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  - Craig Hospital
  - Paralyzed Veterans of America
  - American Kennel Club

- **Xalud Therapeutics:**
  - Co-Founder
  - Co-Chair Scientific Advisory Board
  - Early stage startup; *entirely preclinical*

“Listening” & “Talking” to Neurons:
Clinical Implications of Glial Dysregulation of Pain, Opioid Actions & Drugs of Abuse

**Moving from Concept to Clinical Trials!**

**Linda R. Watkins**

*Psychology & Neuroscience, Univ. Colorado-Boulder*

co-Founder, co-Chair Sci. Advis. Board, Xalud Therapeutics
Global Concepts

- Views of pathological pain are changing
- Recognition of Non-Neuronal players in pain: Glia (microglia & astrocytes) in spinal cord & trigeminal nuc.
- Recognition of Non-Neuronal players in opioid actions: Glia disrupt the clinical efficacy of opioids, including morphine, oxycodone, remifentanil, codeine, etc.
- Clinical implications of glial dysregulation of pain & opioid actions ... *glia targeting therapeutics are approaching clinical trials!*

Global Concepts

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Neuropathic Pain

~ Beyond Pain ~
For Opioids: The Data Support That Blocking Glial/Immune Activation Will:

- Improve opioid analgesia
- Suppress opioid tolerance
- Suppress opioid dependence
- Suppress opioid reward linked to drug craving/drug abuse
- Suppress both opioid-induced respiratory depression & constipation

What Have the Past 25 Years Revealed?
(Watkins et al., Brain Behav Immunity 2007; Grace et al., Nature Reviews Immunology 2014)

Spinal & trigeminal glia (microglia, astrocytes) are activated in every clinically-relevant model of enhanced pain:
- Somatic (sciatic etc.) & trigeminal nerve injury
- TMJD, occlusal interference
- Chronic tooth pulp inflammation
- “Migraine” facial allodynia
- Bone cancer; chemotherapy
- Multiple sclerosis
- Spinal cord injury
- Radiculopathy/herniated discs, and so on...

Suppressing spinal & trigeminal glial activation &/or glial proinflammatory cytokines:
- suppresses pain in every clinically-relevant model, returning pain to normal

Microglia Actively Survey the CNS & Rapidly Respond to Challenge

Microglia Actively Survey the CNS & Rapidly Respond to Challenge

Videos from: Davalos et al., Nature Neuroscience supplements, 8 (2005) 752-758

Glia (microglia, astrocytes) are activated in every clinically-relevant model of enhanced pain:
- Peripheral nerve injury, chemotherapy
- Bone cancer
- Multiple sclerosis
- Spinal cord injury
- Radiculopathy/herniated discs
- Rat migraine, etc.

Suppressing glial activation &/or glial proinflammatory cytokines:
- suppresses pain in every clinically-relevant model
- returns pain to normal

What Have the Past 25 Years Revealed?

But … Wait!

Propentofylline, minocycline, ibudilast, etc also affect neurons!

CHEMO-GENETICS

provides proof of microglial dysregulation of pain

DREADDs: Designer Receptors Exclusively Activated by Designer Drugs

CHEMO-GENETICS

definitive proof of microglial dysregulation of pain

DREADDs: Designer Receptors Exclusively Activated by Designer Drugs

Gene Therapy is used to express, in a cell type specific manner in vivo, either excitatory or inhibitory designer receptors that can only be activated by CNO (Clozapine-N-oxide, the designer drug)

Now, for the first time, we can very specifically turn “on” and turn “off” microglia at will!!

