Dynamics of a Cytokine Storm
Robert Stengel*
MAE, Princeton University
2012

- Cytokine Signaling and Inflammation
- TGN1412 Phase I Clinical Trial
- Modeling the Response of Individual Cytokines
- Integrated Model of Cytokine Response
- Applications of the Dynamic Model
  - Inhibition of Individual Cytokines
  - Effects of TGN1412 Infusion Duration
  - Effects of Model Uncertainty

* with Hao Yiu, CBE, and Andrea Graham, EEB, Princeton University


Cytokines
- Signaling peptides, proteins, or glycoproteins
- Secreted by immune-system cells, epithelial and endothelial cells, smooth muscle
- In turn, cells are regulated by cytokines
- Pro- or anti-inflammatory response to pathogens, “non-self” molecules, tumors, and toxins
Cytokine Storms (Hypercytokinemia) are Central to Many Lethal Infections

- Systemic Inflammatory Response Syndrome (SIRS)
- Spanish Flu of 1918 (~500M, 10% mortality, WW)
- Severe Acute Respiratory Syndrome (SARS, 10% mortality, WW)
- Seasonal influenza (40,000 deaths/yr, US)
- Systemic sepsis (750,000/yr, 25-50% mortality, US)
- Dengue virus (50-100M/yr, 25,000-50,000 deaths/yr, WW)
- Hantavirus (30% mortality)

“Most studies have focused on direct measurements of a few cytokines and chemokines in the peripheral blood compartment and have failed to interrogate the whole of the immune cascade in the context of the infecting pathogen..... while the peripheral blood may not provide an accurate picture of the cytokine profiles in a tissue, in the lungs, the location of the initial infection does not seem to be a determinant of the severity of local and systemic cytokine storms.... all can lead to indistinguishable clinical syndromes of acute lung injury (ALI) with respiratory failure, sepsis, and a cytokine storm.” Tisoncik et al, “Into the Eye of the Cytokine Storm”, MicroMolBioRev, 2012.

TGN1412 Clinical Trial
November 13, 2006

- Phase 1 study of humanized monoclonal antibody engineered as anti-CD28 super-agonist that did not require co-stimulation
- Intended applications of the drug
  - Restore T-cell populations destroyed by cancer chemotherapy
  - Regulate T cells in autoimmune disease (e.g., rheumatoid arthritis)

Beginning of the Trial

- 8 healthy male subjects, 19 to 34 yr
  - 6 received TGN1412
  - 2 received placebo (saline)
- Infusions lasted 3 to 6 min
  - 0.1 mg/kg body weight
  - 2 mg/min
- Clinical measurements began before the infusion and captured the start
  - Clinical trial did not intend to study Cytokine Storms
  - Tragic but unprecedented opportunity to track cytokine storms in disease-free patients
**TGN1412 Clinical Trial, 3/13/2006**

- Within an hour of infusion, subjects experienced:
  - Headaches
  - Muscle pain
  - Nausea
  - Diarrhea
  - Decreased blood pressure
  - Decreased heart rate
- Severe depletion of lymphocytes and monocytes from 4th hour to 4th day
- Multi-organ failure:
  - Infiltrates in the lung
  - Intravascular coagulation
  - Renal failure
  - Lung injury
- Gross swelling of head and body
- Peripheral ischemia requiring surgery (one case)

**Timeline of the 2006 Clinical Trial**

- **Median Cytokine Concentrations in the TGN1412 Clinical Trial**
  - TNF-α
  - IFN-γ
  - IL10
  - IL8
  - IL6
  - IL4
  - IL2
  - IL1
  - IL12

**Measurements**

- Normal cytokine ranges: 3.7-48 pg/mL
- Cytometric Bead Array Measurements:
  - 5-20% assay accuracy compared to ELISA (Elshal, McCoy, 2007)
  - ELISA is 15-30% accurate (Kristiansen et al, 2002)
  - Signal saturation at 5,000 pg/mL (Suntharalingam, 2006)
- Median estimates for 6 TGN1412 patients at each measurement over 5 days
  - Inter-quartile error bars often span measurement range
- Digitized at 6-hr intervals for our study
Median Lymphocyte and Monocyte Concentrations in the TGN1412 Clinical Trial

CD3+ T CD4+ T CD8+ T

Monocytes Neutrophils

Suntharalingam, 2006

Dynamic System with Feedback Control

• Use available information
• To identify system dynamics, subject to “feedback” therapy

What Do We Know?

