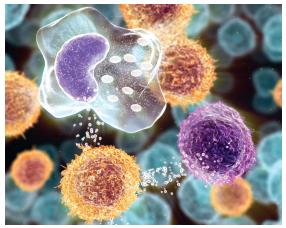
Dynamics of a Cytokine Storm



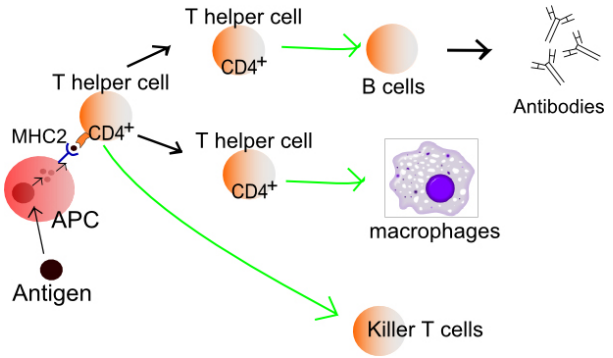
Robert Stengel*
MAE, Princeton University
2012

- 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

PLOS ONE, Oct 1, 2012, http://www.plosone.org/

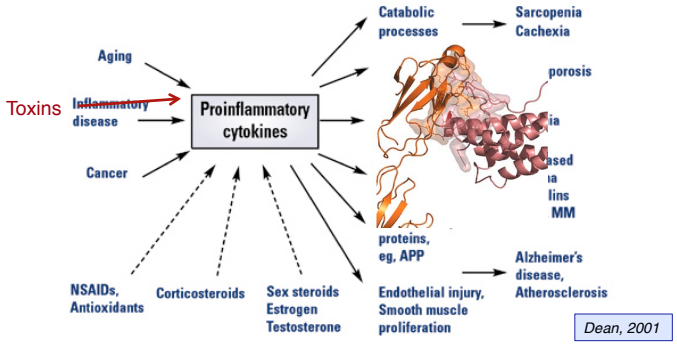
Basic Adaptive Immune Response to Infection



… but what do these arrows represent?

