The Sympathetic Nervous System (SNS)

A not so “sympathetic” regulator of immune function in autoimmune disease:
RA as an example

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Blood Borne Signals
TNF, IL-1, IL-6

Neural-Immune Cross-Talk

Thymus
Bone Marrow
Lymph Node
GALT
Blood
Spleen
Lymphocytes
Macrophages
Dendritic cells
Others

Pathogens

Disease

Sympathetic Nervous System

X
Rheumatoid Arthritis

- Autoimmune Disease
  - Chronic inflammatory response
  - Production of autoantibodies
  - Loss of Tolerance: Imbalance between autoreactive effector T cells (CD4+ Th1 & Th 17) and T reg cells

- Th cell balance regulated by the SNS

- SNS activity is chronically elevated in RA patients

- How this impacts Th cell balance is not known
SNS Regulates Th Cell Differentiation via $\beta^2$-AR Activation of cAMP-PKA Pathway

$\beta_2$

CD4+ $\beta_2$ (200-750 sites/cell)
CD4+ Th1 clones $\beta_2$ (250 sites/cell)
CD4+ Th2 clones (no detectable $\beta_2$)
CD4+ Treg cell $^1$?
CD4+ Th17 cells?

APCs

$\alpha$, $\alpha_1$, $\alpha_2$, $\beta$, $\beta_2$

$\beta_2$-AR Shifts Th0 cell $\rightarrow$ Th2 Differentiation

Guereschi et al., 2013
Hypothesis: Reduce disease severity is due in part to a $\beta_2$-AR driven shift in Th1 vs Th2 cell balance.

(AA: Lorton et al., 1998; 2004) (CIA: Malfait et al., 1999; Härle et al., 2005)
Day 0
Terbutaline (β₂-AR agonist; 1.5 mg/ml/day i.p.)
Saline Vehicle

Day 12-28
Terbutaline (β₂-AR agonist; 1.5 mg/ml/day i.p.)

Adjuvant-Induced Arthritis (AA)
CFA (0.3 mg M. butyricum in 100 µl MO)

Day 28
Outcome Assessments
Th1/2 cell cytokines (ELISAs)
Foot Pad Swelling (data not shown)
X-ray analysis (data not shown)

Spleen PBMCs
Draining Lymph Nodes (DLN)
Spleen: Failure of a $\beta_2$-AR agonist to shift from a Th1 to Th2 cytokine profile

A. IFN-γ (pg/ml)

B. TNF-α (pg/ml)

C. IL-2 (pg/ml)

D. IL-10 (pg/ml)

No Change in IL-4; (40-80 pg/ml)

Anova with Bonferoni post-hoc test, N = 8
DLN: $\beta_2$-AR agonist promotes a Th1 cytokine profile

A. IFN-γ (pg/ml)

B. TNF-α (pg/ml)

C. IL-2 (pg/ml)

D. IL-10 (pg/ml)

No Change in IL-4 (40 -80 pg/ml)

Anova with Bonferoni post-hoc test, N = 8; *P<0.05
PBMC: Failure of a β2-AR agonist to shift Th1 cytokine profiles

A. B. C. D.

Conclusions

- Different responses in each tissue examined: animal models critical for understanding RA
- Stimulating $\beta_2$-ARs after disease onset fails to inhibit Th1 cell driving cytokines
  - Spleen: $\beta_2$-AR agonists produced no change IFN-$\gamma$, IL-2, IL-4 or TNF-$\alpha$, and increased IL-10 (source ?)
  - DLN stimulating $\beta_2$-ARs promotes IFN-$\gamma$ & IL-2, no change in IL-4, IL-10, TNF-$\alpha$
- $\beta_2$-AR stimulation under normal circumstances inhibits IFN-$\gamma$ and IL-2 production via cAMP-PKA
- These findings indicate abnormal $\beta_2$-AR functions
Conclusions

- In spleen cells, the inability of terbutaline to reduce IFN-γ and IL-2 could be easily explained by the well-known down-regulation and desensitization of β₂-AR with repeated stimulation.

- Subsequent, cAMP assays and receptor binding experiments, confirmed this hypothesis (Lorton et al., Clin Dev Immunol., 2013)

- However, the terbutaline-induced increase in IFN-γ and IL-2 were intriguing. not explained by canonical signaling of β₂-AR
Does Altered β2-AR Coupling to Second Messengers Occur in DLNs in AA: cAMP-PKA to ERK1/2?

