PTPN22: What’s not “Toll-like” about “Interferin” with Rheumatoid Arthritis?

1-31-13

Erik J. Peterson, M.D.
Department of Medicine
University of Minnesota Medical School
PTPN22: A Rheumatoid Arthritis Risk Gene at the Intersection Between Infection and Innate Immunity

1-31-13

Erik J. Peterson, M.D.
Department of Medicine
University of Minnesota Medical School
Morning perkiness
Isn't this a GLORIOUS DAY?

Uncalled-for thrift
Dented cans of peas, only thirty-nine cents!

Fitness obsession
My resting heart rate is two beats a minute!

Seven Deadly Virtues

Guilt-mongering
How can we go to the carnival when people are starving?

Gossip prudery
If you can't say anything nice, don't say it at all.

Overseriousness
I only read the classics.
I have the following financial relationships to disclose:

  Consultant for: Bristol-Myers-Squibb

I will not discuss off label use and/or investigational use in my presentation.
Overview:
Selected topics in Rheumatoid Arthritis

• Update on therapy and continuing disease burden
• Role of innate immune cells/pathways
• Insights from study of PTPN22, RA risk gene
• Type 1 Interferon: therapeutic candidate?
A previously-healthy 32-year-old woman, full-time attorney and mother of 2, gradually developed pain and swelling of finger and knuckle joints, wrists, ankles, and forefeet 4 months ago. She now reports that most mornings, joint stiffness/pain prevents her from dressing herself and performing childcare for ~ 2 hours. Marked fatigue forces her to nap by 3:30 each afternoon. She has lost 5 pounds over 2 months.

FHx: Her mother had type 1 diabetes, and 2 sisters are on thyroid replacement Rx.

PE: incomplete fist formation; tenderness of multiple MCPs, PIP, and MTP; R knee effusion.

Lab: CRP 15 (< 8); RF 64 IU; anti-CCP > 250 IU.

Case: “I’m just a ‘working stiff’, Doc”
Case

Course: She started methotrexate 15 mg weekly. Four weeks later, she reported “50%” improvement in hand pain, but still had > 1 hour a.m. stiffness and swollen ankles. Methotrexate dose was increased to 20 mg weekly, but she noted little additional change after another four weeks. Adalimumab 40 mg sq every other week was added. Within 3 weeks, she noted markedly reduced joint pain and stiffness. She was independent in self-cares, and her work capacity improved to pre-illness levels. Exam showed no palpable synovitis. CRP normalized.

12 months after starting continuous combination therapy, her symptoms remained under excellent control, joint exam was benign.
Rheumatoid Arthritis (RA)

- Common (0.5-1.5% prevalence), chronic, systemic syndrome clinically-dominated by symmetrical, inflammatory joint changes.
- Pathology: leukocyte-infiltrated synovial membranes (pannus).
- Pathophysiology: Autoreactive T and B lymphocytes and innate immune cells orchestrate an inflammatory response resulting in connective tissue injury, leading to progressive joint loss of function and deformity.
RA = multisystem and progressive syndrome

TIME
**A Golden Era for Rheumatologists:**
FDA-approved Treatments for Rheumatoid Arthritis
January 2013

<table>
<thead>
<tr>
<th>Biologic Disease-Modifying anti-rheumatic Drugs</th>
<th>Trade Name/Approval Date</th>
<th>Target of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adalimumab</td>
<td>Humira® 2002</td>
<td>TNF-α</td>
</tr>
<tr>
<td>Certolizumab pegol</td>
<td>Cimzia® 2009</td>
<td>TNF-α</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Enbrel® 1998</td>
<td>TNF-α</td>
</tr>
<tr>
<td>Golimumab</td>
<td>Simponi® 2009</td>
<td>TNF-α</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Remicade® 2002</td>
<td>TNF-α</td>
</tr>
<tr>
<td>Abatacept</td>
<td>Ocrevus® 2005</td>
<td>CD28</td>
</tr>
<tr>
<td>Anakinra</td>
<td>Kineret® 2001</td>
<td>IL-1</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Rituxan® 2006</td>
<td>CD20</td>
</tr>
<tr>
<td>Tocilizumab</td>
<td>Actemra® 2010</td>
<td>IL-6 receptor</td>
</tr>
<tr>
<td>Tofacitanib</td>
<td>Xeljanz 2012</td>
<td>JAK inhibitor</td>
</tr>
</tbody>
</table>

Abbreviations: IL = interleukin; TNF-α = tumor necrosis factor-alpha

Tofacitinib (Xeljanz): new kid on the therapeutic block

- Inhibits enzymatic activity of Janus tyrosine kinases (JAK); thought to target cytokine receptor (e.g. IL-2R) signaling, and TNF signaling in macrophages.
- **Effective:** either monotherapy or in combination with methotrexate for TNF inhibitor-resistant patients.
- **Tolerability:** diarrhea, rhinitis, headache, URI (each in < 5% pts).
- **Safety:** “manageable safety profile”
  - Opportunistic infections increased in treated pts (2.7 events per 100 pt-yrs).
  - Increased malignancy (n=12 in 3328 treated pts).
  - Lipids, leukocyte counts showed modest alterations in Phase 3 trials.

