CB2-mediated immunoregulation in Inflammatory Bowel Disease

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Overview

• Why study cannabinoid signaling in intestinal immunoregulation?
• G_i2 -/- mice develop spontaneous colitis (IBD)
• Search for G_i2-linked receptor turns up CB2
• CB2 agonist ameliorates immune colitis
• Cannabinoid signaling:
  – Sources of endocannabinoids
  – Degradation
  – CB receptors link to Gi proteins
  – Adenylyl cyclase, intracellular calcium flux
Endocannabinoids

- Include substances such as anandamide
- Released from N-arachidonoylphosphatidylethanolamine (NAPE) by phospholipase D
- Hydrolyzed by FAAH (cytoplasmic enzyme with strong specificity for anandamide)
- Much research has centered on CB1 in CNS, pain regulation
- _9 THC is a non-specific agonist
- AM1241 is selective CB2 agonist
- SR 144528 is CB2 antagonist
We are blessed with endocannabinoid receptors

- CB2 second endocannabinoid receptor discovered (1993)
- Found on immune cells, less in CNS (as CB1, CB1 also expressed to lesser extent on immune cells)
- Highly expressed on B>NK>CD4+>CD8+>NKT
- 81% nucleic acid identity bet human and rat
- CB2 -/- mouse has no grossly observable phenotype
Cannabinoids and the immune system

• Mice treated w/ 9-THC have impaired resistance to Herpes infection
  – Impaired resistance to Listeria and Legionella
• Defects in neutrophil recruitment
• Defects in macrophage function
  – Phagocytosis, antigen processing, NO generation
• Defects in T cell proliferation
• Decreased antibody production
Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice

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Reduced endocannabinoid immune modulation by a common cannabinoid 2 (CB2) receptor gene polymorphism: possible risk for autoimmune disorders

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Figure 2 | The endocannabinoid system and innate immunity. Bacteria stimulate lymphocytes, dendritic cells (DCs) and macrophages — through pattern-recognition receptors, such as Toll-like receptors (TLRs) — to release cytokines and chemokines, which attract leukocytes to the site of infection. It is now known that stimulation of these cells also induces the release of endocannabinoids — such as arachidonoyl ethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) — that are also chemotactic for leukocytes. Leukocytes — including T cells, B cells, eosinophils, natural killer (NK) cells, DCs and macrophages — invade the tissues, promoting the elimination of the microorganisms and the development of an adaptive immune response. These activated cells seem to upregulate the expression of both types of cannabinoid receptor — cannabinoid receptor 1 (CB₁) and CB₂ — and these are then available for participation in immune regulation.
CB2 agonists have diverse effects on cell signaling

• Several sources present sometimes contradictory data
• Non-cannabinoid receptor mediated effects
• Three main effects:
  – Regulates adenylyl cyclase
  – Regulates MAP kinase
  – Modulates intracellular calcium concentration
G proteins and their GCPRs
CB2 receptor

- Couples efficiently only with Gi proteins
- In concentration dependent manner, inhibit adenylyl cyclase activity (pertussis toxin sensitive-indicates requirement for Gi-mediated signaling)
- cAMP effects may contribute to immunoregulatory role of cannabinoids
  - IL-2 transcription, I_B_degradation
CB2 receptor

- Constitutively activate MAP kinase
- Also pertussis toxin sensitive
- Action depends on ligand and concentration (particular ligands inhibit MAP kinase phosphorylation)
CB2 receptor

- Modulate intracellular ionized calcium concentrations
- Activates phospholipase C
- Subsequent release of calcium from stores that are inositol triphosphate sensitive
Cannabinoid system: Role in gut immunoregulation

- Have discovered several pieces of evidence:
  - G_i2 -/- mouse develops spontaneous colitis
  - It has several key regulatory cell deficiencies
  - These same deficiencies seen in CB2 -/- mouse
  - Colitic mice treated with CB2 agonist get better
B and T cell subset deficiencies

