Mechanism of β-Cell Death/Failure in Diabetes

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Pancreatic Islets with Insulitis in T1D
Apoptosis of $\beta$-cells as the final step in T1D
Islet mass in db/db mice before & after T2D
TUNEL+ β-cells in db/db mice
1. Signal transduction in β-cell apoptosis (type 1 PCD) of T1D
2. Could β-cell death be the initial event in T1D?
3. Signal transduction of lipid injury in T2D
4. Role of autophagy (type 2 PCD) in β-cell mass & function
1. Signal transduction in β-cell apoptosis of T1D
Destruction of Pancreatic Islets in Autoimmune Diabetes

T cells

CD4

CD8

CD8

FasL

TRAIL

TNFβ

IFNγ

TNFα

Islets

MΦ

IFNγ

TNFα

IL-1

Granzyme

Perforin

TNFα
Caspase-dependent Apoptosis of Insulinoma cells by TNFα/IFNγ Synergism
Caspase-dependent Apoptosis of MIN6 insulinoma cells by TNFα/IFNγ Synergism

Untreated

IFNγ+TNFα

DNA content

Cell number

2.3 %

26.3 %

15.0 %
STAT1 in IFNγ/TNFα Synergism

A

30 min

U  T  I  D

24 h

U  I  T  D

48 h

U  I  T  D

Phospho STAT1

STAT1

B

% blue cells

Vec + LacZ

STAT1 + LacZ

None TNFα

None TNFα

None IFNγ

None IFNγ

+TNFα

+TNFα
*In vivo* evidence indicating the role of IFNγ-inducible genes and TNFα in T1D of NOD mice.
DTH-like Model in Pancreatic β-cell Apoptosis
(J Immunol 166:4481, 2001)
No diabetes in STAT1--/- NOD

A. male

- +/+ (n=5)
- +/- (n=18)
- +/- (n=14)

B. female

- +/+ (n=6)
- +/- (n=25)
- +/- (n=18)

Diabetes 56:2651, 2007
TNFα/IFNγ vs. IL-1β/IFNγ in islet cell death

IL-1β + IFNγ + TNFα

STAT1, IRF-1

NF-κB

iNOS

XIAP

NO

Apoptosis

Diabetes

β-cells
Induction of TNFα susceptibility by IκBα-SR

(Diabetes 52:1169-75, 2003)
Decreased death of mIκBα islets by IFNγ/IL-1β
Production of RIP-mlκBα transgenic mice

Diagram showing the production process involving backcrossing of NOD and B6 strains to produce NOD-scid and B6 strains, with the insertion of RIP, IκB-RR-AA-F-PEST, and SV40 poly A. The diagram also shows the detection of mIκBα and endogenous IκBα in Tg- and Tg+ samples.
No degradation of mIκBα by TNFα
Insulin + TNFα

Tg +

Hoechst

Merge
Accelerated Diabetes in RIP-\(\kappa B\alpha\) NOD mice

PNAS 104:1913, 2007
Implication

1. Apoptosis of pancreatic β-cells is the final step in T1D.

2. STAT1 plays an important role in β-cell death by cytokines *in vitro* and in the development of T1D *in vivo*.

3. NF-κB plays an antiapoptotic role in T1D of NOD mice.

4. TNFα or TNFα/IFNγ rather than IL-1β or IL-1β/IFNγ is the dominant death effector in T1D.

5. Caution should be given to the therapeutic application of NF-κB inhibitors for the treatment of T1D.
2. Could $\beta$-cell death be the initial event in T1D?
• Apoptosis is the final step in T1D.
• The initial event of T1D is still elusive.
• Physiologic apoptosis of β-cells (organogenesis) has been reported to prime naïve T cells.
• Apoptotic cells do not induce inflammation, while necrotic cells do.
• However, apoptotic cells might be able to induce inflammation or immune responses on certain conditions (secondary necrosis).
• Apoptosis could be both the initial and final event in T1D.
Apoptosis vs. Necrosis

Phagocytic clearance of apoptotic cells

- Waste disposal
  - Removal of cell corpses
  - Prevention of leakage of contents from dying cells

- New meaning
  - Suppression of inflammation (e.g., TGF-β1, PGE2, TNF-α)
  - Modulation of cell killing (e.g., NO, CD35L)
  - Regulation of immune response (via class I and II MHC)

Danger model (DAMP)

- T helper
- APC
- Distressed cell
- Normal cell
- Sig1
- Sig2 (Co-stimulation)

Presentation of peptides
Degradation and processing
Early exposure of 'eat me' flags
Altered secretion
Alarm signal
Does apoptosis initiate autoimmunity?