Tofacitinib (Xeljanz): features

- As of November, 2012, FDA-approved to “…treat adults with moderately to severely active rheumatoid arthritis (RA) who have had an inadequate response to, or who are intolerant of, methotrexate.”
- Once-daily **oral** administration
- Tolerability comparable to traditional DMARDs

For RA patients and payors, a not-so-Golden Era

• There is no cure for RA; the causes are unknown.
• Predictors of Rx response are lacking.
• ~30-40% show inadequate response to therapy.
• Costs of treating RA are escalating.
  ✓ Direct 2008 U.S. costs specific to RA = $22.3 B
  ✓ In “biologic era,” primary cost driver is pharmacy
  ✓ Per-RA patient pharmacy costs doubled since 1997

Kawatkar et al, *Arthritis Care and Res*; Vol. 64, No. 11, November 2012
## Exhibit 17

2011 Key Metrics for Top 10 Specialty Therapy Classes, PBM-Adjudicated Claims Only, Ranked by PMPY Spend

<table>
<thead>
<tr>
<th>Rank</th>
<th>Therapy Class</th>
<th>PMPY Spend</th>
<th>% of Total Specialty Spend</th>
<th>Prevalence of Use</th>
<th>Cost Per Adjusted Rx</th>
<th>Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inflammatory Conditions</td>
<td>$ 40.70</td>
<td>23.7%</td>
<td>0.23%</td>
<td>$2,066.77</td>
<td>17.7%</td>
</tr>
<tr>
<td>2</td>
<td>Multiple Sclerosis</td>
<td>$ 32.89</td>
<td>19.2%</td>
<td>0.10%</td>
<td>$3,115.93</td>
<td>20.3%</td>
</tr>
<tr>
<td>3</td>
<td>Cancer</td>
<td>$ 25.20</td>
<td>14.7%</td>
<td>0.15%</td>
<td>$3,259.34</td>
<td>15.7%</td>
</tr>
<tr>
<td>4</td>
<td>HIV</td>
<td>$ 18.08</td>
<td>10.5%</td>
<td>0.10%</td>
<td>$ 894.33</td>
<td>4.9%</td>
</tr>
<tr>
<td>5</td>
<td>Growth Deficiency</td>
<td>$  6.72</td>
<td>3.9%</td>
<td>0.03%</td>
<td>$3,103.62</td>
<td>6.6%</td>
</tr>
<tr>
<td>6</td>
<td>Anticoagulants</td>
<td>$  6.42</td>
<td>3.7%</td>
<td>0.31%</td>
<td>$1,013.35</td>
<td>5.1%</td>
</tr>
<tr>
<td>7</td>
<td>Hepatitis C</td>
<td>$  6.34</td>
<td>3.7%</td>
<td>0.02%</td>
<td>$3,370.99</td>
<td>194.8%</td>
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<tr>
<td>8</td>
<td>Transplant</td>
<td>$  5.63</td>
<td>3.3%</td>
<td>0.11%</td>
<td>$ 334.68</td>
<td>-1.7%</td>
</tr>
<tr>
<td>9</td>
<td>Respiratory Conditions</td>
<td>$  4.65</td>
<td>2.7%</td>
<td>0.02%</td>
<td>$2,800.35</td>
<td>17.6%</td>
</tr>
<tr>
<td>10</td>
<td>Pulmonary Hypertension</td>
<td>$  4.23</td>
<td>2.5%</td>
<td>0.01%</td>
<td>$3,507.42</td>
<td>2.6%</td>
</tr>
</tbody>
</table>

2011 Drug Trend Report, Express Scripts, published 4-12, thanks to Jeff McNamara
RA drugs top list of Medicare spending on specialty drugs

<table>
<thead>
<tr>
<th>Rank</th>
<th>Medication</th>
<th>Therapy Class</th>
<th>PMPY Spend</th>
<th>% of Total Specialty Spend</th>
<th>PMPY $ Change from 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Revlimid® (lenalidomide)</td>
<td>Cancer</td>
<td>$18.79</td>
<td>6.1%</td>
<td>$2.65</td>
</tr>
<tr>
<td>2</td>
<td>Enbrel® (etanercept)</td>
<td>Inflammatory Conditions</td>
<td>$17.85</td>
<td>5.8%</td>
<td>$2.76</td>
</tr>
<tr>
<td>3</td>
<td>Humira® (adalimumab)</td>
<td>Inflammatory Conditions</td>
<td>$14.64</td>
<td>4.8%</td>
<td>$2.75</td>
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<tr>
<td>4</td>
<td>Copaxone® (glatiramer)</td>
<td>Multiple Sclerosis</td>
<td>$13.50</td>
<td>4.4%</td>
<td>$3.14</td>
</tr>
<tr>
<td>5</td>
<td>Gleevec® (imatinib)</td>
<td>Cancer</td>
<td>$12.97</td>
<td>4.2%</td>
<td>$2.45</td>
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<tr>
<td>6</td>
<td>Truvada® (emtricitabine and tenofovir)</td>
<td>HIV</td>
<td>$12.26</td>
<td>4.0%</td>
<td>$1.85</td>
</tr>
<tr>
<td>7</td>
<td>enoxaparin</td>
<td>Anticoagulants</td>
<td>$9.62</td>
<td>3.1%</td>
<td>$7.17</td>
</tr>
<tr>
<td>8</td>
<td>Atripla® (efavirenz, emtricitabine and tenofovir)</td>
<td>HIV</td>
<td>$9.33</td>
<td>3.0%</td>
<td>$1.24</td>
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<tr>
<td>9</td>
<td>Avonex® (interferon beta-1a)</td>
<td>Multiple Sclerosis</td>
<td>$7.45</td>
<td>2.4%</td>
<td>$2.32</td>
</tr>
<tr>
<td>10</td>
<td>Tarceva® (erlotinib)</td>
<td>Cancer</td>
<td>$6.94</td>
<td>2.3%</td>
<td>$1.13</td>
</tr>
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</table>