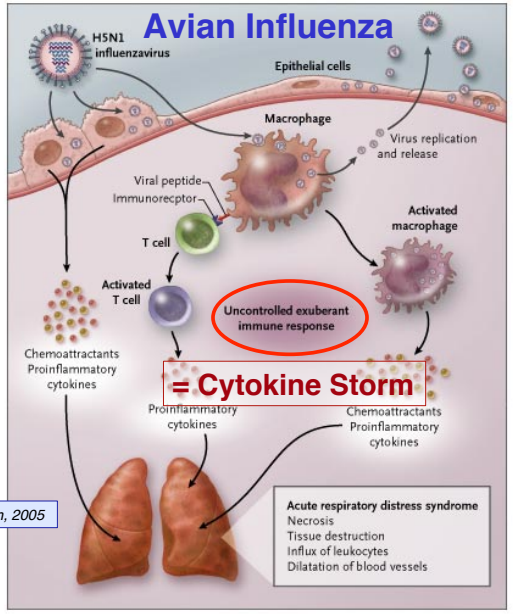
Cytokines

- 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

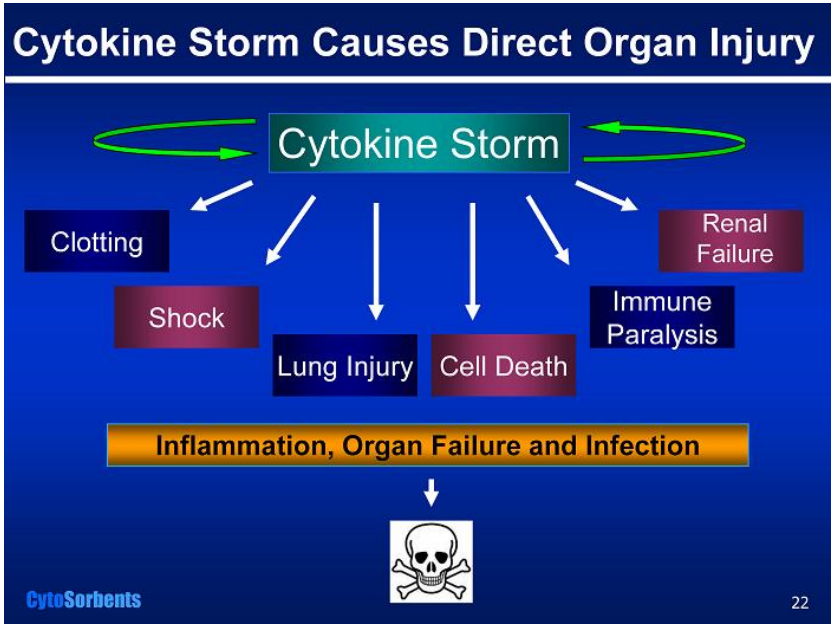


Dean, 2001

Avian Influenza



Osterholm, 2005

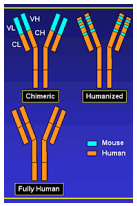


Cytokine Storms (Hypercytokinemia) are Central to Many Lethal Infections

- **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

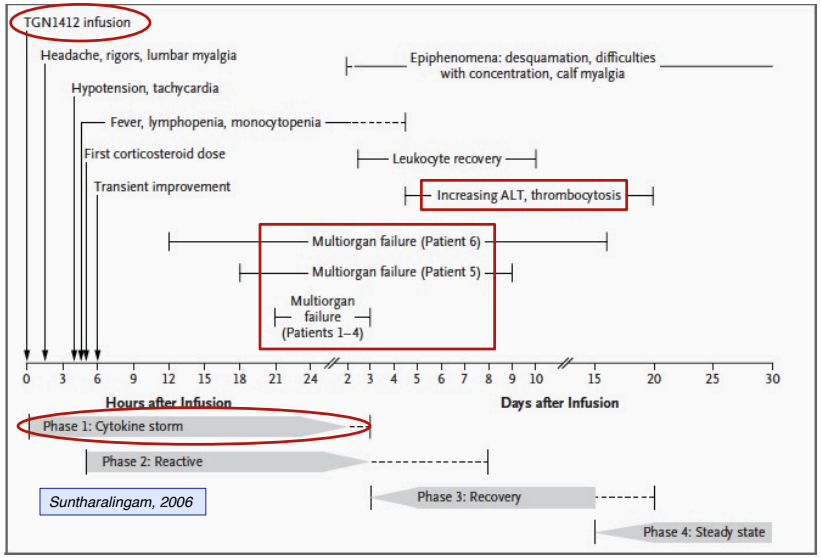
- **8 healthy male subjects, 19 to 34 yr**
  - **6 received TGN1412**
  - **2 received placebo (saline)**
- **Infusions lasted 3 to 6 min**
  - **0.1 mg/kg body weight**
  - **2 mg/min**
- **Clinical measurements began before the infusion and captured the start**

- **Clinical trial did not intend to study Cytokine Storms**
- **Tragic but unprecedented opportunity to track cytokine storms in disease-free patients**