TGN1412

Dynamic Process

Input, u

Cellular Processes

Unmodulated Excitation

Signaling Molecules, e.g., Cytokines, RNA, peptides, ...

Observation Process: Measurement may contain error or be incomplete

Measurement Error, p

Output, y = Measurement, z

x = dynamic state
u = input
w = exogenous disturbance
p = parameter
For k = time or event index

Least-Square-Error Estimates of System Parameters

• More generally, least-squares estimation is used for
  • Higher-degree curve-fitting
  • Multivariate estimation
  • Identification of dynamic system parameters

• Use available information

Error “Cost” Function

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  • Multivariate estimation
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Error “Cost” Function
2nd-Order Model for Response of an Individual Cytokine

- 2nd-order linear, time-invariant ordinary differential equation
- Initial rate of change induced by TGN1412 (~instantaneous infusion)
- Rate of change of cytokine concentration, $x_2(t)$
- "Acceleration", $dx_2/dt$, is proportional to concentration and rate of change through $a$ and $b$
- Concentration is referenced to basal level
- Initial rate of change is induced by TGN1412 (i.e., ~instantaneous infusion)

\[
\begin{align*}
\frac{dx_1(t)}{dt} &= \dot{x}_1(t) = x_2(t) \\
\frac{dx_2(t)}{dt} &= \dot{x}_2(t) = -ax_1(t) - bx_2(t)
\end{align*}
\]

Parameters to be identified from experimental data are $a$, $b$, and $x_2(0)$
Combining equations

\[
\begin{bmatrix}
\dot{x}_1(t) \\
\dot{x}_2(t)
\end{bmatrix} =
\begin{bmatrix}
0 & 1 \\
-a & -b
\end{bmatrix}
\begin{bmatrix}
x_1(t) \\
x_2(t)
\end{bmatrix}
\]

\[
\begin{bmatrix}
x_1(0) \\
x_2(0)
\end{bmatrix} =
\begin{bmatrix}
0 \\
x_2(0)
\end{bmatrix}
\]

\[\dot{x}(t) = Ax(t), \quad x(0) \text{ given}\]

Propagate State from One Sampling Instant to the Next in Discrete Steps

- Incremental integration via state transition matrix

\[
x(t_{k+1}) = e^{At}x(t_k) = \Phi(\Delta t)x(t_k), \quad x(0) \text{ given}
\]

- Elements of $F$ are directly related to the elements of $A$

\[
\Phi(\Delta t) = \text{Inverse Laplace Transform}\left[\frac{1}{(sI - A)^{-1}}\right]
\]
Discrete-Time Model of 2nd-Order System

- Based on eigenvalues of continuous-time system

\[
\begin{bmatrix}
    x_1(t_{k+1}) \\
    x_2(t_{k+1})
\end{bmatrix}
= 
\begin{bmatrix}
    \frac{(\lambda_1 e^{\lambda_1 \Delta t} - \lambda_2 e^{\lambda_2 \Delta t})}{(\lambda_1 - \lambda_2)} \\
    \frac{(\lambda_1 e^{\lambda_1 \Delta t} - \lambda_2 e^{\lambda_2 \Delta t})}{(\lambda_1 - \lambda_2)} \\
    \frac{\lambda_1 \lambda_2 (e^{\lambda_1 \Delta t} - e^{\lambda_2 \Delta t})}{(\lambda_1 - \lambda_2)^2} \\
    \frac{\lambda_1 \lambda_2 (e^{\lambda_1 \Delta t} - e^{\lambda_2 \Delta t})}{(\lambda_1 - \lambda_2)^2}
\end{bmatrix}
\begin{bmatrix}
    x_1(t_k) \\
    x_2(t_k)
\end{bmatrix}
\]

where

\[a = -\lambda_1 \lambda_2, \quad b = \lambda_1 + \lambda_2, \quad \Delta t = 6 \text{ hr}\]

Error Cost Function for Parameter Identification

- Squared error of difference between measurements and model's estimates of cytokine concentration

\[
J = \sum_{k=0}^{20} \varepsilon(t_k)^2 = \sum_{k=0}^{20} [z(t_k) - x_1(t_k)]^2
\]

where

\[z(t_k) : \text{ Measurement data set, } x_1(t_k) : \text{ Concentration estimate propagated by discrete-time model}\]