2011 Drug Trend Report, Express Scripts, published 4-12
HERITABILITY OF “RISK” PROVIDES OPPORTUNITY TO UNDERSTAND RHEUMATOID ARTHRITIS PATHOPHYSIOLOGY

- RA disease susceptibility:
  - siblings bear 3-5 fold increased risk
  - MZ twins 20-40 fold increased risk.
  - genetic factors contribute ~60% of risk
- Disease results from concerted action of numerous “risk genes” interacting with environmental influences.

Genome Wide Association Studies (GWAS) have identified numerous RA susceptibility genes. October 2009: >30 RA risk loci

- CD40
- CCL21
- CD244
- IL2RB
- TNFRSF14
- PRKCQ
- PIP4K2C
- IL2RA
- AFF3
- REL
- BLK
- TAGAP
- CD28
- TRAF6
- PTPRC
- FCGR2A
- PRDM1
- CD2-CD58

HLA DR4 “shared epitope” hypothesis


Courtesy of Peter Gregersen
RA model: genetic factors + environment initiate autoimmunity, setting up joint damage mediated by inflammatory factors.
Tissue-resident “innate immune” cells can sense infection; initiate inflammation and adaptive T cell responses

Innate immune cells produce pro-inflammatory cytokines AND type 1 Interferon after infectious challenge.
TLR link pathogen recognition to cytokine production by myeloid cells

**TLR = Toll-like receptor**: transmembrane sensors for pathogen associated molecules (bacterial products, viral nucleic acids).

TLR3, 4, 7, 8 and 9 signals promote **type 1 IFN**, in addition to proinflammatory cytokines.

The majority of FDA-approved Treatments for Rheumatoid Arthritis Target Innate Immune cell products and pathways

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<td>Rituximab</td>
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<td>Tofacitanib</td>
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Abbreviations: IL = interleukin; TNF-α = tumor necrosis factor-alpha

RA risk loci encoding functions in innate immune cell signaling

October 2009: >30 RA risk loci

HLA “shared epitope” hypothesis
PADI4 PTPN22 CTLA4

CD40 REL
CD244 BLK
CD28 TAGAP
IL2RB CD28
IL2RA PRKCI
TNFRSF14 TRAF6
TNFAIP3 PTPRC
PRC PIP4K2C F-GR2A
AFF3 PRDM1
IL2-IL21 CD2-CD58


Courtesy of Peter Gregersen
RA model: genetic factors + environment initiate autoimmunity, setting up joint damage mediated by inflammatory factors.
RA “risk” gene *PTPN22* promotes *Innate Immune* signaling

- Ptpn22 promotes type 1 IFN production after TLR stimulation of myeloid cells.
- Ptpn22 is required for type 1 IFN-dependent suppression of arthritis.
- RA-associated PTPN22 variant (“LypW”) exhibits “loss-of-function.”
Acknowledgements

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La Jolla Institute for Allergy and Immunology

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Stephanie Stanford
Amanda Campbell

Klaus Ley
Iftach Shaked
Zbigniew Mikulski

Within Our Reach
Finding a Cure for Rheumatoid Arthritis

Rheumatology Research Foundation
Advancing Treatment | Finding Cures

Alliance for Lupus Research
PREVENT. TREAT. CURE.
PTPN22 (*Protein Tyrosine Phosphatase non-receptor #22*) is a susceptibility gene for human autoimmunity

- *PTPN22* variant is strongly associated with increased risk of seropositive Rheumatoid Arthritis (Hazard Ratio = ~2.0 for *PTPN22* variant heterozygotes).
- *PTPN22* variant is also associated with increased risk for
  - Type 1 diabetes
  - Systemic lupus erythematosus
  - Thyroiditis

PTPN22 encodes Lymphoid Phosphatase (Lyp), a cytoplasmic enzyme.

- Expressed in both lymphoid and myeloid cells.
- Negative regulator of T cell receptor (TCR) signaling.
- *PTPn22* “knockout” mice exhibit splenomegaly and T cell hyperresponsiveness.

Why study *Ptpn22* function in innate immune cells?

- *PTPN22* is expressed and regulated in myeloid cells (Arimura, Sci Sig, 2010).
- Myeloid cells stimulated through TLR produce molecules active in *PTPN22*-associated RA: TNFα, IL-1β, IL-6.
Hypothesis

Ptpn22 regulates myeloid cell Toll-like receptor signaling.