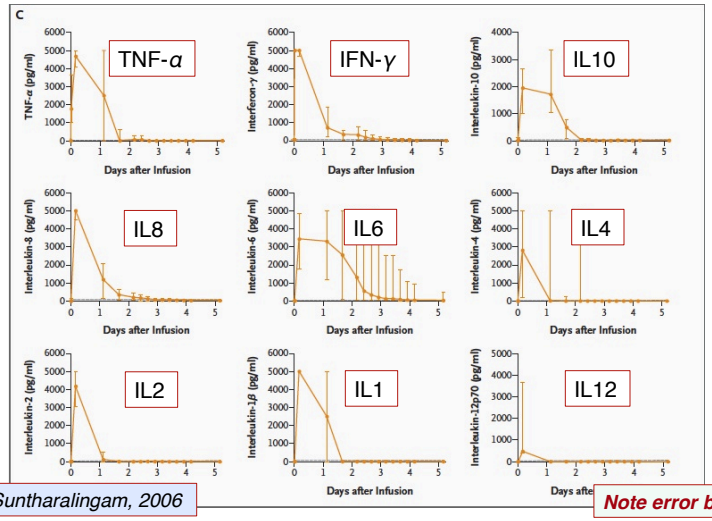
TGN1412 Clinical Trial, 3/13/2006

- **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



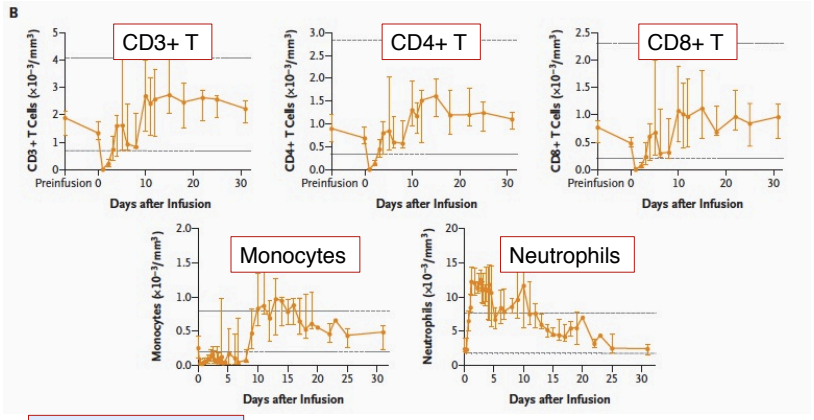
Suntharalingam, 2006

Note error bars

Measurements

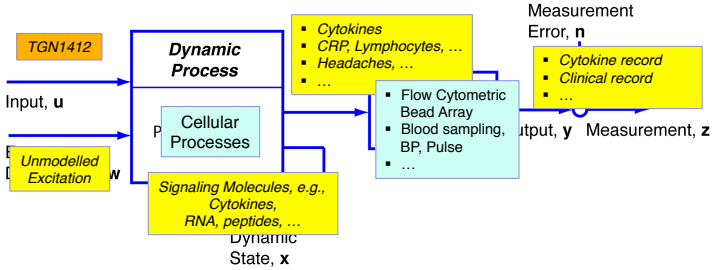
- Normal cytokine ranges: 3.7-48 pg/mL
- Cytometric Bead Array Measurements
  - 5-20% assay accuracy compared to ELISA *(Elshal, McCoy, 2007)*
  - ELISA is 15-30% accurate *(Kristiansen et al, 2002)*
  - Signal saturation at 5,000 pg/mL *(Suntharalingam, 2006)*
- Median estimates for 6 TGN1412 patients 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



Suntharalingam, 2006

What Do We Know?



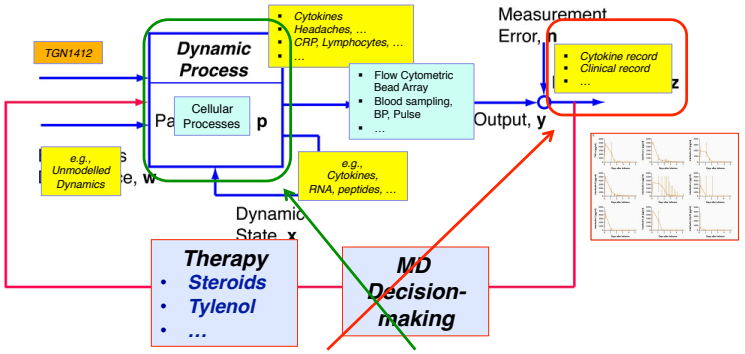
Dynamic Process: Current state depends on prior state

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

Observation Process: Measurement may contain error or be incomplete

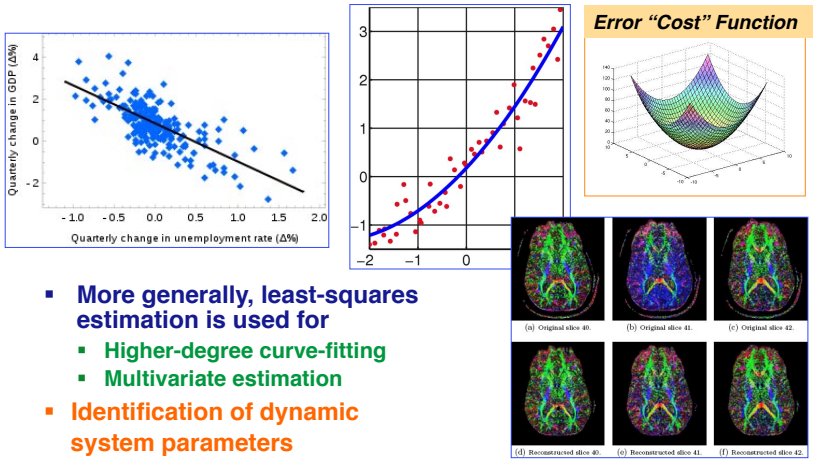
y	**= output (error-free)**
z	**= measurement**
n	**= measurement error**