Gradient-Free Search for Parameter Identification

- Error minimized by choice of \(a\), \(b\), and \(x_2(0)\)

\[
\min_{a, b, x_2(t_0)} J = \min_{a, b, x_2(t_0)} \sum_{k=0}^{20} [z(t_k) - x_1(t_k)]^2
\]

using Nelder-Mead (Downhill Simplex) algorithm [MATLAB's \texttt{fminsearch}]

Comparison of Median Cytokine Histories and 2nd-Order Responses
2nd-Order Models of Response to Unit Initial Rates of Change

- Same response shapes as experimental data

2nd-Order Models of Response to Unit Initial Concentrations

- Novel wave forms unlike experimental data
- New insights about relative cytokine response

Eigenvalues ($\lambda_1$, $\lambda_2$), Time Constants ($\tau_1$, $\tau_2$), Periods ($P$), Damping Ratios ($\zeta$), and Initial Rates of Separate 2nd-Order Models

### Eigenvalues, Time Constants, Periods, Damping Ratios, and Initial Rates

<table>
<thead>
<tr>
<th>Component</th>
<th>$\lambda_1$, d'</th>
<th>$\lambda_2$, d'</th>
<th>$\tau_1$, d</th>
<th>$\tau_2$, d</th>
<th>$P$, d</th>
<th>$\zeta$</th>
<th>$x_0(0)$, pg/mL.d</th>
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</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>-2.63</td>
<td>-2.63</td>
<td>0.38</td>
<td>0.38</td>
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<tr>
<td>IFN-γ</td>
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<td>0.14</td>
<td>0.49</td>
<td>1.63</td>
<td>1.2</td>
<td>55328</td>
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<tr>
<td>IL10</td>
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<td>-2.08</td>
<td>0.48</td>
<td>0.48</td>
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<td>12047</td>
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<td>0.15</td>
<td>0.54</td>
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<td>IL6</td>
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<td>-1.55</td>
<td>0.65</td>
<td>0.65</td>
<td>4.05</td>
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<td>-4.17</td>
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<td>0.24</td>
<td>1.51</td>
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<td>-4.08</td>
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<td>0.25</td>
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<td>IL1</td>
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<td>0.37</td>
<td>2.32</td>
<td>1</td>
<td>35535</td>
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<tr>
<td>IL12</td>
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<td>-4.13</td>
<td>0.24</td>
<td>0.24</td>
<td>1.52</td>
<td>1</td>
<td>4947</td>
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</tbody>
</table>

Combine Nine Models into a Single Uncoupled 18th-Order Model

Verify that results are same as those for low-order models

$$x(t) = \begin{bmatrix} x_1(t) & x_2(t) & x_3(t) & x_4(t) & \ldots & x_{17}(t) & x_{18}(t) \end{bmatrix}^T$$

$$A = \begin{bmatrix} a_{1,1} & 0 & 0 & \ldots & 0 & 0 \\ 0 & a_{2,2} & 0 & \ldots & 0 & 0 \\ 0 & 0 & a_{3,3} & \ldots & 0 & 0 \\ \vdots & \ldots & \ldots & \ddots & \ldots & \ldots \\ 0 & 0 & 0 & \ldots & a_{17,17} & 0 \\ 0 & 0 & 0 & \ldots & 0 & a_{18,18} \end{bmatrix}$$
Discrete-Time 18th-Order Model

- Propagation equation and initial conditions

\[ x(t_{k+1}) = \Phi(\Delta t)x(t_k), \quad k = 0, 20 \]

\[ x(0) = \begin{bmatrix} 0 & x_2(0) & 0 & x_4(0) & \cdots & 0 & x_{16}(0) & 0 & x_{18}(0) \end{bmatrix}^T \]

- State transition matrix

\[ \Phi(\Delta t) = e^{A\Delta t} = \begin{bmatrix} \phi_{1,1} & \phi_{1,2} & \cdots & 0 \\ \phi_{2,1} & \phi_{2,2} & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \phi_{18,18} \end{bmatrix} \]

- Uncoupled 18th-order response is identical to that of 9 separate 2nd-order models