Approach: Utilize Ptpn22-deficient cells to examine TLR signaling.
*Ptpn22* is DISPENSABLE for pro-inflammatory cytokines induced by TLR stimulation

Macrophages, 4 hr Lipopolysaccharide (LPS; TLR4 agonist) stimulation

Bone marrow cultured with M-CSF for 6 days

Bone marrow-derived macrophages (BMM)

Quantitative PCR on total RNA

Wang, Y. et al, *submitted*
Ptpn22 is SELECTIVELY REQUIRED for TLR-induced upregulation of type 1 Interferon

Macrophages

WT ■ Ptpn22\(^{-/-}\) □

Ifnb

Ifna4

4 hr stimulation

LPS

poly(I:C)

(TLR3 agonist that mimics viral RNA)

Quantitative PCR on total RNA

Wang, Y. et al, submitted
Ptpn22 is required for TLR-driven, type 1 IFN-dependent host defense

Wang, Y. et al, submitted 12-12
Type 1 Interferons mediate protection against infection-induced tissue damage

• “Type 1” IFN = IFNβ, IFNα:
  ✓ Discovered (1957, by Isaacs and Lindenmann) as “interference” factors that render cells resistant to viral infection
  ✓ Induced by viral nucleic acids signaling through TLR
  ✓ Signal through Interferon alpha receptors (IFNAR)

• Type 1 IFN promote anti-viral host defense:
  ✓ Upregulate anti-viral genes
  ✓ Induce myeloid cell activation; promote T cell action against infected host cells

The role of type 1 Interferons in autoimmunity is complex

- However, type 1 IFN may promote immunoregulation:
  - Inhibit disease in animal models of diabetes, lupus, colitis
  - IFN-β suppresses animal and human multiple sclerosis
  - IFN-β inhibits TNFα and IL-1, limits cellular proliferation, and retards inflammation in animal models of arthritis

Crow, MK. Arthritis Research & Therapy 2010
Is Ptpn22 is required for type1 IFN-dependent regulation of inflammation?
Could decreased type 1 IFN production in *Ptpn22* deficiency result in unrestrained inflammation?

Fever, leukocyte recruitment, reactive oxygen species

Increased inflammation

Tissue Damage (e.g. inflammatory arthritis, arthritis)
Hypothesis: PTPN22 is required for anti-inflammatory effects of type 1 IFN

Approach:
Examine role of Ptpn22 in inflammatory arthritis

FEATURES, “Serum transfer” arthritis model
• Small-joint polyarthritis develops after injection of auto-antibody-containing serum.
• Requires IL-1 > TNF for full inflammation.
• Poly(I:C), TLR3 ligand, suppresses arthritis through type 1 IFN signaling.
• Arthritis suppression by poly(I:C) does not require T or B lymphocytes.

Korganow, Immunity (1999)
Yarilina, J. Immunology (2007)
Assay: TLR-mediated suppression of arthritis

Measurements
- Arthritis severity scores, joint swelling
- Serum IFN levels
- Synovial gene expression

Collaboration with Bryce Binstadt, MD/PhD, UMN
PTPn22 is required for arthritis suppression driven by TLR agonist poly(I:C)

Not shown: poly(I:C) fails to induce type 1 IFN or suppress synovial IL-1 in Ptpn22/-/- mice.

Wang, Y. et al, submitted
**PTPn22 mediates poly(I:C)-driven anti-inflammatory effects**

**Day 11 after arthritogenic serum transfer**

H & E-stained ankle sections, 40X.

Wang, Y. et al, *submitted*
*Ptpn22* is required for both type1 IFN-dependent host defense and immunoregulation.

**Diagram:**
- TLR
- Myeloid cell Membrane
- PTPn22
- IFN
- IFNAR
- Nucleus
- NFκB
- Type 1 Interferons (IFN)
- Type 1 IFN stimulated genes
- "Pro-inflammatory" cytokines
- "Arthritis Suppression"
- "Antiviral host defense"

Type 1 Interferons (IFN) stimulate genes that lead to antiviral suppression and arthritis suppression. PTPn22 is involved in this process, regulating the signaling cascade initiated by IFN binding to IFNAR.
Hypothesis 2

Human RA-associated PTPN22 variant “LypW” differentially regulates Toll-like receptor signaling to type 1 IFN promoters.

Approach 1: Determine the role of human Lyp in regulating inflammatory arthritis.
RA-associated \textit{PTPN22} variant harbors an Arginine (R) \text{→} Tryptophan (W) substitution

Lyp

Major variant

RA-associated variant

\(620R\) \text{“LypR”}

Proline-rich regions

\(620W\) \text{“LypW”}

~10\% of Caucasian Minnesotans

Method: Compare responses of LypW and “LypR” transgenic mice

BREED TO PTPN22-DEFICIENT MICE

LypR.Ptpn22-/-  LypW.Ptpn22-/-

Wang, Y. et al, submitted
Mice expressing LypR, but not LypW, suppress arthritis after poly(l:C) treatment

Data Not Shown: In LypW-/- mice, poly (l:C) fails to induce type 1 IFN or suppress synovial IL-1 in.

