Using Biosimulation to Identify a Biological Basis for Poor Response to TNF-α Neutralizing Therapies

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ABSTRACT

Introduction: Roughly a third of all rheumatoid arthritis (RA) patients fail to achieve an ACR20 response to TNF-α neutralizing therapies. However, differences in pathophysiology that distinguish these nonresponders from responders and the implications for alternate treatment strategies remain unclear. To address these issues, we used computer simulation to identify biological features that might explain why some RA patients respond poorly to TNF-α neutralizing therapies.

Methods: Three potential classes of TNF-α blockade nonresponders were defined, specifically, those lacking improvement in (1) synovial hyperplasia, (2) cartilage degradation, or (3) both. The Entelos® Rheumatoid Arthritis PhysioLab® platform, a large-scale computer model of a rheumatoid joint, was used to identify possible biological explanations for these responses. The model simulates cell population dynamics, mediator production, and cell contact of synovial fibroblasts, macrophages, T cells, and chondrocytes, and predicts synovial hyperplasia and cartilage degradation. Parameter values associated with cytokine/mediator synthesis in the joint were explored to identify and characterize virtual patients (VPs) with high disease activity before and after TNF-α blockade.

Results: Analysis of a reference VP revealed that synovial hyperplasia and cartilage degradation were differentially affected when TNF-α was varied within different ranges, laying the basis for identifying VPs in the three nonresponder classes. Specifically, VPs with low initial TNF-α activity show minimal reduction in degradation in response to TNF-α blockade, while VPs with negligible initial TNF-α activity show poor response in both outputs. Alternatively, insufficient neutralization of TNF-α yields improvement in degradation but poor response in hyperplasia. Mechanistically, in VPs with low TNF-α, disease was perpetuated by increased activity of alternate macrophage activating pathways (e.g., CD40-ligation), reduced activity of anti-inflammatory cytokines (e.g., IL-10), and increased activity of degradation-promoting cytokines (e.g., IL-1β). Nonresponder VPs also showed altered responses to other therapies such as IL-1Ra.

Conclusions: Biosimulation results predicted that two key features that can lead to a poor clinical response to TNF-α blockade are insufficient neutralization of TNF-α and low pre-treatment TNF-α activity. Disease activity in VPs with low pre-treatment TNF-α showed greater reliance on alternate inflammatory and degradative pathways and weaker anti-inflammatory activity. Increased emphasis on IL-1β-dependent pathways in particular was important to maintain disease activity, and thus improved efficacy of IL-1Ra was seen in many nonresponder VPs. Validation of one or more of these patient types could help to identify nonresponders and select alternate treatment strategies.

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Nonstandard Abbreviations
RA - rheumatoid arthritis; VP - virtual patient; RP – reference (virtual) patient; NR – non-responder; HNR - hyperplasia non-responder; CNR - cartilage non-responder; DNR - double non-responder.
INTRODUCTION & MODELING METHODS

Roughly a third of all rheumatoid arthritis (RA) patients fail to achieve an ACR20 response to TNF-α neutralizing therapies (Lipsky et al. 2000). The purpose of this study was to use biosimulation techniques to identify virtual RA patients that would resemble clinically reported patients with poor response to TNF-α neutralizing therapies. A large scale mathematical model comprised of differential and algebraic equations was used to simulate the conditions in a rheumatoid joint. The resulting RA PhysioLab® platform incorporates cell functions, biochemical interactions, and tissue structure to reproduce known characteristics of RA and enables the prediction and evaluation of clinical outcomes in response to molecular level interventions.

I. Model Scope

• The RA PhysioLab platform focuses on the area surrounding the cartilage-pannus junction in a prototypical rheumatoid joint (Figure 1)

• The model includes a representative patient type called the reference patient, which matches clinical reports of RA patients with respect to:
  - cartilage degradation rate
  - synovial tissue cellularity
  - mediator concentrations
  - responses to therapies

• The model includes a synovial tissue compartment which includes:
  - fibroblast-like synoviocytes (FLS)
  - macrophages
  - CD4+ T cells

• The model includes a cartilage compartment consisting of:
  - chondrocytes
  - type II collagen
  - aggrecan

• The model encompasses functions such as cell life-cycles, cell-cell contact, mediator production and effects, matrix metabolism, mediator transport as

• Mediators, matrix molecules, and therapeutics represented include:

<table>
<thead>
<tr>
<th>Cytokines &amp; Mediators.</th>
<th>Matrix Molecules &amp; Mediators</th>
<th>Therapeutics</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β FGF-2 MCP-1</td>
<td>type II collagen aggrecan</td>
<td>methotrexate (MTX)</td>
</tr>
<tr>
<td>IL-2 GM-CSF MIP-1α</td>
<td>MMP-1</td>
<td>glucocorticoids</td>
</tr>
<tr>
<td>IL-4 IGF-I IL-8</td>
<td>MMP-3</td>
<td>NSAIDs</td>
</tr>
<tr>
<td>IL-6 IFN-γ PGE2</td>
<td>MMP-13</td>
<td>sTNF-RII</td>
</tr>
<tr>
<td>IL-10 PDGF sTNF-R1</td>
<td>TIMP-1</td>
<td>anti-TNFα mAb</td>
</tr>
<tr>
<td>IL-12 TGF-β sTNF-R1I</td>
<td></td>
<td>IL-1Ra</td>
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<tr>
<td>IL-13 TNF-α IL-1Ra</td>
<td></td>
<td></td>
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<tr>
<td>IL-17</td>
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</tbody>
</table>

• Note: This investigation was conducted on the first generation RA PhysioLab platform. Subsequent development has included additional components such as angiogenesis and a bone erosion index
II. Model Development and Validation

- **Data Used**: Quantitative data on cellular functions and mediator activity was obtained from published in vivo and in vitro data

- **Calibration**: Soluble mediator levels, cell populations, and cell activity were compared and calibrated against published data on rheumatoid cells/joints

- **Validation**: The model was validated by testing predicted results against clinical results for various therapeutic interventions (Figure 3)
Figure 2. The PhysioLab Effect Diagram encompasses the entire model. The biological components represented in each row are indicated. The red box outlines the macrophage TNF-α and IL-1β synthesis regulation shown magnified in Figure 3.

Figure 3. Left: detail of effect diagram showing regulation of macrophage TNFα and IL-1β synthesis. Black-headed arrows represent stimulation/upregulation. White-headed arrows represent inhibition/down-regulation. Cumulative effect is determined mathematically by combining dose-dependencies given by curves as shown on right. Many of the regulatory cytokines are produced by T cells and FLS in addition to macrophages.
Figure 3: Response of the reference patient to various therapies, administered beginning at month 6 (arrow) of an 18 month simulation. Responses were used to validate the reference patient.
BIOSIMULATION RESULTS

I. Non-Responder Patient Rationale
Investigation of the sensitivity of the reference patient (RP) disease activity to changes in TNF-α revealed that cartilage degradation is more sensitive at medium/high TNF-α activity, whereas hyperplasia is more sensitive at lower TNF-α (Figure 4). This suggests the potential for three classes of non-responders, outlined in Table 1: partial neutralization of TNF-α selectively yields a cartilage response (hyperplasia non-responder, HNR); neutralization of low TNF-α favors hyperplasia response (cartilage non-responder, CNR); and neutralization of minimal initial TNF-α yields poor response in both outputs (double non-responder, DNR).

![Figure 4. Curves showing sensitivity of hyperplasia and degradation in the reference patient to synovial tissue TNF-α. Arrows represent change in TNF-α with therapy in different patient types.](image)

Table 1. Comparison of reference and potential non-responder patients. TNF-α levels are normalized to pre-treatment TNF-α in the reference patient. Post-treatment levels refer to TNF-α activity after therapy with sTNF-RII. Differences from reference patient are indicated in red type.

<table>
<thead>
<tr>
<th></th>
<th>RP: Reference Patient</th>
<th>HNR: Hyperplasia Non-responder</th>
<th>CNR: Cartilage Non-responder</th>
<th>DNR: Double Non-responder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>med (1x)</td>
<td>med/high (≥1x)</td>
<td>low (≤0.1x)</td>
<td>very low (≤0.01x)</td>
</tr>
<tr>
<td>TNF-α activity</td>
<td>&lt;0.01x</td>
<td>≥0.1x</td>
<td>&lt;0.01x</td>
<td>&lt;0.01x</td>
</tr>
<tr>
<td>Post-treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease in</td>
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<td>Weak (&lt;20%)</td>
<td>Strong (&gt;20%)</td>
<td>Weak (&lt;20%)</td>
</tr>
<tr>
<td>hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease in</td>
<td>Strong (30%)</td>
<td>Strong (&gt;20%)</td>
<td>Weak (&lt;20%)</td>
<td>Weak (&lt;20%)</td>
</tr>
<tr>
<td>degradation rate</td>
<td></td>
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II. Biological Basis for Non-Responder Virtual Patients

Virtual patients were created with disease activity comparable to the reference patient, but with differential responses to therapy due to altered TNF-α activity or neutralization. Modifications in degradative and pro/anti-inflammatory pathways perpetuate disease activity in patients with reduced TNF-α, and contribute to non-response in patients with med/high TNF-α. Multiple virtual patients were identified in each non-responder class. In Figure 5, each colored line represents a different patient within a non-responder class. The intersection of the line with the axes indicates the synthesis/activity of factors relative to the untreated reference patient.