18th-Order Stability Matrix with Concentration Coupling

- 90 unknown coefficients
  - 18 coefficients in diagonal blocks
  - 72 coefficients in off-diagonal blocks

\[ A = \begin{bmatrix} 0 & 1 & 0 & 0 & \cdots & 0 & 0 \\ a_{2,1} & a_{2,3} & 0 & 0 & \cdots & 0 & 0 \\ 0 & 0 & 0 & 1 & \cdots & 0 & 0 \\ a_{4,1} & 0 & a_{4,3} & a_{4,4} & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & 0 & \cdots & 0 & 1 \\ a_{18,1} & 0 & a_{18,3} & 0 & \cdots & 0 & a_{18,18} \end{bmatrix} \leq A_c \]

- Reasonable to assume that off-diagonal blocks are small

Parameter Estimates for 18th-Order Uncoupled Model

- Minimize weighted error cost function with respect to 27 parameters (18 coefficients + 9 initial rates of change)
- Diagonal weighting matrix, \( Q \), normalizes the errors by each cytokine’s typical values

\[ J = \sum_{k=0}^{20} e^T(t_k)Qe(t_k) = \sum_{k=0}^{20} [z(t_k) - x_c(t_k)]^T Q [z(t_k) - x_c(t_k)] \]

where

\[ q_i = \frac{1}{\sum_{k=0}^{20} z^2_i(t_k)}, \quad i = 1, 9 \]

\[ x_c = \begin{bmatrix} x_1 & x_3 & \cdots & x_9 \end{bmatrix}^T \quad (9 \times 1) \]

- 18th-order Downhill-Simplex algorithm
- Same parameter estimates as individual 2nd-order models to at least 3 significant digits

Parameter Estimates for Coupled 18th-Order Model

- Downhill-Simplex minimization of

\[ J = \sum_{k=0}^{20} [z(t_k) - x_c(t_k)]^T Q [z(t_k) - x_c(t_k)] \]

with respect to 90 parameters (assuming same initial conditions as before) produces unreasonable results

- Regularize error cost function to keep off-diagonal parameters, \( p_c \), small

\[ J = \sum_{k=0}^{20} [z(t_k) - x_c(t_k)]^T Q [z(t_k) - x_c(t_k)] + r_c p_c^T p_c \]

- Error cost is reduced by 20%, implying that coupling effects are significant
Parameter Estimates for Coupled 18th-Order Model

- Regularize error cost function to keep “total damping” (i.e., the trace of $A$) the same as uncoupled results

$$ J = \sum_{k=0}^{n} \left[ \mathbf{z}(t_k) - \mathbf{x}(t_k) \right]^T Q \left[ \mathbf{z}(t_k) - \mathbf{x}(t_k) \right] + r_c \mathbf{p}_c^T \mathbf{p}_c + r_T \left[ \text{Tr}(\mathbf{A}_c) - \text{Tr}(\mathbf{A}_c) \right]^2 $$

- Error cost is reduced by an additional 1%

Concentration Coefficients of the Coupled 18th-Order Model

- Odd columns and even rows of $A$

<table>
<thead>
<tr>
<th></th>
<th>TNF</th>
<th>IFN</th>
<th>IL10</th>
<th>IL8</th>
<th>IL6</th>
<th>IL4</th>
<th>IL2</th>
<th>IL1</th>
<th>IL12</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>-0.413</td>
<td>0.345</td>
<td>-0.383</td>
<td>-0.186</td>
<td>-0.632</td>
<td>-0.680</td>
<td>-0.206</td>
<td>0.072</td>
<td>-0.818</td>
</tr>
<tr>
<td>IFN</td>
<td>-0.554</td>
<td>-0.184</td>
<td>1.576</td>
<td>1.542</td>
<td>0.128</td>
<td>0.184</td>
<td>0.696</td>
<td>-0.903</td>
<td></td>
</tr>
<tr>
<td>IL10</td>
<td>-0.487</td>
<td>0.846</td>
<td>-3.329</td>
<td>0.145</td>
<td>-0.727</td>
<td>-0.111</td>
<td>-0.030</td>
<td>-0.017</td>
<td>0.617</td>
</tr>
<tr>
<td>IL8</td>
<td>0.992</td>
<td>-0.207</td>
<td>1.566</td>
<td>-13.571</td>
<td>0.058</td>
<td>-0.823</td>
<td>-0.316</td>
<td>0.046</td>
<td>-3.356</td>
</tr>
<tr>
<td>IL6</td>
<td>0.412</td>
<td>-1.688</td>
<td>-0.303</td>
<td>0.042</td>
<td>-2.784</td>
<td>0.640</td>
<td>0.769</td>
<td>0.955</td>
<td>0.065</td>
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<tr>
<td>IL2</td>
<td>-1.129</td>
<td>-1.072</td>
<td>-0.278</td>
<td>0.271</td>
<td>0.101</td>
<td>-16.305</td>
<td>0.776</td>
<td>0.778</td>
<td>-0.237</td>
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<tr>
<td>IL1</td>
<td>0.053</td>
<td>-0.090</td>
<td>-0.376</td>
<td>0.891</td>
<td>-0.575</td>
<td>0.227</td>
<td>0.289</td>
<td>-7.571</td>
<td>0.604</td>
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<tr>
<td>IL12</td>
<td>-0.877</td>
<td>-0.075</td>
<td>0.275</td>
<td>-0.228</td>
<td>0.320</td>
<td>0.343</td>
<td>1.554</td>
<td>-0.271</td>
<td>-19.448</td>
</tr>
</tbody>
</table>