Wang, Y. et al, submitted
Hypothesis 2

Human RA-associated PTPN22 variant “LypW” differentially regulates Toll-like receptor signaling to type 1 IFN promoters.

Approach 2: Compare TLR signaling in human LypW carriers and non-carriers.
“LypW” carriers display reduced type 1 IFN-inducible gene expression

Blood-derived dendritic cells
(Healthy donors)

\[ IRF7 \quad P = 0.011 \]
\[ MX1 \quad P = 0.013 \]
\[ ISG15 \quad P = 0.033 \]

LPS stimulation, 4 hours

Wang, Y. et al, submitted
Summary

1. During TLR signal-driven inflammatory arthritis suppression, Ptpn22 deficiency or LypW expression results in:

   - \[\uparrow\] Arthritis severity
   - \[\downarrow\] Type 1 IFN production
   - \[\uparrow\] Synovial IL-1β

2. LypW carrier humans show impaired signaling through type 1 IFN-inducing TLR.
Loss of PTPN22 function engenders a type 1 IFN deficiency

Pathogen → Myeloid cell from a PTPN22-deficient animal or a LypW carrier → Pro-inflammatory cytokines → Type I Interferon

Host defense

Uncontrolled infection → Uncontrolled inflammation → Joint damage
Model: *Ptpn22* loss of function = “broken fire extinguisher”

**PTPN22 sufficient**

- **Myeloid cell trigger:** e.g. infection, endogenous TLR ligand
- Type 1 IFN antagonizes pro-inflammatory cytokines, limits cellular infiltration, injury

**PTPN22 loss-of-function (LypW carriage)**

- Poorly-restrained pro-inflammatory cytokines are leads to increased cellular infiltration and injury

**Inflamed RA joint**

**Myeloid cell trigger:** e.g. infection, endogenous TLR ligand

**Type 1 IFN** antagonizes pro-inflammatory cytokines, limits cellular infiltration, injury

**Poorly-restrained pro-inflammatory cytokines** are leads to increased cellular infiltration and injury
Implications for Rheumatoid Arthritis

_LypW and Disease Mechanism_

• RA-associated LypW may exert “risk”-enhancing effects via altered regulation of TLR signaling in innate immune cells.

• *Speculation:* Therapies that boost type 1 IFN signaling (e.g. recombinant type 1 IFN) could benefit RA patients who are also PTPN22-LypW carriers.
Preliminary data: type 1 IFN holds promise as Rheumatoid Arthritis Rx

AMELIORATION OF COLLAGEN-INDUCED ARTHRITIS AND SUPPRESSION OF INTERFERON-γ, INTERLEUKIN-12, AND TUMOR NECROSIS FACTOR α PRODUCTION BY INTERFERON-β GENE THERAPY


Treatment with recombinant interferon-β reduces inflammation and slows cartilage destruction in the collagen-induced arthritis model of rheumatoid arthritis

Arthritis Res Ther 2004, 6:R239-R249

Intraarticular Interferon-β Gene Therapy Ameliorates Adjuvant Arthritis in Rats

HUMAN GENE THERAPY 17:985–996 (October 2006)
Type 1 IFN and Rheumatoid Arthritis: Phase II Trial of IFN-β

A multicentre, randomised, double blind, placebo controlled phase II study of subcutaneous interferon beta-1a in the treatment of patients with active rheumatoid arthritis


- 209 RA patients, on methotrexate but with active disease; treated with IFN-b or placebo, given subcu. 3X per week for 24 weeks.
- Result: No significant differences in clinical responses.
So, type 1 IFN does not work in Rheumatoid Arthritis?

Limitations of van Holten et al:
- Biopsies showed no Rx effect on synovial histology, suggesting possible ineffective dosing or route of administration.
- Patients selected by clinical criteria without regard for genetic factors.
- Response variance magnitude unclear.

Before we throw in the towel:
- Pilot trial of type 1 IFN to treat RA, comparing LypW carriers and non-carriers
- Utilize longer-acting interferon-beta (PEGylated)
Summary

• Knowledge of RA causes or cure is lacking
• But, improved understanding of contributing cells and molecules has resulted in more effective and better-tolerated combination treatment, including a new orally-administered JNK inhibitor.
• Most current RA therapies target cytokines and signals operating in innate immune cells.
• RA heritability is multigenic, based upon genes with putative function in either lymphoid or innate immune system cells.
• Risk gene PTPN22 promotes TLR signaling and balance of type 1 IFN and proinflammatory cytokine production by myeloid cells.
The role of type 1 Interferons in autoimmunity is complex

• Type 1 IFN exacerbate/accelerate autoimmunity:
  ✓ Lupus-prone mice lacking IFNAR are protected from renal disease
  ✓ SLE risk genes are associated with higher serum IFNa levels
  ✓ IFN-α Rx for HCV or malignancy may develop SLE-like symptoms

• However, type 1 IFN may promote immunoregulation:
  ✓ Inhibit disease in animal models of diabetes, lupus, colitis
  ✓ IFN-β suppresses animal and human multiple sclerosis
  ✓ IFN-β inhibits TNFα and IL-1, limits cellular proliferation, and retards inflammation in animal models of arthritis

