The fundamental parameters of mitochondrial function are depicted in the scheme: basal respiration, ATP turnover, proton leak, and maximal respiration, or spare respiratory capacity. The decrease in the oxygen consumption rate (OCR) upon addition of oligomycin is proportional to ATP production. FCCP uncouples proton movement from ATP production, and therefore induces the maximal metabolic potential of a cell. Rotenone abolishes mitochondrial oxygen consumption; the calculated proton leak indicates mitochondrial damage due to protons that escape the mitochondria.
Results

- Non-infected wild type MEFs had minimal changes, 3 hr CVB3 had increased basal respiration and spare respiratory capacity.
- c-FLIP\textsubscript{L} had unchanged maximal respiratory capacity with 3 hr CVB3 infection.
- c-FLIP\textsubscript{S}, and v-FLIP infected with CVB3 for 3 hr had increased basal respiration, proton leak and maximal respiratory capacity.
- 24 hr CVB3 c-FLIP\textsubscript{L} and c-FLIP WT had decreased overall oxygen consumption, and c-FLIP\textsubscript{S} and v-FLIP had unchanged oxygen consumption.

Figure 2. The OCR of non-infected (black circles) and CVB3-infected (red circles) mouse embryonic fibroblasts, expressing various forms of FLIP, was measured 3 hours post infection. In wild-type MEF, CVB3 infection led to an increase of overall metabolism. FLIP overexpression did not change the basal OCR compared to wild-type cells, but increased the spare respiratory capacity after infection. By contrast, both short forms of FLIP (c-FLIP\textsubscript{L} or MCV) increase metabolism, which intensifies upon viral infection.

Figure 3. After 24 hours, the metabolic rates, and particularly the spare respiratory capacity, of infected wild-type and FLIP overexpressing cells decreased below the levels of their respective non-infected controls. The metabolic rate of infected FLIP\textsubscript{L} and MCV overexpressing cells paralleled the levels of their non-infected counterparts.
Conclusion

1. Overexpression of either form of FLIP effects cellular metabolism during viral infection

2. During the early stages of viral infection, c-FLIP$ _S$ and v-FLIP promote higher metabolic rates, whereas c-FLIP$ _L$ does not.
   - This observation coincides with the fact viruses usurped only c-FLIP$ _S$
   - Increasing metabolic rate could support an elevated demand for energy during viral infection

3. Later stages of infection, c-FLIP$ _L$ decreases cellular metabolism
   - Increased IFN may cause this effect
Summer Time in Vermont

Berry Pick’n

Beach Bum’n

Adventures Out!

Cook Out!

Burlington 4th of July Party!
Special Thanks

- Dr. Ralph Budd
- Dr. Iwona Buskiew
- Dr. Andreas Koenig
- Ms. Elizabeth Yasewicz

Questions?
Please feel free to contact any of our presenters with questions regarding their research experience

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