- All cytokines are self-regulatory (negative coefficients)
  - Caveat: intensive therapy contributed to results
- Self-regulation sensitivity is stronger than inter-cytokine sensitivity in all but one case
- 1:1 Coupling > 5-10% in many instances, 60% in one case (IFN -> IL6)

Cytokine Sensitivity to Coupling

- Row-wise comparison of coupling coefficients to self coefficient

$$ C_i = \sum_{j=1}^{n} \left| \frac{a_{i,j} - a_{i,j-1}}{a_{i,j-1}} \right| \times 100 \% , \quad i = 1, 9 $$

<table>
<thead>
<tr>
<th>Receiver</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>5%</td>
</tr>
<tr>
<td>IFN</td>
<td>1%</td>
</tr>
<tr>
<td>IL10</td>
<td>2%</td>
</tr>
<tr>
<td>IL8</td>
<td>1%</td>
</tr>
<tr>
<td>IL6</td>
<td>12%</td>
</tr>
<tr>
<td>IL4</td>
<td>0%</td>
</tr>
<tr>
<td>IL2</td>
<td>1%</td>
</tr>
<tr>
<td>IL1</td>
<td>2%</td>
</tr>
<tr>
<td>IL12</td>
<td>1%</td>
</tr>
</tbody>
</table>

Coupled Eigenvalues (Response Modes) and Three Most Significant Response (Eigenvector) Components

- 11 response modes
  - 7 are oscillatory
  - 4 are real

<table>
<thead>
<tr>
<th>Mode</th>
<th>$\lambda$, d$^+$</th>
<th>$P$, d$^-$</th>
<th>$\phi$, d$^\prime$</th>
<th>EV #1</th>
<th>EV #2</th>
<th>EV #3</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.84</td>
<td>-</td>
<td>-</td>
<td>IL10</td>
<td>IL6</td>
<td>IL8</td>
</tr>
<tr>
<td>2</td>
<td>-1.4 ± 0.75</td>
<td>3.93</td>
<td>0.89</td>
<td>IL6</td>
<td>TNF</td>
<td>IL10</td>
</tr>
<tr>
<td>3</td>
<td>-1.88</td>
<td>-</td>
<td>-</td>
<td>IL8</td>
<td>TNF</td>
<td>IL1</td>
</tr>
<tr>
<td>4</td>
<td>-2.27 ± 0.61</td>
<td>2.66</td>
<td>0.97</td>
<td>IL1</td>
<td>IL8</td>
<td>IFN</td>
</tr>
<tr>
<td>5</td>
<td>-3.28 ± 0.60</td>
<td>1.89</td>
<td>0.98</td>
<td>IL1</td>
<td>IL10</td>
<td>IFN/IL4</td>
</tr>
<tr>
<td>6</td>
<td>-3.22 ± 0.98</td>
<td>1.86</td>
<td>0.96</td>
<td>IL1</td>
<td>IL4</td>
<td>TNF</td>
</tr>
<tr>
<td>7</td>
<td>-3.75</td>
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<td>IL10</td>
<td>IL12</td>
<td>TNF</td>
</tr>
<tr>
<td>8</td>
<td>-4.02 ± 0.80</td>
<td>1.56</td>
<td>0.97</td>
<td>IL4</td>
<td>IL12</td>
<td>IL2</td>
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<tr>
<td>9</td>
<td>-4.41 ± 0.71</td>
<td>1.40</td>
<td>0.99</td>
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<td>IL12</td>
<td>IFN/IL8</td>
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<td>1.17</td>
<td>0.99</td>
<td>IL8</td>
<td>IFN</td>
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<td>11</td>
<td>-5.82</td>
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<td>IL8</td>
<td>IFN</td>
<td>IL12</td>
</tr>
</tbody>
</table>