Dynamic System with Feedback Control



- **Use available information**
- **To identify system dynamics, subject to "feedback" therapy**

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**

2nd-Order Model for Response of an Individual Cytokine

- 2nd-order linear, time-invariant ordinary differential equation
  - 1st –order model inadequate for representation of dynamics
- Two solution variables
  - Cytokine concentration, $x_1(t)$
  - 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*

$$\frac{dx_1(t)}{dt} = \dot{x}_1(t) = x_2(t)$$
$$\frac{dx_2(t)}{dt} = \dot{x}_2(t) = -ax_1(t) - bx_2(t)$$

- Concentration is referenced to basal level
- Initial rate of change is induced by TGN1412 (i.e., ~instantaneous infusion)

$$\begin{bmatrix} x_1(0) \\ x_2(0) \end{bmatrix} = \begin{bmatrix} 0 \\ x_{2_0} \end{bmatrix}$$

2nd-Order Model for Response of Individual Cytokine

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

$$\begin{bmatrix} \dot{x}_1(t) \\ \dot{x}_2(t) \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -a & -b \end{bmatrix} \begin{bmatrix} x_1(t) \\ x_2(t) \end{bmatrix} \qquad \begin{bmatrix} x_1(0) \\ x_2(0) \end{bmatrix} = \begin{bmatrix} 0 \\ x_{2_0} \end{bmatrix}$$

or

$$\dot{\mathbf{x}}(t) = \mathbf{A}\mathbf{x}(t), \quad \mathbf{x}(0) \text{ given}$$

Characteristic Equation and Eigenvalues of the Second-Order Model

$$\Delta(s) \triangleq |s\mathbf{I} - \mathbf{A}| = \begin{vmatrix} s & -1 \\ a & (s+b) \end{vmatrix} = s^2 + bs + a$$
$$= (s-\lambda_1)(s-\lambda_2) = s^2 - (\lambda_1 + \lambda_2)s + \lambda_1\lambda_2 = 0$$

- Consequently

$$\begin{bmatrix} \dot{x}_1(t) \\ \dot{x}_2(t) \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -\lambda_1\lambda_2 & (\lambda_1 + \lambda_2) \end{bmatrix} \begin{bmatrix} x_1(t) \\ x_2(t) \end{bmatrix}$$

$\begin{bmatrix} x_1(0) \\ x_2(0) \end{bmatrix}$ given

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

- Incremental integration via state transition matrix

$$\mathbf{x}(t_{k+1}) = e^{\mathbf{A}\Delta t}\mathbf{x}(t_k) = \mathbf{\Phi}(\Delta t)\mathbf{x}(t_k), \quad \mathbf{x}(0) \text{ given}$$

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

$$\Phi(\Delta t) = \text{Inverse Laplace Transform}\left[(sI - A)^{-1}\right]$$

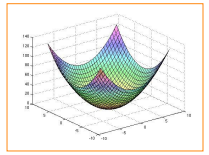
Discrete-Time Model of 2nd-Order System

- Based on eigenvalues of continuous-time system

$$\begin{bmatrix} x_1(t_{k+1}) \\ x_2(t_{k+1}) \end{bmatrix} = \begin{bmatrix} \dfrac{\left(\lambda_1 e^{\lambda_2 \Delta t} - \lambda_2 e^{\lambda_1 \Delta t}\right)}{(\lambda_1 - \lambda_2)} & \dfrac{\left(e^{\lambda_1 \Delta t} - e^{\lambda_2 \Delta t}\right)}{(\lambda_1 - \lambda_2)} \\ \dfrac{\lambda_1 \lambda_2 \left(e^{\lambda_2 \Delta t} - e^{\lambda_1 \Delta t}\right)}{(\lambda_1 - \lambda_2)} & \dfrac{\left(\lambda_1 e^{\lambda_1 \Delta t} - \lambda_2 e^{\lambda_2 \Delta t}\right)}{(\lambda_1 - \lambda_2)} \end{bmatrix} \begin{bmatrix} x_1(t_k) \\ x_2(t_k) \end{bmatrix}$$

where $a = -\lambda_1 \lambda_2$ $b = \lambda_1 + \lambda_2$ $\Delta t = 6$ hr $\begin{bmatrix} x_1(0) \\ x_2(0) \end{bmatrix}$ given