Peripancreatic lymph node, not Spleen
Aims of the Study

1. To study whether apoptotic $\beta$-cell accumulation could stimulate APC on certain conditions

2. To explore the receptors for apoptotic $\beta$-cells and signal transduction

3. To study their roles in the initiation of T1D
Generation and classification of early, late apoptotic cells and necrotic insulinoma cells

- Viable cells
- Early apoptotic cells
- Late apoptotic cells
- Necrotic cells

PI

Annexin V-FITC
Production of proinflammatory molecules by B6 Mφ after contact with LA (secondary necrotic) cells but not EA cells

![Graphs showing production of proinflammatory molecules (TNF-α, NO, IL-12, p70, IL-1β) by B6 Mφ after contact with LA (secondary necrotic) cells but not EA cells.](image)
Proinflammatory responses by LA cells are mediated by NF-κB pathway.
Transfection with DN MyD88, a common adaptor for TLR abolished NF-κB activation by LA β-cells.
Proteinase K abolished TNF$_\alpha$ release by LA cells.
(proteinaceous nature of LA cell ligands)
No induction of IFNβ by LA β-cells

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<th>LPS</th>
<th>PGN</th>
<th>LA</th>
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- **TNFα**
- **IL1-β**
- **IFNβ**
- **Actin**
No STAT1 Tyr phosphorylation by LA β-cells
TNFα release after LA β-cell encounter was inhibited in TLR2 KO mice.
No significant role of TLR4 in LA β-cell response
*In vivo* priming of diabetogenic CD4+ T cells by DC was studied by measuring CSFE dilution with FACS.
β-cell-selective T cell priming *in vivo* was impaired in TLR2 KO mice.
Figure 4

A

TLR2+/+

TLR2−/−

% Diabetes

Days

B

TLR4+/+(HeN)

TLR4−/−(HeJ)

% Diabetes

Days

D

NOD.TLR2+/+

NOD.TLR2−/

% Diabetes

Weeks of birth

E

TLR2

WT

KO

% Insulitis
LA $\beta$-cells trigger TLR2-dependent stimulation of APC and priming of autoreactive T cells in PLN

**TLR2-dependent priming of autoreactive T cells**

**Immunity** 27:321, 2007
Summary

1. LA insulinoma cells induced production of TNFα, IL-12 and NO, like necrotic cells.

2. IκBα-SR abrogated proinflammatory cytokine production by Mφ in response to LA cells.

3. Cytokine production in responses to LA cells was attenuated in TLR2−/− Mφ but not in TLR4−/− Mφ.

4. DC maturation by LA cells was abrogated in TLR2−/− mice.

5. The incidence of multiple STZ-induced diabetes or natural T1D was lower in TLR2−/− mice compared to wild-type mice.

6. TLR2 blockade could be a modality to inhibit T1D.
3. Signal transduction of lipid injury in T2D
Normal Islet

Islet Hyperplasia

Insulin Resistance

Leptin, Metformin

AMPK → AMPK(P)

ACC → ACC(P)

Acetyl coA → Malonyl CoA

Islet Failure

What proteins?

What proteins?

Apoptosis? Oxidative stress?

TG

Fatty acid

PPAR

CPT
Possible Mediators of β-cell Apoptosis in T2D

- **Zinc**
- **Tobacco Nicotinic Acid (TNF)***
- **Amylin**
- **Insulin**
- **Fatty acid**
- **ROS**

**AGE**

**TNFR**

**SR33557**

**GD3**

**Sphingomyelinase**

**Peroxidation**

**Ceramide**

**Ceramide synthetase**

**Fatty acyl-CoA synthetase**

**Acyl-CoA synthetase**

**TriacsinC**

**PDMP**

**Acyl-CoA synthetase**

**ACBP**

**CPT**

**Etomoxir**

**Nucleus**

**Ψm (?)**
β-cell lipoapoptosis by PA (palmitic acid)
Saturated FFA induces JNK activation

Primary Hepatocytes

Time: 0 5 15 30 60 120

GSTcJun
JNK1

BSA
Elaic

Linoleic
Arachidonic

Unsaturated FFA

Saturated FFA

Liver in-vivo Perfusion

Dodecanolic
Myristic
Palmitic
Stearic
PA-induced insulin resistance in primary hepatocytes thru JNK activation
PA-induced IRS-1 serine phosphorylation thru JNK activation in primary islets
JNK activation attenuates insulin transcription