+ : Pro-inflammatory; others are mixed

Net Coupling Effect

$$ C_i = \sum_{j=1}^{n} \left| \frac{a_{i,j} - a_{i,j-1}}{a_{i,j-1}} \right| \times 100 \% , \quad i = 1, 9 $$

Gross Coupling Effect

$$ C_i = \sum_{j=1}^{n} \left| \frac{a_{i,j} - a_{i,j-1}}{a_{i,j}} \right| \times 100 \% , \quad i = 1, 9 $$

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<th>Percent</th>
</tr>
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<tbody>
<tr>
<td>TNF</td>
<td>5%</td>
<td>TNF</td>
<td>61%</td>
</tr>
<tr>
<td>IFN</td>
<td>1%</td>
<td>IFN</td>
<td>30%</td>
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<td>IL10</td>
<td>2%</td>
<td>IL10</td>
<td>90%</td>
</tr>
<tr>
<td>IL8</td>
<td>1%</td>
<td>IL8</td>
<td>54%</td>
</tr>
<tr>
<td>IL6</td>
<td>12%</td>
<td>IL6</td>
<td>175%</td>
</tr>
<tr>
<td>IL4</td>
<td>0%</td>
<td>IL4</td>
<td>28%</td>
</tr>
<tr>
<td>IL2</td>
<td>1%</td>
<td>IL2</td>
<td>24%</td>
</tr>
<tr>
<td>IL1</td>
<td>2%</td>
<td>IL1</td>
<td>41%</td>
</tr>
<tr>
<td>IL12</td>
<td>1%</td>
<td>IL12</td>
<td>20%</td>
</tr>
</tbody>
</table>
Cytokines That Drive Coupling

- Column-wise comparison of coupling coefficients to self coefficient

\[ C_j = \frac{1}{a_{2j-1}} \sum a_{2j-1} \times 100 \% \] \( j = 1, 9 \)

Net Coupling Effect

Gross Coupling Effect

\[ C_j = \frac{1}{|a_{2j-1}|} \sum |a_{2j-1}| \times 100 \% \] \( j = 1, 9 \)

Effecter | Percent
--- | ---
TNF | 5%
IFN | 1%
IL10 | 9%
IL6 | 2%
IL6 | 2%
IL6 | 2%
IL4 | 0%
IL2 | 1%
IL1 | 5%
IL12 | 1%

Effecter | Percent
--- | ---
TNF | 78%
IFN | 27%
IL10 | 111%
IL6 | 151%
IL4 | 18%
IL2 | 27%
IL1 | 48%
IL12 | 39%

Implications for control (i.e., treatment)

Most Significant Cytokine Interactions
(from concentration coupling matrix)

- “T” indicates inhibition
- “->” indicates excitation

Coupled Response to Unit Initial Cytokine Concentrations

Motifs of Response to Unit Initial Cytokine Concentrations over 5 Days

- Unit initial condition on individual cytokines (z axis)
- Most significant coupling on remaining cytokines (x-y axes)
Principal Components Identify Similarities in Wave Forms of Cytokine Responses

- Covariance Matrix of Measurements
  \[ Z = z(t_k)z^T(t_k) \]

- Singular-Value Decomposition of Z produces the Principal Components
  \[ y(t_k) = Cz(t_k), \quad k = 0, k_f \]

- Principal components identify similarity but not causality

Shapes of Three Most Significant Principal Components

99% explanation of measured wave shapes in 1st 3 components

Shapes of Three Most Significant Principal Components

99% explanation of measured wave shapes in 1st 3 components

Coefficients of the 1st Three Principal Components

Dendrogram Identifies Three Cytokine Clusters By Distance

- Principal Component Analysis identifies similarity in wave forms without regard to causality
Modeled Responses for Three Cytokine Clusters