**Excitatory DREADDs**: CNO binds to expressed receptors in vivo, activating only microglia (CD68 promoter).

Grace et al. *Proc Soc Neurosci* ’14; Grace et al. MS in review, ’15

**Excitatory DREADD**: Selective microglia activation in spinal cord creates Interleukin-1 mediated pain.

Grace et al. *Proc Soc Neuroscience* 2014; Grace et al. MS in review 2015
**DREADDs**: **Designer Receptors Exclusively Activated by Designer Drugs**

**Inhibitory DREADDs**: CNO binds to expressed receptors in vivo, inhibiting only microglia (CD68 promoter)

Grace et al. Proc Soc Neurosci '14; Grace et al. MS in review, '15

**Inhibitory DREADD**: First definitive proof that microglial inhibition suppresses neuropathic pain

**Glia Release Neuroexcitatory, Pain Enhancing Substances**
(Watkins et al., Brain Behav Immun 2007)

**Activated glia release**:
- Arachidonic acid & prostaglandins
- Excitatory amino acids
- Nerve growth factors
- Proinflammatory cytokines, chemokines
- Enkephalinases
- Reactive oxygen species; NO

- Amplify pain signaling to spinal cord
- Amplify pain transmission to brain:
  - upregulate AMPA & NMDA receptor number/function
  - downregulate GABA & outward K+ currents
  - downregulate glial glutamate transporters & GRK2

Glial Proinflammatory Cytokines: Major Players in Neuroexcitation in Pain ... as well as Response to Opioids

Proinflammatory Cytokines:
- Tumor Necrosis Factor
- Interleukin-1
- Interleukin-6

Neuroexcitation!
By Enhancing pain, Opposes opioid analgesia

What Activates Glia?
- Proinflammatory cytokines
- Heat shock proteins
- CGRP
- Fractalkine
- Substance P
- ATP
- Prostaglandins
- Glutamate
- Nitric oxide
- Alcohol, Cocaine
- Opioids
- Endogenous danger signals

Hutchinson et al., Pharmacol Reviews 2011
Glial Activation by Endogenous Danger Signals

Endogenous danger signal activation of glia (microglia, astrocytes) implicated in pain in multiple rodent models, such as:
- Peripheral nerve injury
- Medication overuse headache, migraine
- Streptozotocin diabetic neuropathy
- Spinal cord injury
- Bone cancer
- Arthritis
- Pancreatitis

When bad things happen... endogenous danger signals are created... glia are activated... pain is amplified by glial pain-enhancing proinflammatory cytokines

What Activates Glia?

- Proinflammatory cytokines
- Heat shock proteins
- CGRP
- Fractalkine
- ATP
- Nitric oxide
- Prostaglandins
- Alcohol, Cocaine
- Opioids

Endogenous danger signals

Hutchinson et al., Pharmacol Reviews 2011

Spinal Glial Activation Opposes Both Intrathecal Morphine & Intrathecal Methadone Analgesia

Hutchinson et al., Brain Behavior & Immunity, '08

Blocking Spinal Interleukin-1 Unmasks Morphine Analgesia

Morphine

Behavioral output = Morphine + IL-1

Interleukin-1: Neuroexcitation which Opposes analgesia

Hutchinson et al., Brain Behav Immunity 2008
Blocking Spinal Interleukin-1 Unmasks Morphine Analgesia

Blocking glial activation improves the efficacy of opioids for pain control

Hutchinson et al., Brain Behav Immunity 2008

Opioid effects are different for neurons & glia

Opioids exist as mirror-image stereo-isomers

(-)-Morphine
- Binds to µ-opioid receptors
- Powerful analgesic

(+)-Morphine
- NO binding to µ-opioid receptors
- NO analgesia

Mirror Image Molecules
.... but, for neurons, not the same!

Neuronal Receptors are Stereoselective

[(-)-Morphine: Active Agonist at Classical Opioid Receptors on Neurons

[+]-Morphine: INActive Agonist at Classical Opioid Receptors on Neurons

thanks to Dr. Kenner Rice
Opioid Effects are Different for Neurons vs. Glia

**Neuronal Receptors are Stereoselective**

- [-]-Naloxone & [-]-Naltrexone: Active Antagonists at Classical Opioid Receptors on Neurons
- [+] -Naloxone & [+] -Naltrexone: INactive Antagonists at Classical Opioid Receptors on Neurons

This Point is KEY

**Glial Non-Stereoselectivity Extends to Opioid Antagonists!**

- [-]-Naloxone & [-]-Naltrexone: Active Antagonists at Glial Opioid receptor
- [+] -Naloxone & [+] -Naltrexone: Active Antagonists at Glial Opioid receptor

[+]-Naloxone should POTENTIATE morphine analgesia by:
(a) NOT blocking morphine effects on neurons, yet
(b) Removing glial activation that OPPOSES analgesia!