Error Cost Function for Parameter Identification

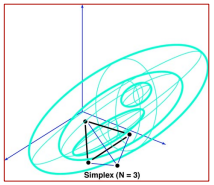


- Squared error of difference between measurements and model's estimates of cytokine concentration

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

$z(t_k)$: **Measurement data set**

$x_1(t_k)$: **Concentration estimate propagated by discrete-time model**



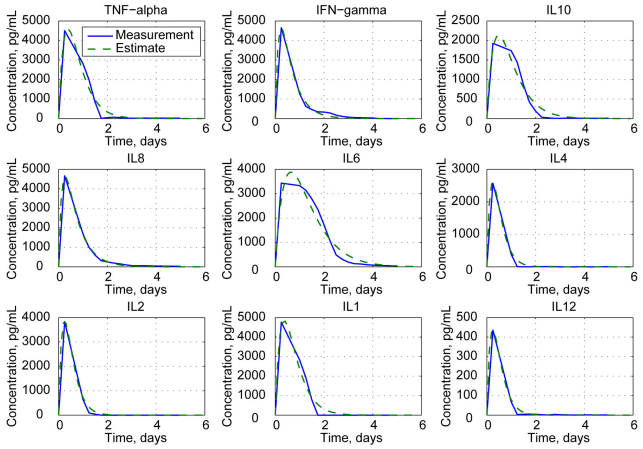
Gradient-Free Search for Parameter Identification

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

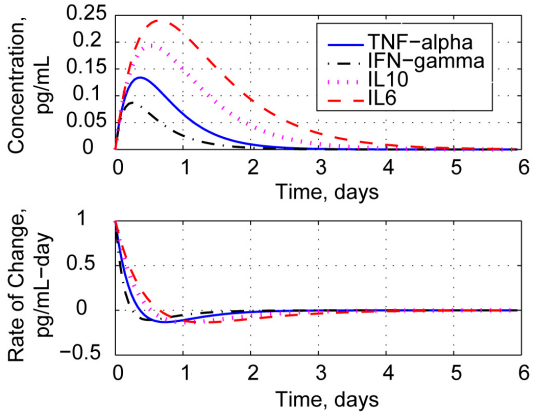
$$\min_{a,b,x_2(t_0)} J = \min_{a,b,x_2(t_0)} \sum_{k=0}^{20} \left[z(t_k) - x_1(t_k) \right]^2$$

using Nelder-Mead (Downhill Simplex) algorithm [MATLAB's *fminsearch*]

Comparison of Median Cytokine Histories and 2nd-Order Responses



2nd-Order Models of Response to Unit Initial Rates of Change

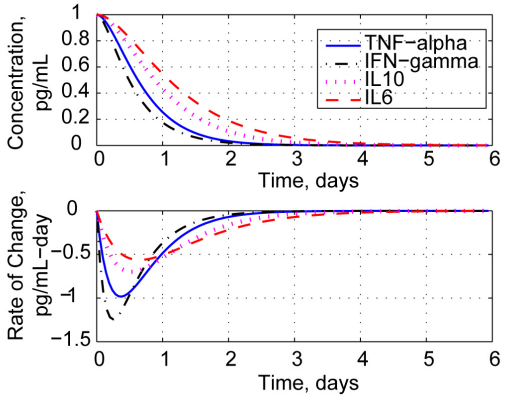


$x_1(t)$

$x_2(t)$

- **Same response shapes as experimental data**

2nd-Order Models of Response to Unit Initial Concentrations



$x_1(t)$

$x_2(t)$

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

Eigenvalues (λ_1, λ_2), Time Constants (τ_1, τ_2), Periods (P), Damping Ratios (ζ), and Initial Rates of Separate 2nd-Order Models