Hyperplasia Non-Responders

- Characteristics & Response:
  - Hyperplasia improvement <20%, degradation improvement >20%
  - Partial neutralization (e.g. - 2x Kd, 66% dose sTNF-RII) of med/high TNF-α
  - Increased T-cell activation (more antigen, reduced TCR disruption)
  - Decreased synthesis of anti-inflammatory mediators (IL-10)

- Clinical Relevance:
  - Similar to ACR non-responders with chondroprotection (Lipsky et al. 2000)
  - Possible improvement with dose-escalation (St. Clair et al. 2002)
  - Consistent with association between high TNF producing allele -308A, and poor response (Mugnier et al. 2003, Padyukov et al. 2003)
  - Consistent with association between high IL-10 producing allele -1087G, and good response (Padyukov et al. 2003)

Cartilage Non-Responders

- Characteristics & Response:
  - Hyperplasia improvement >20%; degradation improvement <20%
  - Efficient neutralization of low pre-treatment TNF-α
  - Increased synthesis of degradation-enhancing mediators (IL-1β, PDGF)
  - Increased synthesis of other inflammatory mediators (IL-1β, IL-12)
  - Decreased synthesis of anti-inflammatory mediators (IL-10, TGFb, IL-13)

- Clinical Relevance:
  - Consistent with association between pre-treatment TNF-α activity and ACR response (Ulfgren et al. 2000, Alex et al. 2003)
  - Consistent with association between high IL-10 producing allele -1087G, and good response and between low TGF-β1 producing allele and poor response (Padyukov et al. 2003)

Double Non-Responders

- Characteristics & Response:
  - Hyperplasia improvement <20%; degradation improvement <20%
  - Efficient neutralization of very low pre-treatment TNF-α
  - Increased synthesis of degradation-enhancing mediators (IL-1β, PDGF)
  - Increased synthesis of other inflammatory mediators (IL-1β, CD40L)
  - Decreased synthesis of anti-inflammatory mediators (IL-10, TGFβ, IL-13)

- Clinical Relevance:
  - Consistent with association between pre-treatment TNF-α activity and ACR response (Ulfgren et al. 2000, Alex et al. 2003)
  - Consistent with association between high IL-10 producing allele -1087G and good response, and between low TGF-β1 producing allele and poor response (Padyukov et al. 2003)
III. Responses to sTNF-RII Therapy: Pre- and post-treatment disease activity

**Figure 6.** Hyperplasia and cartilage degradation rate pre- (solid) and post-treatment (shaded) for the reference patient and a representative non-responder patient from each class. Disease activity in the reference and non-responder patients are comparable, but response to TNF neutralization differs.

![Graph showing hyperplasia and degradation rate](image)

* indicates > 20% improvement

**SUMMARY**

*Biosimulation with the RA PhysioLab platform suggests:*

- Insufficient neutralization of med/high pre-treatment TNF-α can result in hyperplasia NR, while low pre-treatment TNF-α may yield cartilage and double NRs

- Compensatory activity of multiple alternate degradative and pro/ anti-inflammatory pathways perpetuate disease and contribute to poor response in non-responders

- Non-responders may benefit from dose-escalation (HNR) or alternate treatment strategies based on the specific alterations in pathology identified here

- Non-responder virtual patients developed here can be used for:
  - Identification of biomarkers predicting response to TNF-α neutralization
  - Evaluation of alternate or combination treatment strategies for each NR type
  - Identification of novel therapeutic approaches for use in NRs

**ACKNOWLEDGEMENTS**

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Figure 5. Comparison of therapeutic reduction in synovial hyperplasia, cartilage degradation rate, and inflammatory cytokine levels in response to: (1) MTX, (2) sTNF-RII, (3) IL-1Ra, and (4) sTNF-RII + IL-1Ra. Continuous administration of biologics was assumed at concentrations chosen to give the same area under the curve for serum concentration compared with clinical studies of the corresponding therapy (25 mg 2x/week sTNF-RII, 100 mg/day IL-1Ra). Effective IL-1β reflects reduction in efficacy due to presence of IL-1Ra. Combination of the biologics is more efficacious than monotherapy because blockade of one cytokine does not eliminate the disease-promoting activity of the other.

Figure 6. Cartilage degradation rate and synovial hyperplasia as a function of sTNF-RII and IL-1Ra dose in continuously administered combined therapy. Solid white points represents monotherapy and combination therapy at doses used in monotherapy (see Figure 5 caption). Significant therapeutic effect can be obtained at reduced concentrations of these agents.
REFERENCES

Alex et al. 2003: Abstract, ACR 2003