**Opioid Effects are Different for Neurons vs. Glia**

**GLIAL Receptors are Not Stereoselective**

- [-]-Morphine: Active Agonist at Glial Opioid Receptor
- [+] -Morphine: Active Agonist at Glial Opioid Receptor

Glial opioid receptor -- Fits BOTH [-] & [+] enantiomers

**Neuronally INACTIVE (+)-Naloxone Potentiates Morphine Analgesia!**

Hutchinson et al., *Brain Behav. Immun.‘09*
Why is This Important? This Difference Predicts: 

**Effects on neurons & glia should be separable**

To increase the efficacy of opioids:
* structurally modify opioids to prevent glial activation, or  
* create a long-lasting version of (+)-naloxone that only blocks glia

---

Opioid Activation of Glia Suppresses Analgesia

---

Opioid Activation of Glia Suppresses Analgesia: Blocked by (+)-Naloxone

---

So .... What is this Mystery Receptor?  
To target it, one must know what it is

Toll-Like Receptor 4 (TLR4):

Classically .......

“not me, not right, not OK” receptors

TLR4 detects:
* Bacteria (lipopolysaccharide; LPS)
* endogenous danger signals (stress/damage/death)
* All classes of opioids used clinically

---

Hutchinson et al., TSWJ 2007; Br Behav Immun 2008
Glial TLR4

~ the “not me, not right, not okay” receptor ~

is also activated by Endogenous Danger Signals that drive Neuropathic Pain

If that is True, then....

* Might that suggest that blocking TLR4 can do more than just potentiate opioid analgesia?

* Might (+)-Naloxone also be a stand-alone treatment for neuropathic pain?

---

Toll Like Receptor-4 (TLR4):

(+)-Naloxone, a non-opioid TLR4 antagonist, reverses neuropathic pain and inhibits glial activation

---

Glia & Opioid Reward:
Conditioned Place Preference

Mor: Saline/ Saline

Saline paired

Saline paired


---

(+)-Naloxone Blocks Morphine Reward:
Blockade of Morphine-Induced Conditioned Place Preference

Suppresses opioid:
* nuc. accumbens dopamine elevation
* conditioned place preference
* self-administration
* incubation of craving
* reinstatement/relapse

~ and true for cocaine, and methamphetamine as well

---

**Beyond Pain!** (+)-Naltrexone Inhibits: Brain Neuronal Death after Heart Attack/CPR (CA1 region of Hippocampus)

**Taken Together, the Data Predict that Blocking Glial / Immune Activation will:**

- Suppress pathological pain due to: neuropathy, multiple sclerosis, bone cancer, etc.
- Improve opioid analgesia
- Suppress opioid tolerance
- Suppress opioid dependence
- Suppress opioid reward linked to drug craving/drug seeking
- Suppress respiratory depression, constipation, & (likely) itch

.....and it won’t just be for opioids (e.g. effects of alcohol, cocaine, methamphetamine are all amplified by glia!!)

**States of Glial Activation: Not Just “Off” or “On” Anymore!**

Basal State: Boring but Vigilant
States of Glial Activation: Not Just “Off” or “On” Anymore!

Activated State: Proinflammatory

* Can occur for a period of time after prior activation
* No longer producing proinflammatory products...but....Ready for Action!


States of Glial Activation: Not Just “Off” or “On” Anymore!

Reactivation from the “Primed” State: *Explodes* into Action in Response to a New Challenge!

Aging
Stress
Trauma
Opioids

FIRST “HIT”

Critical Window of Time

SECOND “HIT”

2-Hit Hypothesis: A 2nd “Hit” Can Create a Faster, Strong, Longer Glial Response

Sets the Stage For Chronic Pain??