(A) Palmitate 0.5 mM

(GSTcJUN) JNK1/2

(B) wt islets vs Jnk1^−/− islets

Rel. Insulin 1 3.2 1.1 1.2 2.8 0.9 3.0 1.3 2.9

Glucose 5.5 mM + + + + + + + +
Glucose 16.7 mM + + + + + + + +
Palmitate + + + + + + + +
BSA + + + + + + + +
D-JNKi + + + + + + + +

(C) wt islets vs Jnk1^−/− islets

Rel. Insulin 1 3.1 1.2 1.3 1 3.0 1.1 3.2 1.2 2.9

β-Actin + + + + + + + + +
Insulin + + + + + + + + +

(D) p-Akt + + + + + + + +
Akt + + + + + + + +

Insulin + + + + + + + +
Palmitate + + + + + + + +
BSA + + + + + + + +
D-JNKi + + + + + + + +
JNK1 phosphorylates IRS2 at Thr287, the equivalent of IRS1-Ser307

Solinas G et al. PNAS 103:16454, 2006
JNK induces both insulin resistance & β-cell dysfunction.

(ER Stress, FFA)

NIT1 Cell death & JNK Phosphorylation

[Graph showing viability and JNK phosphorylation levels with different concentrations of PA and SP600125]

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<td>10</td>
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• Saturated FFA induces JNK activation in insulin target tissue. ( -> insulin resistance)

• JNK activation leads to decreased insulin gene transcription. ( -> β-cell dysfunction)

• JNK activation induces IRS-2 phosphorylation at Thr287 leading to decreased β-cell proliferation or β-cell death directly. ( -> decreased β-cell mass)
UP binds & inhibits Grp

Grp78 releases

ER lumen

Ire1

Phosphorylation

TRAF2
(K63 E3 ligase)

SEK1/MKK4 (?)

Ask1

Other kinases

JNK

p38 MAPK

IKK

IKK

NF-κB

NF-κB

XBP-1 mRNA splicing & maturation

ATF4 mRNA translation

Induces XBP-1 & Ire1

Induces genes related to UP retrograde-transport & degradation (ERAD of EDEM)

Induces XBP-1 & Ire1

ATF4

CHOP, Bip, ERp72, GADD34

ATF3, 4E-BP1 (mTOR target)

TRB3 (?)

ATF6

Relocates to Golgi, cleaved by site 1/2 proteases

Dephosphorylate eIF2a as a cofactor for PP1

Translation machinery

eIF4E

Induces genes related to UP retrograde-transport & degradation (ERAD of EDEM)

Dephosphorylate eIF2α as a phosphatase for PP1

3 Arms of UPR. Reed J. J Clin Invest 115:2656, 2005
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<tr>
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<tr>
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<tr>
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<td>db/db</td>
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Diabetes 58:329, 2009
Merksamer P et al.
Cell 135:1, 2008
4. Role of autophagy in β-cell mass & function
(Macro)autophagy

- **Definition**
  - “Self-eating”: a process of degradation of dysfunctional or senescent organelles & protein aggregates for rejuvenation of organelles and supply of nutrients
  - Characterized by organelles surrounded by double membrane (autophagosome)
  - Lead to cell death when excessive

- **PCD type 1 -> apoptosis**
  - **PCD type 2 -> autophagy**
  - Necrosis

JCI, 2005
Autophagy (II)

- **Regulation**
  - Induction by fasting, rapamycin (mTOR/S6K inhibitor)
  - Inhibition by nutrients such as AA (thru Vps34?), growth factor

- **Effects**
  - Cell survival during nutrient/growth factor deficiency
  - Essential during development & organogenesis
  - Removal of dysfunctional cellular organelles (mitochondria, ER, peroxisome)
  - Removal of damaged proteins
  - Programmed cell death when apoptosis is blocked
  - Cancer cell death
  - Suppressor oncogene
  - Antigen presentation to immune system
  - Killing of microbes
1. Insulin and its downstream signal mTOR/S6K1 are well-known inhibitors of autophagy. (cf: glucagon)

2. Organelles such as mitochondria and ER that are closely related to survival/death of β-cells and insulin action/sensitivity rely on autophagy for proper function.