Crow, MK. Arthritis Research & Therapy 2010
Differential location/effects of IFNα and IFNβ may explain opposing roles of type 1 IFN in autoimmunity and inflammation

Crow, MK. Arthritis Research & Therapy 2010
Cytokine imbalance is associated with tissue injury

**TNF, IL-1**

- Fever, leukocyte recruitment, reactive oxygen species
- Innate immune cells (DC, macrophage)

**Type 1 IFN**

- ↑Anti-viral genes
- CD8 T cell activation
- Pathogen Clearance
- Tissue Damage (e.g. Rheumatoid Arthritis)

Molecules associated with infection and tissue damage are potent activators of innate immune cells

Mills, K., Nat Rev. Immunology, 2011
Conclusion

- Ptpn22 promotes type 1 IFN production after TLR activation.
- Ptpn22 is critical for type 1 IFN-dependent suppression of arthritis.
- Disease-associated PTPN22 variant ("LypW") exhibits "loss-of-function" for type 1 IFN-dependent responses.
PTPN22 promotes TLR-driven type 1 Interferon-dependent responses
Potential immune response sites of PTPN22 action in developing autoimmunity

Genetic predisposition

Environmental insult

Response to Infection

Tolerance breakdown

Symptoms/Tissue injury

Chemoattraction
Potential immune response sites of PTPN22 action in developing autoimmunity

- Genetic predisposition
- Environmental insult
- Tolerance breakdown
- Symptoms/Tissue injury

TIME

Response to Infection

Chemoattraction
RA model: genetic factors + environment initiate loss of tolerance, setting up joint damage mediated by inflammatory factors
**PTPn22 is a susceptibility gene for human autoimmunity**

- A *PTPn22* genetic variant confers increased RA risk
  - Relative Risk = ~2.0 for single allele carriers
  - Relative Risk = 3-4 for homozygotes.
- *PTPn22* variant is also strongly associated with increased risk for
  - Type 1 “autoimmune” diabetes
  - Systemic lupus erythematosus
  - Autoimmune thyroiditis.
- Disease allele prevalence in Caucasians: 7-15%

Hypothesis

*Ptpn22* regulates TLR signaling
**Ptpn22** is DISPENSABLE for cytokine upregulation after TLR stimulation

- Bone marrow cultured with M-CSF for 6 days
- Bone marrow-derived macrophages (BMM)

Macrophages, 4 hr LPS stimulation

**Il1b**

**Tnfa**

Quantitative PCR on total RNA

Wang et al, *under review*
ptpn22 is REQUIRED for TLR-induced upregulation of type 1 Interferon

Macrophages, 4 hr stimulation

WT ■ Ptpn22−/− □

**Ifnb**

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**Ifna4**

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LPS (TLR4)

poly (I:C) (TLR3)

Quantitative PCR on total RNA

Wang et al, *under review*
Ptpn22 selectively promotes myeloid cell-produced type 1 IFN *in vitro* and *in vivo*

*Ptpn22* is **required** for:
- IFNβ protein secretion after TLR3/4 stimulation.
- Dendritic cell *ifnb* induction after TLR7 or 9 stimulation.
- *Ifnb* induction in liver after systemic TLR3 agonist.

*Ptpn22* is **dispensable** for:
- TLR stimulated cytokines *TNFa, IL6, IL1 in vivo*. 

Hypothesis: *PTPN22* is required for anti-inflammatory effects of type 1 IFN

**Approach:**

K/BxN serum-transfer model of arthritis

- Mimics pathology of human rheumatoid arthritis.
- Arthritis development is independent of T and B cells.
- Poly(I:C), a TLR3 ligand, suppresses arthritis progression through type 1 IFN.

---

Method: TLR-driven suppression of serum-transfer model of arthritis

Measurements
- Arthritis severity scores, joint swelling
- Serum IFN levels
- Synovial gene expression
*PTPn22* is required for arthritis suppression driven by poly(I:C), a type 1 Interferon-inducing TLR agonist.
poly(I:C) drives type 1 IFN, and suppresses IL-1, in a PTPn22-dependent manner during arthritis

**Arthritic mice**

**WT** ■ **ptpn22**

**Serum, Day 4**

- IFNα: saline < p(I:C) (P = 0.0033)
- IFNβ: saline < p(I:C) (P = 0.0102)

**Synovium, Day 11**

- *Isg15*: saline < p(I:C) (P < 0.0001)
- *Il1b*: saline < p(I:C) (P = 0.0118)
PTPN22 promotes TLR-induced host defense and anti-inflammatory function of type 1 IFN
Defects in Type 1 IFN associated with PTPN22 “loss of function” may cause tissue injury

Pathogen

Ptpn22-null macrophage or DC

Pro-inflammatory cytokines

Uncontrolled inflammation

Impaired host defense

Type I Interferon

Tissue Injury
Downstream effector pathways activated by arthritogenic anti-GPI autoantibodies.
The anti-glucose-6-phosphate isomerase (GPI) antibody transfer model.