So.... Does Prior glial activation alter the pain response to a NEW challenge?

Changing “no pain” to “pain”;
Changing “pain” to “chronic pain”

1. **Aging**
2. **Stress**
3. **Trauma/Inflammation**
4. **Opioids**

FIRST “HIT”

Critical Window of Time

SECOND “HIT”

And this is TRUE!

**Prior Surgery (Laparotomy):** Changes “no pain” to “pain”

**Hains et al. J. Pain ’10; J. Neuroimm. ’11; Loram et al. BBI ’11; Grace et al., in review ’15**
Prior Surgery: Changes “pain” to “chronic pain” ~ prevented by glial activation inhibitor

**But wait a minute...** this makes a scary prediction about opioids given post-trauma

Since ~
Trauma (Hit 1) leads to Opioids being given to treat the acute pain (Hit 2)

And ~
Trauma and Opioids both activate glia

Then ..... If glial priming (Hit 1 → Hit 2) amplification of pain is true, then this predicts that opioids (Hit 2) given after trauma (Hit 1) might have an evil side: a negative long-term consequence of opioids on pain

**And this is True!**

So.... Does Prior glial activation alter the pain response to a NEW challenge?

**Morphine in the early post-trauma period changes “pain” to “chronic pain”**

**FIRST “HIT”**
Trauma versus Sham

**SECOND “HIT”**
Short Course of Morphine
Early Post-Trauma

TRUE for FEMALES as well as males, and across rodent strains, and across multiple models (every one studied to date)

Grace et al. *Proc Soc Neuroscience* 2014; Grace et al. MS in prep; Ellis et al. MS in prep

Peri-Trauma Morphine: Changes “pain” to “chronic pain” after peripheral nerve injury

**This is *NOT* Due to Opioid receptors:**
- Recapitulated by Non-Opioid, TLR4 agonist: (+)-Morphine
- Recapitulated in confirmed siRNA Knockdown of Mu Opioid Receptors: not dependent on Mu Opioid Receptors

Grace et al., *Soc. for Neuroscience* 2014; Grace et al. *MS in review 2015*
Peri-Trauma Morphine: Changes “pain” to “chronic pain” after peripheral nerve injury

This **IS** Due to Microglia and TLR4:
*PREVENTED by DREADD inhibition of microglia only during morphine dosing
*PREVENTED by intrathecal TLR4 antagonist (+)-Naloxone only during morphine dosing

Long Duration Maintenance of Morphine-Amplified Pain **IS** Due to Microglia and TLR4: PERMANENTLY REVERSED by treatment >1 month later

Fall in pain threshold: Touch becomes pain

Grace et al., Soc. for Neuroscience 2014; Grace et al. MS in review 2015

Based on the Strength of the 25 year Glial Story for Pain, Across Labs & Across Diverse Animal Models: Translation to Clinical trials for Osteoarthritis & Neuropathic Pain

**Xalud Therapeutics** (entirely pre-clinical/pre-IND to date)

**XT202:** (+)-Naltrexone; blood brain barrier permeable TLR4 antagonist;
Awarde d a Depart. of Defense grant to expedite moving to Investigational New Drug status in 2016 for neuropathic pain and drug abuse; DoD grant for spinal cord injury pain

**XT101, XT150:** non-viral interleukin-10 (IL-10) gene therapies;
Awarded an NIH U44 grant to move to Investigational New Drug status in 2015; applying for osteoarthritis (OA; intra-articular) & neuropathic pain (intrathecal); positioning for Clinical Trials in humans and veterinary populations; NIH SBIR grant for multiple sclerosis; American Kenner Club grant for translation to dogs

A Focus on Interleukin-10 (IL-10)
a potent endogenous Anti-inflammatory cytokine

The importance of central pro-inflammatory cytokines across many neuropathic pain models, across so many independent labs, suggested that an anti-inflammatory cytokine approach to suppress glial activation might prove successful for neuropathic pain control

Plus – proinflammatory cytokines are important in diseases like ARTHRITIS: might local, intra-articular IL-10 help arthritis as well?