3. Disruption of autophagy in neurons leads to neurodegeneration.

4. However, the role of autophagy in diabetes or metabolic disorders has been hardly studied.
INFLUENCE OF GLUCAGON, AN INDUCER OF CELLULAR AUTOPHAGY, ON SOME PHYSICAL PROPERTIES OF RAT LIVER LYOSOMES

RUSSELL L. DETER and CHRISTIAN DE DUVE

From The Rockefeller University, New York

ABSTRACT

The response of rat liver lysosomes to an intraperitoneal injection of glucagon has been evaluated from studies on the mechanical fragility, osmotic sensitivity, and sedimentation properties of these subcellular particles. It has been found that about ½ hr after the injection of glucagon the hepatic lysosomes exhibit a fairly sudden increase in their sensitivity to mechanical stresses and to exposure to a decreased osmotic pressure. At the same time, their sedimentation properties undergo complex changes characterized mainly by a significant increase in the sedimentation coefficient of a considerable proportion of the total particles. In addition, glucagon causes an increase in the proportion of slowly sedimenting particles, with the result that the distribution of sedimentation coefficients within the total population tends to become bimodal. The latter change is more pronounced for acid phosphatase, less so for cathepsin D, and barely detectable for acid deoxyribonuclease. All these modifications are maximal between 45 and 90 min after injection and regress to normal within approximately 4 hr. With the exception of the increase in the slow component, for which no explanation can be advanced at the present time, they are consistent with the hypothesis that glucagon causes an increase in lysosomal size, and may be related to the autophagic-vacuole formation known to occur after glucagon administration.
Um SH et al. Nature 431:201, 2004
RIP

Sauer B. Method 14:381, 1998
**A** Regulation of autophagy induction

- Nutrients → TOR active → Vac8, Atg13, P, Atg1, P → No autophagy
- No nutrients, Rapamycin → TOR inactive → Vac8, Atg13, Atg17, P → Autophagy induction

**B** Vesicle nucleation

- Cytoplasmic membrane
- PI → PI 3-P
- Vps15, Vps34, Atg14, Atg6

**C** Vesicle expansion and completion

- Ub-like
- Atg12, Atg10, E2
- Atg5, Atg12, E3
- Guides LC3 to PE
- Outer LC3-II
- Delipidation by Atg4 (deubiquinating enzyme-like)

**D** Retrieval (recycling)

- Atg9, Atg2, P, Atg18

Yuan & Levin. JCI 115:2679, 2005
Glucose-induced Ca\textsuperscript{2+} transients in isolated islets (n = 5 each).
p62: binds polyUb & LC3 (Atg8)

Large Ub+ aggregates in Atg7−/− β-cells
A

Ubiquitin  p62

Atg7^F/F

Atg7^F/F, Cre^+  

Atg7^Δβ-cell

B

Ubiquitin  p62  Hoechst  Merge

100 μm

20 μm

Atg7^F/F

Atg7^F/F, Cre^+  

Atg7^Δβ-cell
RIP-Cre/Atg7 UPR Genes

9.7.09
19 wks

Blood glucose (mg/dl)

+p/f (ob/ob) - f/f (ob/ob) - f/f (ob/w)

p=0.047
Figure 2 | Macroautophagy as a default pathway for proteasome-inaccessible substrates. When proteins are accessible to both the ubiquitin (Ub)–proteasome and autophagy pathways, the greater efficiency of the ubiquitin–proteasome system makes it the favoured and dominant clearance route. When a cytosolic protein is aggregate prone and a poor proteasome substrate, then autophagy becomes the main clearance route by default — under these circumstances, the autophagy route becomes more effective than the proteasome.
1. β-cell-specific Atg7 knockout led to decreased β-cell mass due to increased apoptosis & decreased proliferation.
2. Atg7Δβ-cell mice had decreased serum insulin and hyperglycemia.
3. Ca\(^{2+}\) transient and insulin secretion in response to glucose were diminished in Atg7 knockout β-cells.
4. Ubiquitin aggregates co-localized with p62 accumulated in Atg7 knockout β-cells.
5. Swelling of mitochondria, distension of rough ER and Golgi complex, and loss of insulin granules were observed in Atg7 knockout β-cells.
6. Role of autophagy in the pathogenesis of diabetes remains to be investigated.
Conclusion

1. β-cell death could be both the final and initial steps in T1D.