β-Arrestin-dependent, G Protein-independent ERK1/2 Activation by the β2 Adrenergic Receptor*

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Altered Receptor Signaling in the DLN?

**Canonical**

- **nerve terminal**
  - Gs
  - AC
  - ATP → cAMP → PKA → Transcription Factors → Gene Regulation

**Noncanonical**

- **nerve terminal**
  - Gs
  - AC
  - ATP
  - GRK5/6
  - PKA
  - ERK1/2 MAPK
  - β-Arrestin
  - Transcription Factors → Gene Regulation

**Legend**
- ATP
- cAMP
- PKA
- Transcription Factors
- Gene Regulation
- β-Arrestin
- Gs
- AC
- ERK1/2 MAPK
Hypothesis: Terbutaline induces a shift in β₂-ARs signaling from cAMP-PKA to ERK 1/2 in the DLN

Adjuvant-Induced Arthritis (AA)

Day 1
- CFA (0.3 mg M. butyricum in 100 µl MO)
- Mineral Oil (MO)
- M. Butyricium (in saline; SMB)

Day 12-28
- Terbutaline (β₂-AR agonist; 1.5 mg/ml/day i.p.)
- Saline Vehicle

Day 21 or 28

Outcome Assessments
- DLN: β₂-AR Western Blots
  (antibodies to detect β₂-ARs, and β₂-ARs phosphorylated by PKA and or GRK)

Draining Lymph Nodes (DLN)
Unchanged DLN $\beta_2$-AR Density Late Disease

A. D21

B. D28

ANOVA; Bonferoni Post-Hoc Test N=4; *P < 0.05; **P < 0.01, ***P<0.001
Conclusions: These findings along with increased IFN-γ indicate that β2-ARs in DLN are NOT down-regulated or desensitized.
β₂-AR phosphorylated by PKA and GRK in DLN

A. D21

\[\frac{\beta_2-AR_{PKA:\beta_2-AR_T}}{\beta_2-AR_{GRK:\beta_2-AR_T}}\]

B. D28

\[\frac{\beta_2-AR_{PKA:\beta_2-AR_T}}{\beta_2-AR_{GRK:\beta_2-AR_T}}\]

C. D21

D. D28

ANOVA; Bonferoni Post-Hoc Test N=4; *P < 0.05; **P < 0.01, ***P < 0.001
Conclusions: These findings coupled with increased IFN-γ, provide support β2-AR signaling via ERK1/2.
Summary

➢ Findings support a shift in $\beta_2$-AR receptor signaling from cAMP-PKA to ERK1/2 in DLN

• $\beta_2$-AR agonist elevated IFN-$\gamma$ and IL-2
• No change in $\beta_2$-AR density,
• Receptor phosphorylation by PKA increased PKA (day 21) and GRK phosphorylation (day 21 and 28)
Future Studies

• Are GRK5/6 and ERK 1/2 elevated in DLN cells?
• Can production of IFN-γ be blocked by inhibitors of ERK1/2 pathway?
• Why the different profiles in the spleen and DLN?
  – Inflammatory cytokine levels
  – CFA distribution/concentration
• Does the SNS regulate balance between Th17 and Treg cells?
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SNS Function: Respond to stress & maintain normal body functions (homeostasis)

The SNS integrates the functions of many systems required to mount an immune response.
SNS Inhibition of Th1 Cytokines (IFN-γ and IL-2) to Push Th2 Cell Differentiation Occurs via β2-AR of cAMP-PKA Pathway

CD4+ β2 (200-750 sites/cell)
CD8+ β2 (500-2500 sites/cell)
CD4+ Th1 clones β2 (250 sites/cell)
CD4+ Th2 clones (no detectable β2)
CD4+ Treg cell
CD4+ Th17 cells?

The SNS integrates the functions of many systems required to mount an immune response.