- Groupings suggested by dendrogram identify similar responses

Group A

Group B

Group C

- Consistent with trends suggested by Tisoncik et al, 2012

Effects of Inhibiting Pro-Inflammatory Cytokines

- Respective rows of A set to zero
- Remaining cytokine responses computed as before

Effects of Inhibiting Anti- and Mixed Inflammatory Cytokines
8 mg dose of TGN1412 would be unsafe at any dosage rate
Possible safe dose of TGN1412: < 8/300 mg, \( t_{dose} > 1 \) day
However, linear model prediction may be inaccurate

\[
B_1 = 2880 \text{ mg/d}, B_2 \text{ indeterminate without additional information}
\]
Evolution of the Mean State Vector

**Continuous-Time Model**

\[
E\left[\dot{x}(t)\right] = E[A\bar{x}(t)] = AE\left[x(t)\right]
\]
\[\Delta \dot{x}(t) = A\bar{x}(t) \quad E[x(t)] = \mathbf{x}(0) \text{ given}\]

**Discrete-Time Model**

\[
\bar{x}(t_{k+1}) = e^{A\Delta t} \bar{x}(t_k) = \Phi(\Delta t) \bar{x}(t_k)
\]

Propagation of the State Covariance Matrix from Initial Condition

**P(0) given**

\[
E\left[[x(t_{k+1}) - \bar{x}(t_{k+1})][x(t_{k+1}) - \bar{x}(t_{k+1})]^T\right] = \Phi(\Delta t) E\left[[x(t_k) - \bar{x}(t_k)][x(t_k) - \bar{x}(t_k)]^T\right] \Phi^T(\Delta t)
\]

\[\Delta P(t_{k+1}) = \Phi(\Delta t) P(t_k) \Phi^T(\Delta t)\]

- Evolution of uncertainty covariance is linear
- Diagonal elements are square roots of standard deviations

Propagation of the State Covariance Matrix with Uncertain Disturbance

- For this evaluation, neglect initial uncertainty \[P(0) = 0\]
- Focus on exogenous effects

\[
P(t_{k+1}) = \Phi(\Delta t) P(t_k) \Phi^T(\Delta t) + W(t_k)
\]

where

\[
W(t_k) = L(t_k) W_D L^T(t_k) \Delta t
\]

- \(W_D\): Covariance matrix of exogenous disturbance
- \(L(t_k)\): Disturbance-effect matrix for continuous model
- \(\Delta t = 0.01\) days for calculation

Effects of Uncertainty on Cytokine Concentration Standard Deviation

- **A:** Initial Concentration Uncertainty
- **B:** Initial Rate Uncertainty
- **C:** Disturbance Uncertainty
- **D:** Model Parameter Uncertainty
Cellular-Cytokine Associations (from the literature)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>IFN-γ</th>
<th>IL1α</th>
<th>IL1β</th>
<th>IL2</th>
<th>IL4</th>
<th>IL8</th>
<th>IL12</th>
<th>IL6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Macrophage</td>
<td>S, R</td>
<td>S, R</td>
<td>S, R</td>
<td>R</td>
<td>R</td>
<td>S, R</td>
<td>S, R</td>
<td>S</td>
</tr>
<tr>
<td>Dendritic Cell</td>
<td>S, R</td>
<td>S, R</td>
<td>S, R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Mast Cell</td>
<td>S</td>
<td>S, R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S, R</td>
<td>S</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>S, R</td>
<td>S, R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S, R</td>
<td>S</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>S</td>
<td>S, R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S, R</td>
<td>S</td>
</tr>
<tr>
<td>Basophil</td>
<td>S</td>
<td>S, R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>NK</td>
<td>S, R</td>
<td>S, R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

Adaptive system:

Mixed: S, R

Data types that secrete the cytokine are denoted by S; those that are regulated by the cytokine are indicated by R.