2. TLR2-dependent recognition of apoptotic β-cells undergoing secondary necrosis may be the initial step in the development of T1D.

3. Saturated fatty acids could induce death/dysfunction of β-cells and insulin resistance in T2D thru JNK.

4. Autophagy is essential for the maintenance of β-cell structure, mass and function.
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- **Harvard University**
  Diane Mathis
  Christophe Benoist
Production of β-cell-specific Atg7 Knock-out (Atg7Δβ-cell) Mice
(Upper) PCR analysis using genomic DNA from isolated pancreatic islet cells
(Lower) RT-PCR using RNA from primary islet cells.
eIF2α phosphorylation is required to maintain expression of essential beta cell factors

No & area of autophagosomes with double-membrane vacuole-like structure representing steady-state autophagy level
No change in body wt of $Atg7^{\Delta \beta \text{-cell}}$ mice
Random blood glucose level
IPGTT at 20 wk of age
Fasting serum insulin level *in vivo*
$\text{Atg7}^{+/+}, \text{Cre}^+$

$\Delta$ β-cell mass

$\text{Atg7}^{+/+}$

$\text{Atg7}^{+/+}, \text{Cre}^+$

$\text{Δ} \beta$-cell

$\text{H} \& \text{E staining}$

Relative $\beta$-cell mass (%)

- Atg7+/+
- Atg7+/+, Cre+
- Δβ-cell

*
(Lt) TUNEL$^+$ cells representing apoptotic β-cells. (Rt) BrdU$^+$ cells representing β-cell replication.
(Lt) Insulin content in the pancreas.  (Rt) Insulin secretion in response to glucose ex vivo.
Glucose-induced Ca\(^{2+}\) transients in isolated islets (n = 5 each).
Accumulation of Ubiquitin Aggregates in β-cells of Atg7Δβ-cell Mice
IRS1-Ser307 phosphorylation alone, cannot explain the effects of JNK on insulin transcription
Marker for ER stress

**p-eIF2α**

<table>
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<th>0</th>
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<td>36 kD</td>
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<td>40 kD</td>
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**ATF4**

**LC3 I/II (Marker for Autophagy)**

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<tr>
<td>16 kD</td>
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**Triggers?**

PA (uM) - 200 400 600

**pJNK**

**JNK**
Phosphorylcholine +

Sphingomyelin

Deacylase

SPC (Increased in Niemann-Pick)

Cerebroside, Ganglioside (GM, GD)

Gaucher’s ds: β-glucosidase
Niemann-Pick: acidic sphingomyelias
Tay-Sachs: N-acetylglactosaminidase

Sphingolipid ds
Effect of agents inhibiting conversion to ceramide

![Bar chart showing the effect of agents on cell viability](image)

Palmitoyl Co A → Serine → **Myriocin** → 2S-3-Ketosphinganine → 2S,3R-Shinganline → Sphingosine → Ceramide

**Agents:**
- Palmitate
- Fumonisin B1
- Myriocin
- Serine
- 2S-3-Ketosphinganine
- 2S,3R-Shinganline
- Sphingosine
- Ceramide
Changes in LPC contents by PA & PLA$_2$ inhibitors

Phosphatidic acid $\xrightarrow{}$ DAG $\xrightarrow{}$ PC $\xrightarrow{\text{PLA}_2}$ LPC
LPC 20 (μM)

0 15 30 60 180 360 (min)