Component	λ_1, d^{-1}	λ_2, d^{-1}	τ_1, d	τ_2, d	P, d	ζ, -	$x_2(0)$, pg/mL-d
TNF-α	-2.63	-2.63	0.38	0.38	2.39	1	32821
IFN-γ	-7.21	-2.05	0.14	0.49	1.63	1.2	55328
IL10	-2.08	-2.08	0.48	0.48	3.02	1	12047
IL8	-6.71	-1.84	0.15	0.54	1.79	1.22	50804
IL6	-1.55	-1.55	0.65	0.65	4.05	1	16437
IL4	-4.17	-4.17	0.24	0.24	1.51	1	29489
IL2	-4.08	-4.08	0.25	0.25	1.54	1	42780
IL1	-2.71	-2.71	0.37	0.37	2.32	1	35535
IL12	-4.13	-4.13	0.24	0.24	1.52	1	4947

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

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

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

$$= \begin{bmatrix} TNF_{\alpha(t)} & \dfrac{d\left[TNF_{\alpha(t)}\right]}{dt} & IFN_{\gamma(t)} & \dfrac{d\left[IFN_{\gamma(t)}\right]}{dt} & \cdots \end{bmatrix}^T$$

$$\mathbf{A} = \left[\begin{array}{cc|cc|c|cc} 0 & 1 & 0 & 0 & \cdots & 0 & 0 \\ a_{2,1} & a_{2,2} & 0 & 0 & \cdots & 0 & 0 \\ \hline 0 & 0 & 0 & 1 & \cdots & 0 & 0 \\ 0 & 0 & a_{4,1} & a_{4,1} & \cdots & 0 & 0 \\ \hline \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ \hline 0 & 0 & 0 & 0 & \cdots & 0 & 1 \\ 0 & 0 & 0 & 0 & \cdots & a_{18,17} & a_{18,18} \end{array}\right] \triangleq \mathbf{A}_{UC}$$

Discrete-Time 18th-Order Model

- Propagation equation and initial conditions

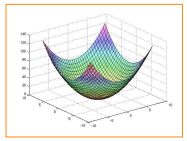
$$\mathbf{x}(t_{k+1}) = \mathbf{\Phi}(\Delta t)\mathbf{x}(t_k), \quad k = 0,20$$

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

- State transition matrix

$$\mathbf{\Phi}(\Delta t) = e^{\mathbf{A}\Delta t} = \begin{bmatrix} \phi_{1,1} & \phi_{1,2} & \cdots & 0 \\ \phi_{2,1} & \phi_{2,2} & \cdots & 0 \\ \cdots & \cdots & \cdots & \cdots \\ 0 & 0 & \cdots & \phi_{18,18} \end{bmatrix}$$

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



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

$$J = \sum_{k=0}^{20} \boldsymbol{\varepsilon}^T(t_k)\mathbf{Q}\boldsymbol{\varepsilon}(t_k) = \sum_{k=0}^{20} \left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right]^T \mathbf{Q}\left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right]$$

where

$$q_{ii} = \frac{1}{\sum_{k=0}^{20} z_i^2(t_k)}, \quad i = 1,9$$

$$\mathbf{x}_c = \begin{bmatrix} x_1 & x_3 & \cdots & x_{17} \end{bmatrix}^T \quad (9\times 1)$$

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

18th-Order Stability Matrix with Concentration Coupling

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

$$\mathbf{A} = \left[\begin{array}{cc|cc|c|cc} 0 & 1 & 0 & 0 & \cdots & 0 & 0 \\ a_{2,1} & a_{2,2} & a_{2,3} & 0 & \cdots & a_{2,17} & 0 \\ \hline 0 & 0 & 0 & 1 & \cdots & 0 & 0 \\ a_{4,1} & 0 & a_{4,3} & a_{4,4} & \cdots & a_{4,17} & 0 \\ \hline \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ \hline 0 & 0 & 0 & 0 & \cdots & 0 & 1 \\ a_{18,1} & 0 & a_{18,3} & 0 & \cdots & a_{18,17} & a_{18,18} \end{array}\right] \triangleq \mathbf{A}_C$$

- Reasonable to assume that off-diagonal blocks are small

Parameter Estimates for Coupled 18th-Order Model

- Downhill-Simplex minimization of

$$J = \sum_{k=0}^{20} \left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right]^T \mathbf{Q}\left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right]$$

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

- Regularize error cost function to keep off-diagonal parameters, $\mathbf{p}_C$, small

$$J = \sum_{k=0}^{k_f} \left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right]^T \mathbf{Q}\left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right] + r_C \mathbf{p}_C^T \mathbf{p}_C$$