*1992: Our University of Colorado-Boulder research lab began studying spinal glial dysregulation of pain by pro-inflammatory cytokines; pure basic science

* 2000: We began studying Interleukin-10 for its potential in pain control, spearheaded by Dr. Erin Milligan (now Associate Professor, Univ. New Mexico)

* 2009: Therapeutic potential for Neuropathic Pain of peripheral and central origin led to the formation of Xalud Therapeutics to move IL-10 gene therapy to clinical trials

* 2015: After 10 generations of improvements since 2000, and testing in mice, rats, and pet dogs, now preparing Investigational New Drug application to the FDA
**Xalud Therapeutics**

pronounced “Salud!” ~ “To your health!”; “X” for IL-10

Non-Viral Gene Therapy to Induce Interleukin-10, your Body’s Own ANTI-inflammatory Cytokine

---

**Acute Intrathecal Injection of IL10 Gene Therapy**

INTERLEUKIN-10:

1. Suppresses TNF, IL1 & IL6:
   - Transcription
   - Translation
   - Post-Translational Processing
   - Release

2. Down-regulates Receptors for Pro-Inflammatory Cytokines

3. Up-regulates Antagonists of Pro-Inflammatory Cytokines

---

**Non-viral IL-10 gene therapy**

**CCI/Sham**

**3 MONTHS!!**

**Equally effective across multiple chronic pain models**

- Sham-Ipsilateral hindpaw
- Sham - Mirror image hindpaw
- CCI-Ipsilateral hindpaw
- CCI-Mirror image hindpaw

---

**Extending XT-101 to Pet Dogs in chronic pain**

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**Dogs, Dogs and more Dogs!**

Subjects in the initial Blinded Osteoarthritis Study to date

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**IL-10 gene therapy treats real disease – disease NOT controlled by any currently available pain drugs – not just rodent models of pain**

---

**pet dogs otherwise euthanized as nothing else works**
**Canine Neuropathic Pain**

*Single XT-101 dose*

**Canine Osteoarthritis**

*Single XT-101 dose*

**Amos: Owner Assessment: Intra-articular (elbow)**

Increase in Disability

50% Reduction in Concomitant Medications

Pain interference with:

**Increase in Disability**
Blockade of Rat Multiple Sclerosis (EAE) Paralysis by i.t. Non-Viral IL-10 Gene Therapy (XT101)

Grace et al., 2014 MS in prep

Blockade of Rat Multiple Sclerosis (EAE) Paralysis by i.t. Non-Viral IL-10 Gene Therapy (XT101)

MOG to induce EAE (rat “MS”)

-2 0 2 4 6 8 10 12 14 16 18 20

0 2 4 6

Time post onset of motor symptoms (days)

Motor score

EAE+XT-101
EAE+vehicle

Intrathecal injections

0=normal; 1 = tail tip paralysis; 2 = full tail paralysis;
3 = hindleg weak; 4 = partial hindleg paralysis;
5 = full hindleg paralysis; 6 = partial foreleg paralysis

Before EAE-treatment

After EAE-Treatment

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Grace et al., 2014 MS in prep

Positive controls (marked paralysis)

Post-treatment (normal)
Blockade of Rat Multiple Sclerosis (EAE) Paralysis by i.t. Non-Viral IL-10 Gene Therapy (XT101)

How Does i.t. IL-10 Gene Therapy Work?

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Conclusions

- Immunology is important; glial cells: volume controls
- Glial cells do not care about normal pain
- Glial responses can create and maintain enhanced pain:
  - Physiologically as part of the ancient Sickness Response
  - Pathologically when triggered by neuropathy, cancer, etc
  - Pharmacologically by clinically relevant opioids
- Glial activation now also linked to opioid tolerance, opioid dependence/withdrawal, opioid reward
- Proinflammatory cytokines are key
- Targeting glia & glial products may provide a novel approach to pain control & increases opioid efficacy
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