- - - + + +

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<th>PA (μM)</th>
<th>-</th>
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<td>PACOCF₂</td>
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<td>-</td>
<td>+</td>
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<td>PACOCF₂</td>
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Lipin (PAP) → DAG
Phosphatidic acid → DAG
CPT → PC
PLA₂ → LPC

pJNK2
pJNK1
JNK

pS307
IRS-1
pJNK2
pJNK1
JNK
Ketosphinganine + Serine → Sphingosine → Ceramide

G-3-P

GPAT + Acyl-CoA → Lysophosphatidic acid

LPAAT + Acyl-CoA → Phosphatidic acid

CPT + CDP-choline → PC

PLA₂ → FFA, LPC

Autotaxin

FFA, LPC → Cell death

LPS, TNFα → DAG acyltransferase

DAG → TG → Fatty changes

LPAAT → FFA, LPAAT enzymatic activity
Macrophage-specific IKKβ knockout

High glucose-palmitate-induced ER stress in NIT-1
(phospho-eIF2(alpha) expression)

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<th>24</th>
<th>48</th>
<th>72 (hr)</th>
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phospho eIF2 alpha (39 kD) ——

Glucose: 30 mM
Palmitate: 200 µM

결과:
• ER stress가 24시에 발생
Palmitate-induced autophagy in NIT-1
(LC3 conversion and expression)

Western blot analysis

control 200 µM palmitate (24 h)

18 kD (LC3-I)

16 kD (LC3-II)

결과:

• 24시에 autophagy induction이 발생
(A) IPGTT

![Graph showing blood glucose levels over time for young and old mice during an intraperitoneal glucose tolerance test (IPGTT)].

- Young mice (open circles)
- Old mice (filled circles)

* * p<0.005 vs. young mice at the same time point

(B) ITT

![Graph showing blood glucose levels over time for young and old mice during an intravenous tolbutamid test (ITT)].

- Young mice (open circles)
- Old mice (filled circles)

* * p<0.01, ** p<0.001 vs. young mice at the same time point

Below the graphs:

**Insulin levels (pM)**

- Young group:
  - Baseline: p<0.005
  - IP 15min: p<0.05 vs. young-baseline

- Old group:
  - Baseline
  - IP 15min: ** p<0.05 vs. young-IP 15min
CD4^+ BDC T cell proliferation in STZ-treated adult mice

**TLR2+/+**
- Citrate: 6% proliferation
- STZ: 57% proliferation

**TLR2-/-**
- Citrate: 7% proliferation
- STZ: 22% proliferation

**Gated on CD4^+ V\beta4^+ cells**

**Proliferation index**
- STZ treatment comparison:
  - WT
  - KO

**FLow cytometry graphs**
- CFSE staining
Muscle                             Liver

Y        O                          Y        O

P–JNK

Total JNK

actin
Role of JNK in obesity-induced diabetes

Is glucotoxicity the leading stressor triggering JNK activation? 
Ans) No!
Conversion from Apoptosis to Necrosis by Caspase Inhibitor (EM)
Induction of Necrotic Death by IFNγ/TNFα
by caspase inhibitor (Hoechst/PI double staining)
An improved model for negative regulation of insulin signaling by JNK through IRS1 and 2 Ser/Thr phosphorylation

Solinas G et al. PNAS 103:16454, 2006
Phosphorylation of S6K1

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<td>S6K1</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
</tr>
<tr>
<td>α-actin</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
</tr>
</tbody>
</table>

Phosphorylation of p70 S6K1 (fasting) vs. Total p70 S6K1 (fasting)

- Young (n=7) vs. Old (n=7)
  - pT^{389}-S6K1: p=0.080
  - S6K1/α-actin: p<0.001
p70 S6K1

• Association with islet development
  – Islet hypoplasia and hypoinsulinemia in S6K1(-/-) mouse

• Association with insulin resistance
  – Activation of p70 S6K1 in animal model of aging/obesity
  – Increased insulin sensitivity in S6K1(-/-) mouse
  – S6K1(-/-) mice are protected against obesity- or aging-induced fat accumulation.

  – Mitochondrial activity is increased in S6K1(-/-) mice.

• Regulation of autophagic activity
  – As a sensor of nutrition
Type 1 diabetes

develops in juveniles
absolute insulin deficiency
lean
autoimmune
insulin for life saving

Type 2 diabetes

develops in adults > 40
insulin resistance
relative insulin deficiency
obese
diet, oral hypoglycemics
Role of TLR2 in Cancer-induced inflammation & Metastasis

(Kim S & Karin M. Nature, in press)

- Pulmonary inflammation after tail vein injection of lung cancer cells is decreased in TLR2 KO mice.

- TLR2 KO mice are resistant to death by metastatic cancer after tail vein injection of lung cancer cells.