Splenic B cell subset %

$\% B\ Cells$

SI IEL NKT cell %

$\% CD3+\ cells$

P=0.0001

CB2 $+/-$

CB2 $-/-$
Galp1h2 −/− T cell transfer

Days after T cell tx

% starting weight

P=0.04 (paired t test)
Galphi2-/- T cell tx

Histologic Colitis Score

AM1241  DMSO

P=0.004
Mid-talk summary

- CB2 -/- mouse phenocopies G_i2 -/-
  - CB2 receptor may be the Gi-coupled receptor to G_i2
- CB2 agonist given in setting of G_i2 -/- T cell transfer ameliorates colitis: immune inflammation
Genetics results

- OmpC = outer membrane porin C (E coli)
- Found in 55% of patients with CD
- Patients with CD who are OmpC+ have higher incidence of complicated, perforating disease
- OmpC+ patients with IBD may have defective CB2-mediated signaling
Proposed future studies

• Determine CB2 receptor protein level expressed, functional implications of having CB2 receptor gene mutation
• Will stain EBV transformed immortalized B cell lines made from these patients with CB2 receptor monoclonal antibody
• Will perform functional testing of CB2 receptor ligation
Secondary ab only
Mouse anti human CB2
CB2 agonist effect on [Ca]i mobilization in EBV-immortalized human cell lines

Ionomycin-induced [Ca]i flux (positive control)

AM1241-induced [Ca]i flux
Testing CB2 receptor function

• CB2 receptor is linked to Gi proteins
• Can induce cAMP formation via AC with forskolin
• Ligation with CB2 should dampen cAMP signal
Fig. 5. Stereoselective inhibition of forskolin-stimulated cAMP production by cannabimimetics in CHO-K1 cells expressing the CB₂ receptor. CB₂-CHO-K1 cells were incubated in HEPES-buffered Krebs-Ringer solution containing 1 mg/ml HSA, 5 μM forskolin, and 100 μM RO-201724, as described in Materials and Methods. The incubation also contained HU-210 (●), HU-211 (○), (-)-CP-55940 (■), (+)-CP-55940 (□), Win 55212–2 (▲), or Win 55212–3 (△) over a concentration range up to 1 μM. The reaction was initiated by addition of 1.5 x 10⁶ cells, and the samples were incubated at 37⁰ for 15 min before termination of the reaction by immersion of the samples in boiling water for 3 min. Measurement of cAMP in the samples was performed by radioimmunoassay. Results have been expressed as the percentage of cAMP production obtained in the presence of 5 μM forskolin remaining at each ligand concentration. Data points are the mean ± standard error of three to five experimental values measured in duplicate.

Fig. 6. Sensitivity to PTX treatment of agonist-induced inhibition of forskolin-stimulated cAMP production in CHO-K1 cells expressing the CB₂ receptor. CB₂-CHO-K1 cells were grown in culture for 12 hr in the presence of 0–100 ng/ml PTX. CB₂-CHO-K1 cells were then harvested and incubated in HEPES-buffered Krebs-Ringer solution containing 1 mg/ml HSA, 100 μM RO-201724, and 5 μM forskolin, in the absence (▲) and presence (○) of 100 nm HU-210, as described in Materials and Methods. The reaction was initiated by addition of 10⁶ cells, and the samples were incubated at 37⁰ for 15 min before termination of the reaction by immersion of the samples in boiling water for 3 min. Measurement of cAMP in the samples was performed by radioimmunoassay. Results have been expressed as the cAMP produced (in pmol/10⁶ cells) as a function of PTX concentration. Data points are the means ± standard errors from three to five experimental values.
Cre expression system

- For higher sensitivity and reliability
- Transfect Cre reporter gene into EBV cell line, activate with forskolin, readout by luciferase or secreted alkaline phosphatase-fluorescence plate reader
- Possible drawbacks:
  - Feasibility
  - Validity
Acknowledgements

• Jonathan Braun, MD, PhD
  – UCLA Chair Pathology
  – Mentor, IBD Immunology

• Nancy Buckley, PhD
  – Cal Poly Pomona Dept of Biology
  – CB2 researcher