IMMUNOTOOLS:
EFFECT OF NOTCH-DEFICIENT MACROPHAGES TO AUTOIMMUNE DISEASE

22-02-2017
WIPAWEE WONGCHANA
WHAT DO YOU SEE?
Immune system

Allergy
Ref: https://en.wikipedia.org/wiki/Anaphylaxis
Ref: http://home.allergicchild.com/less-common-allergies-environmental-pet/

Rheumatoid Arthritis

Over reaction

Under reaction

Chronic hepatitis
Cirrhosis
Hepatocellular carcinoma (with cirrhosis)
Smallpox
Breast cancer

Ref: http://www.medthical.com/hepatitis-b.html
Ref: http://carrington.edu/blog/medical/vaccines/smallpox-and-smallpox-vaccine/
Ref: https://en.wikipedia.org/wiki/Breast_cancer
HOW TO DEFENSE AGAINST FOREIGN BODIES?

Ref: https://universe-review.ca/R10-40-Immune.htm
CELLS MEDIATED IMMUNE RESPONSE

(Source: the Human Immune Response System www.uta.edu/chagas/images/immunSys.jpg)
CROSSTALK BETWEEN INNATE AND ADAPTIVE IMMUNE RESPONSE

DCs, Macrophage, B cell

Distinguish between “self” (body) and “non-self” (foreign bodies)
WHAT HAPPENS WHEN IMMUNE SYSTEM FAIL?

Autoimmune disease

Recognize and defense against self

http://drpinna.com/auto-immune-diseases-15353
RESULTS OF AUTOIMMUNE RESPONSE

Inflammation

- Tissue damage
FOCUS

Macrophage

- Regulation of cytokine production
- Co-stimulatory molecule

Autoimmune disease

- Progressive of disease
MACROPHAGES

Cell line

Primary cells

Wipawee W. Unpublished data
GENERATION OF MACROPHAGE PRIMARY CELLS

C57/BL6 mice

- Wild type
- Knockout

Day 0
DMEM high glucose with FBS

Day 3
5% (v/v) horse serum

Day 7
20% (v/v) L929- conditioned media

Collect bone marrow cells
FLOW CYTOMETRY: BIOTECHNOLOGY ANALYSIS OF CELL MARKER

http://cvr.pitt.edu/facilities/vaccine-research-laboratory/shared-resources
PHENOTYPE OF KNOCKOUT MACROPHAGES

IFNγ/LPS 6hr

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<thead>
<tr>
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<tr>
<td>β-actin</td>
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% of Max

Notch1

Ctrl

N1KO

IFNγ+LPS 18 hr

unstimulated 18 hr

isotype
CONCEPT OF THE STUDY

Transcription

Regulation of transcription
mRNA

Translation

Expression protein
Secreted cytokine

MOLECULAR MECHANISM OF CYTOKINE PRODUCTION

Regulation of transcription

- Notch signaling pathway
- NF-κB signaling pathway

[Diagram of molecular mechanism]
INTERLEUKIN-6

[Image of a diagram showing transcription factors and histones interacting with DNA to activate gene expression.]

[Graph showing fold enrichment relative to input for RAW264.7 with conditions: unstimulated, LPS+IFNγ 12 hr, rabbit IgG, and anti-Notch1. The graph also shows significant p < 0.05 difference.]
INTERLEUKIN-12

- IFN-γ
- IL-4/PGE₂
- STAT1/2
- NF-κB
- p50, c-REL
- MyD88
- PAMPs/TLR
- Plasma membrane
- Nucleus
- ICSBP, IRF1/c-REL, PU.1/ET12, GAP12, p50, c-REL, C/EBPβ, AP1
- ETS, GATA, GAAATTCCC, ATGGTGCAACAAAGTCAG, TATA
- (-211/-207), (-156/-153), (-115/-106), (-75/-64), (-28/-22)
CO-STIMULATORY MOLECULE

**Fig. 2.** T cell activation requires 2 types of signals, which are 1) TCR to peptide within MHC molecule and 2) binding of co-stimulatory molecules (i.e., CD80 and CD28).
SUMMARY- MACROPHAGES

- Generate genetic modified macrophages from hematopoietic stem cells
- Confirm phenotype of the knockout trait
- Promise data on the reduction of function cytokine production related T cell polarization
- Decrease in the co-stimulation molecule (CD80) involved in T cell activation
MULTIPLE SCLEROSIS

Auto reactive T cells; Th1 and Th17 CD4+ T cell
DCs and macrophages
HOW TO STUDY MULTIPLE SCLEROSIS?

Animal model:

Use self-peptide, CFA, Pertussis toxin to induce multiple sclerosis in mouse

Experimental Autoimmune Encephalomyelitis
STUDY STRATEGY

1. Transfer activated macrophages
2. Induce EAE disease
3. Score at Day 7 after immunization (daily)
4. Sacrifice and collect spleen at Day 14-15
5. Re-stim splenocyte for 3 days
   - ELISA
   - Intracellular cytokine staining for FACS

MOG$_{35-55}$ peptide + CFA
Pertussis toxin

C57BL/6
**SCORING**

The progression and severity of disease will be monitored and scored from 0-5 as follows:

0 - no disease
1 - limp tail
2 - hind limb weakness
3 - hind limb paralysis
4 - hind and fore limb paralysis
5 - morbidity and death
PROMISING ON PROGRESSIVE OF DISEASE
IFNγ AND IL-17 PRODUCTION IN RE-STIM-SPLENOCYTES

Fig. 2. T cell activation requires 2 types of signals, which are 1) TCR to peptide within MHC molecule and 2) binding of co-stimulatory molecules (ie CD80 and CD28).
SUMMARY- MACROPHAGES IN AUTOIMMUNE DISEASE

- Delay onset and progressive of EAE after transferring knockout macrophages
- Transfer knockout macrophage affect Interleukin-17 expression in study model
OVERVIEW

LPS/TLR4

Notch

NF-κB

MAPKs

Gene transcription

IL-12p40

IL-6

CD80
Fig. 2. T cell activation requires 2 types of signals, which are 1) TCR to peptide within MHC molecule and 2) binding of co-stimulatory molecules (ie CD80 and CD28).
PUBLICATIONS


ACKNOWLEDGEMENTS
THANK YOU