Discussion
TGN1412 Clinical Trial
- Cytokine storm was an unintended over-reaction of immune systems in response to challenge
- Comments on trial:
  - Small number of subjects
  - Limited number of measurements
  - Large variability in individual responses
  - Unanticipated “experiment”
  - Distinct effects of therapy are inseparable from natural response without additional information

Discussion
Data-Driven vs. Theory-Driven Modeling
- Parsimony, at all costs; however, model reduction is not useful
- Linear vs. nonlinear models
- Limitations of linear models
  - Local approximation
  - Products (e.g., mass action) or limiting (e.g., Michaelis-Menten, Hill effects) not represented, except in piecewise fashion
- No reason to incorporate nonlinear effects without cause
- Freedoms of linear models
  - Broad array of analytical methods
  - Definition of modal characteristics
  - Simplicity of addressing high-order models
  - Can be expanded for approximation of nonlinearity
- Analytical difficulties associated with nonlinear models
  - Multiple equilibria
  - Amplitude-dependent response
  - Substitute for higher-order unmodeled dynamics
  - Implicit need for model reduction

Discussion
Analytical Results
- Cytokine Group B had fastest response, peaking 6 hr after infusion
  - During this time T-cell, monocyte, and platelet concentrations crashed (sacrificial response to activation?)
  - Group B returned to normal after 2 days, as did concentrations of these cells
- Neutrophil profile similar to IL6 profile, which was the slowest of the three groups
Discussion

- IL2, IL8, and IL10 had the greatest inductive effect on other cytokines
- IFNγ and IL12 had the greatest inhibiting effect
- Three clusters of similar cytokine response revealed by Principal Component Analysis
- IL1, IL6, IL10, and TNFα had greatest variability in response to uncertainty
- Pro-inflammatory IL8 most likely secreted by innate immune cells and non-immune system tissue
- Opportunity remains to extend present study to measured T cells, monocytes, and platelets

Opinion

- Available clinical results are sparse and fail to reveal important dynamic coupling
  - Variability in 1st appearance of patients
  - Uncertainty in starting point
- Clinical trials focus on treatment of abnormal conditions
  - Safety
  - Efficacy
  - Dosage schedule and level
  - Often restricted to salvage of terminally ill patients
- To better understand cytokine storms, there is a need to better understand normal cytokine dynamics in humans
  - New clinical challenge studies
  - Distinctly different goals from typical pharmacological studies
  - Further studies of human cytokine dynamics using “safe” drugs, e.g., those used for post-infusion therapy

Conclusions

- Dynamic modeling of temporal data provides new insights into cytokine response
- Early, synchronized measurements are important
  - Know the start time for stimulus and immune response
  - Make closely spaced measurements during the first 48 hr of response
- Practical value in linear modeling
  - 2nd-order system as the basic building block for modeling concentration
- For the given total dose, TGN1412 is unsafe at any plausible dosage rate
  - Safe total dose given over one day no greater than ~ 1/300 of the clinical trial dose
  - Prediction based on linear model is uncertain
- Adaptive immune response had dominant effect on the cytokine storm

Acknowledgments

- Hao H. Yiu, currently Staff Engineer, Integra Life Sciences
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Supplemental Material

Immune Cell-Cytokine Associations

Signaling pathways derived from diverse experiments

Cellular Secretion of and Regulation by Cytokines

Joyce, 2000

Spanish Flu Pandemic of 1918

- 500 million cases worldwide
- 50 to 130 million died
Severe Acute Respiratory Syndrome (SARS) Epidemic of 2003
- 8,422 cases worldwide
- 10.9% mortality

T-Cell Activation
- Typically requires
  - Antigen MHC complex
  - Co-stimulatory signal to CD28 receptor
- TGN1412 would not require co-stimulatory signal
- Extensive pre-human testing of TGN1412

Post-Infusion Medications
- Corticosteroids (anti-inflammation)
- Chlorpheniramine (antihistamine)
- Acetaminophen (analgesic for headache)
- Ondansetron (anti-nausea and vomiting)
- Metaraminol (prevention of hypotension)
- Methylprednisolone (anti-inflammation)
- Anti-IL2 receptor antagonist antibody
Eigenvectors for 2\textsuperscript{nd}-Order Model

- Eigenvectors portray participation of each state element in each response mode

\[
(\lambda_i I - A)e_i = 0, \quad i = 1, n
\]

\[
e_1 = \alpha \begin{bmatrix} 1 \\ \lambda_1 \end{bmatrix}; \quad e_2 = \alpha \begin{bmatrix} 1 \\ \lambda_2 \end{bmatrix}
\]