Transfer of serum from K/BxN mice
Host viral sensors signal upregulation of type 1 IFN

Type I interferons signal through the Jak-STAT pathway to regulate gene transcription

Interferon signaling in the liver during hepatitis C virus infection

Cytokine Volume 59, Issue 3 2012 460 - 466
Loss of Type 1 IFN signaling is incompatible with survival

• 3 known patients with IFN receptor mutations:
  ➢ 2 died in infancy of viral infection
  ➢ 1 died of BMT complications

PTPN22 (Protein tyrosine phosphatase non-receptor 22) is a “risk” gene for human autoimmune disease syndromes

Rheumatoid arthritis (#2 gene in risk “potency”)
Type 1 diabetes (#3 in risk “potency”)
Systemic Lupus
Graves’ (thyroid) disease
Others (vitiligo, myasthenia, scleroderma)

PTPN22 encodes Lymphoid phosphatase: TCR regulator
PTPN22 promotes innate immune signaling and potentiates type 1-interferon-dependent immunity.

*Disease-associated LypW is a “loss of function” variant.
MODEL 1: Loss of PTPN22 function = immune deficiency

- Genetic predisposition
- Environmental insult
- Tolerance breakdown
- Symptoms/Tissue injury

TIME

Lymphocyte Autoreactivity

Response to Infection

PTPn22 deficiency

- Aberrant antigen presentation
- CD4+ T cell expansion
- Autoantibody production
- Cytokines
- Chemoattraction
- Inflammation
- Effector T cell action

PTPn22 deficiency
MODEL 2: Loss of PTPN22 function = impaired innate immunoregulation

- Genetic predisposition
- Environmental insult
- Tolerance breakdown
- Symptoms/Tissue injury

TIME

- Lymphocyte Autoreactivity
- Increased innate responsiveness
- Response to Infection
- Aberrant antigen presentation
- CD4 T cell expansion
- Autoantibody production

- Cytokines
- Chemoattraction
- Inflammation
- Effector T cell action
LypW in human Rheumatoid Arthritis

• **Pathogenesis:** Decreased type 1 IFN in LypW carrier RA synovium ➔ decreased immunoregulation ➔ increased synovial inflammation and tissue injury in LypW-associated disease.

• **Prevention/therapy:** Strategies that boost type 1 IFN production or type 1 IFN effects may provide benefit in LypW carriers with rheumatoid arthritis.
Pathogenesis of most autoimmune diseases can be viewed primarily as:

1. Tissue damage results from “overactive” lymphocytes that inappropriately attack an immunocompetent host.

2. Infection causes injury in an immune-deficient host, leading to loss of lymphocyte tolerance and chronic tissue damage.
$PTPN22$ SNP encodes variant “LypW” protein

- $PTPN22$ major allele encodes arginine at amino acid position 620 ("LypR").
- Disease-associated $PTPn22$ allele encodes tryptophan ("LypW").

Questions

• What is the role of *Ptpn22* in host defense and inflammation?
• Does autoimmunity-associated LypW differentially regulate myeloid cell function?
Hypothesis

*Ptpn22* regulates TLR signaling
Ptpn22 selectively promotes myeloid cell-produced type 1 IFN \textit{in vitro} and \textit{in vivo}

\textit{Ptpn22} is \textbf{required} for:

- IFNβ protein secretion after TLR3/4 stimulation.
- Dendritic cell \textit{ifnb} induction after TLR7 or 9 stimulation.
- \textit{Ifnb} induction in liver after systemic TLR3 agonist.

\textit{Ptpn22} is \textbf{dispensable} for:

- TLR stimulated cytokines \textit{TNFa, IL6, IL1 in vivo}. 
How is PTPn22 selectively required for TLR signals?
PTPn22 associates with TRAF3 and promotes TRAF3 K63-linked polyubiquitinylation.

TLR4

Plasma membrane

PTPn22

TRAF3

IRF3

Type 1 IFNs
Type 1 IFN mediate protection against pathogen-induced tissue injury

1. Type 1 IFN in anti-viral host defense:
   ✓ Signal through IFNAR to activate anti-viral genes
   ✓ Induce myeloid cell activation
   ✓ Promote “killer” T cell proliferation and effector function

2. Type 1 IFN in immunoregulation:
   ✓ Suppress IL-1 upregulation and induce IL-10 in macrophages
   ✓ Antagonize Th17 differentiation, through IL-27
   ✓ Inhibit TNF production and disease activity in inflammatory arthritis and multiple sclerosis; protect colonic mucosa against irritant injury.

Lymphochooriomeningitis virus (LCMV) infection model

• TLR signaling plays a critical role in type 1 interferon production after LCMV infection.

• Type 1 interferon is required for expansion of viral-specific cytotoxic T cell in response to LCMV.