**Function:**
Respond to stress & maintain normal body functions (homeostasis) (allostasis - a new "adaptive" normal)
Reciprocal Immune System to SNS Communication in RA

- Mechanism for emotional distress to impact health & disease
- ~ 80% of patients associate disease onset with a severe emotional life stressor (Trigger?)
- Stroke Victims: no RA in paralyzed limbs (↓ vs ↑ SNS nerve activity)
Splenocyte $\beta_2$-AR Receptor Binding in Arthritic Rats: Saturation Curves

Saline Rx

- $B_{\text{max}} = 4497$
- $K_d = 12.08$

SMB Rx

- $B_{\text{max}} = 2023$
- $K_d = 8.216$

Mineral oil Rx

- $B_{\text{max}} = 5405$
- $K_d = 25.82$

CFA Rx

- $B_{\text{max}} = 3828$
- $K_d = 23.41$
SNS-IS Cross-Talk Pathology in RA: Reduced Spleen $\beta_2$-AR Density Late Disease

A. D21

B. D28

ANOVA; Bonferoni Post-Hoc Test
N=4; *P < 0.05; **P < 0.01, ***P<0.001

β₂-AR Phosphorylation Patterns in the Spleen

ANOVA; Bonferoni Post-Hoc Test
N=4; *P < 0.05; ** P < 0.01, ***P<0.001
Hypothesis: Chronic high SNS activity in RA induces β_2-AR down regulation and desensitization

Day 1
- a0.3 mg Mycobacterium butyricum in 0.1 ml sterile mineral oil

Day 28
- aCFA/ICA (vehicle)
- Autoantigen: HSP 65

Day 14

Day 28
- cAMP assay
- β_2-AR Receptor Binding Assays
- β_2-AR Western Blots
- Harvest Spleen & DLN cells
- using antibodies to detect phosphorylated receptor
Adjuvant-Induced Arthritis Rat Model

CFA (0.3 mg M. butyricum in 100 µl MO)

Disease Induction
Autoreactive T cells in DLNs
Autoreactive T cells in spleen
Disease Onset
Peak Disease
Chronic Disease

0 3 7 12 21 28 Days

Day 1
Day 14
Day 28
Hypothesis: Chronic high SNS activity in RA induces β₂-AR down regulation and desensitization in splenocytes.
Hypothesis: Chronic high SNS activity in RA induces $\beta_2$-AR down regulation and desensitization in the spleen.

**Day 1**
- CFA (0.3 mg M. butyricum in 100 µl MO)
- Mineral Oil (MO)
- M. Butyrlicium (in saline; SMB)

**Day 12-28**
- Terbutaline ($\beta_2$-AR agonist; 1.5 mg/ml/day i.p.)
- Saline Vehicle

**Day 21 or 28**
**Outcome Assessments**
- Spleen: cAMP assay (spleen)
- $\beta_2$-AR Binding Assays
- DLN: $\beta_2$-AR Western Blots (antibodies to detect $\beta_2$-ARs)
SNS-IS Cross-Talk Pathology in RA: β₂-AR Agonist Fails to Induce cAMP in Splenocytes

Anova with Bonferoni post-hoc test, Day 28; N = 8; *P<0.05

SNS-IS Cross-Talk Pathology in RA: Splenocyte $\beta_2$-AR have Reduced Agonist Affinity and Density

A. Kd (ICYP (pM))

- Saline
- Mineral Oil
- SMB
- CFA

B. Bmax (sites/cell)

- Saline
- Mineral Oil
- SMB
- CFA

ANOVA; Bonferoni Post-Hoc Test
N=6; $\ast$ P < 0.05; $\#$ P < 0.01

Hypothesis: Chronic high SNS activity in RA induces \(\beta_2\)-AR down regulation and desensitization in the spleen

**Day 1**
- CFA (0.3 mg M. butyricum in 100 \(\mu\)l MO)
- Mineral Oil (MO)
- M. Butyricium (in saline; SMB)

**Day 12-28**
- Terbutaline (\(\beta_2\)-AR agonist; 1.5 mg/ml/day i.p.)
- Saline Vehicle

**Day 21 or 28**

**Outcome Assessments**
- Spleen: cAMP assay (spleen)
- \(\beta_2\)-AR Binding Assays
- DLN: \(\beta_2\)-AR Western Blots
  (antibodies to detect \(\beta_2\)-ARs)
Altered Receptor Signaling in the DLN?

Nerve terminal

↑↑↑ activity

PKA

GRK2

Gs

AC

Desensitization

or

Dephosphorylated
Recycled to membrane

Transported to lysosome
Degraded

β-Arrestin

Canonical

PKA

GRK5/6

Gs

AC

ERK1/2 MAPK

β-Arrestin

Transcription Factors

Gene Regulation