Borrow P, JEM, 2010
**Ptpn22 is required for early type 1 IFN after viral infection**

24 hr after LCMV

**Serum**
- WT
- *Ptpn22*^−/−^ (P < 0.0001)

**Spleen**
- LCMV

**Graphs**
- IFNβ (pg/ml)
- IFNα (median ± IQR, % of pDC)

**Legend**
- Intraperitoneal LCMV
*Ptpn22* is required for early myeloid cell activation

Day 1 after LCMV, spleen

**CD8α⁺ Dendritic Cells**

WT

Ptpn22⁻/⁻

**Counts**

CD86

**Fold induction (MFI)**

CD86

CD40

$P < 0.0001$  

$P = 0.0002$
*Ptpn22* is required for efficient virus-induced cytolytic T cell expansion

Day 7 after LCMV, spleen
Ptpn22 is required for type 1 IFN-driven host defense

TLR

IFN

IFN

IFN

IFN

IFNAR

PLA

PTPn22

TRAF3

IRF3

Cytoplasm

Nucleus

Type 1 IFNs

STAT1

Type 1 IFN

stimulated genes

Anti-viral

host defenses
Is *Ptpn22* required for type 1 IFN-dependent immunoregulation?

**Diagram:**
- TLR
- PTPn22
- TRAF3
- IRF3
- IFN
- IFNAR
- STAT1
- Type 1 IFNs
- Type 1 IFN stimulated genes

**Pathways:**
- Antiviral host defenses
- Immuno-regulation

**Questions:**
- Is *Ptpn22* required for type 1 IFN-dependent immunoregulation?
Innate immune cells are critical in the serum-transfer rheumatoid arthritis model.

Effector phase: macrophages, neutrophils required

Courtesy Bryce Binstadt, UMN
TLR agonist poly(I:C) suppresses serum-transfer arthritis through type 1 IFN

Yarilina et al., JI 2007
poly(I:C) drives type 1 IFN, and suppresses IL-1, in a *PTPn22*-dependent manner during arthritis
Ptpn22 required for type 1 IFN-dependent immunoregulation?
Summary: Question 1
What is \textit{Ptpn22} function in myeloid cells?

- \textit{Ptpn22} is selectively required for TLR signaling leading to efficient type 1 IFN induction.
- \textit{Ptpn22} associates with TRAF3, and promotes its polyubiquitination.
- Myeloid and lymphoid responses to viral infection are defective in \textit{Ptpn22}-/- mice.
- \textit{Ptpn22} is required for TLR3 agonist-dependent suppression of inflammatory arthritis.
Question 2: Hypothesis

Autoimmunity-associated variant “LypW” differentially regulates TLR signaling.

Approach

Study LypW effects on TLR signaling:
- primary human cells
- PTPN22 transgenic mice.
LypW carriers exhibit reduced TLR4-stimulated type 1 IFN-dependent genes

HEALTHY DONOR PBMC-derived dendritic cells; LPS (1 µg/ml) stimulation, 4 hr

Fold increase in mRNA levels over unstimulated conditions by qPCR, normalized to GAPDH.
Proinflammatory cytokine levels do not correlate with \textit{Ptpn22} genotype

HEALTHY DONOR PBMC; LPS (1 \(\mu\)g/ml) stimulation, 4 hr

\textit{Fold increase in mRNA levels over unstimulated conditions by qPCR, normalized to GAPDH.}
BAC transgenic mice express physiologic levels of human *PTPN22*

![Image](image-url)
LypR, but not LypW, transgenic myeloid cells show augmented TLR4-driven type 1 IFN

BMMs, 4 hr LPS

![Graph showing IFNb expression with P values](image)
Summary

• LypW is associated with defects in TLR-driven:
  1. Type 1 IFN-dependent gene upregulation
  2. Macrophage activation
  3. Arthritis suppression.
Consequences of reduced type 1 IFN production: diminished host defense

Virus

Innate immune cells (DC, macrophage)

TNF, IL-1

↑ Anti-viral genes
CD8 T cell activation

Pathogen persistence

Infectious Tissue Damage (Type 1 Diabetes)
The “broken fire extinguisher”: impaired immunoregulation in *PTPN22*-associated RA

**PTPN22 sufficient**

- IL-1
- TNF

TLR trigger: e.g. infection, endogenous TLR ligand

Type 1 IFN antagonizes pro-inflammatory cytokines, limits cellular infiltration, injury

**PTPN22 loss-of-function**

- IL-1
- TNF

Unrestrained pro-inflammatory cytokines are leads to increased cellular infiltration and injury
Implications for Human Disease

*PTPn22W and Disease Mechanism*

- Autoimmunity-associated LypW may exert risk enhancing effects through TLR signaling “loss of function” by innate immune cells.

- *Speculation:* Therapies that boost type 1 IFN signaling (e.g. recombinant IFN) could be beneficial for autoimmune syndromes associated with LypW.

Thank you

Questions?
Future directions—RA and PTPn22

• Determine the molecules/pathways regulated by *Ptpn22* in inflamed synovium:
  - *Utilize microarray to characterize differential gene expression in arthritic, TLR agonist-treated Ptpn22-null mice.*
  - *Examine candidate type 1 IFN-dependent immunoregulatory pathways: IL-27, IL-10, IL-1Ra*

• Characterize interaction between Lyp and TRAF3:
  - *Is physical interaction between TRAF3 and Lyp required?*
  - *Can signaling defects in LypW-expressing cells be rescued by activators of TRAF3 ubiquitination?*
PTPn22 mediates poly(I:C)-driven anti-inflammatory effects

H & E-stained ankle sections, Day 11 after K/BxN serum transfer. Bar, 0.2 mm.