- 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

$$J = \sum_{k=0}^{k_f} \left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right]^T \mathbf{Q}\left[\mathbf{z}(t_k) - \mathbf{x}_c(t_k)\right] + r_C \mathbf{p}_C^T \mathbf{p}_C + r_T \left[\mathrm{Tr}(\mathbf{A}_{UC}) - \mathrm{Tr}(\mathbf{A}_C)\right]^2$$

$$Tr(\mathbf{A}_C) = \sum_{i=1}^{9} a_{2i,2i} = sum(-5.2,-8.6,-4.4,-8.0,-3.3,-8.1-8.0,-5.5,-8.8,)$$

- Error cost is reduced by an additional 1%

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

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

	Mode	λ, d^{-1}	*P*, d	ζ, -	EV #1	EV #2	EV #3
	1	–0.84	-	-	IL10	IL6	IL8
	2	–1.4 ± j0.75	3.93	0.89	IL6	TNF	IL10
+	3	–1.88	-	-	IL8	TNF	IL1
+	4	–2.27 ± j0.61	2.66	0.97	IL1	IL8	IFN
	5	–3.28 ± j0.60	1.89	0.98	IL1	IL10	IFN/IL4
+	6	–3.22 ± j0.98	1.86	0.96	IL1	IL4	TNF
	7	–3.75	-	-	IL10	IL12	TNF
	8	–4.02 ± j0.20	1.56	0.99	IL4	IL12	IL2
	9	–4.41 ± j0.71	1.40	0.99	IL4	IL12	IFN/IL8
	10	–5.29 ± j0.82	1.17	0.99	IL8	IFN	IL12
	11	–5.82	-	-	IL8	IFN	IL12

+: Pro-inflammatory; others are mixed

Concentration Coefficients of the Coupled 18th-Order Model

- Odd columns and even rows of A

	TNF	IFN	IL10	IL8	IL6	IL4	IL2	IL1	IL12
TNF"	-6.413	0.345	-0.383	-0.186	-0.632	-0.680	-0.206	0.672	-0.818
IFN"	-0.554	-18.641	0.078	1.576	1.542	0.128	0.184	0.696	-0.903
IL10"	-0.487	0.846	-3.320	0.145	-0.727	-0.111	-0.030	-0.017	0.617
IL8"	0.992	-0.207	1.566	-13.571	0.058	-0.823	-0.316	0.046	-3.356
IL6"	0.412	-1.688	-0.303	0.042	-2.784	0.640	0.769	0.955	0.065
IL4"	-1.129	-1.072	-0.278	0.271	0.101	-16.305	0.776	0.778	-0.237
IL2"	-0.503	-0.775	0.422	0.506	-0.242	-0.022	-15.226	-0.181	-0.957
IL1"	0.053	-0.090	-0.376	0.891	-0.575	0.227	0.289	-7.571	0.604
IL12"	-0.877	-0.075	0.275	-0.228	0.320	0.343	1.554	-0.271	-19.448

- 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

Net Coupling Effect

$$C_i = \left| \frac{\sum_{j=1}^{9} a_{2i,2j-1} - a_{2i,2i-1}}{a_{2i,2i-1}} \right| \times 100\,(\%), \quad i = 1,9$$

Receiver	Percent
TNF	5%
IFN	1%
IL10	2%
IL8	1%
IL6	12%
IL4	0%
IL2	1%
IL1	2%
IL12	1%

Gross Coupling Effect

$$C_i = \frac{\sum_{j=1}^{9} \left|a_{2i,2j-1}\right| - \left|a_{2i,2i-1}\right|}{\left|a_{2i,2i-1}\right|} \times 100\,(\%), \quad i = 1,9$$

Receiver	Percent
TNF	61%
IFN	30%
IL10	90%
IL8	54%
IL6	175%
IL4	28%
IL2	24%
IL1	41%
IL12	20%

Cytokines That Drive Coupling

- Column-wise comparison of coupling coefficients to self coefficient

Net Coupling Effect

$$C_j = \left| \frac{\sum_{i=1}^{9} a_{2i,2j-1} - a_{2i,2i-1}}{a_{2i,2i-1}} \right| \times 100\,(\%), \quad j = 1,9$$

Effecter	Percent
TNF	5%
IFN	1%
IL10	9%
IL8	2%
IL6	2%
IL4	0%
IL2	1%
IL1	5%
IL12	1%

Gross Coupling Effect

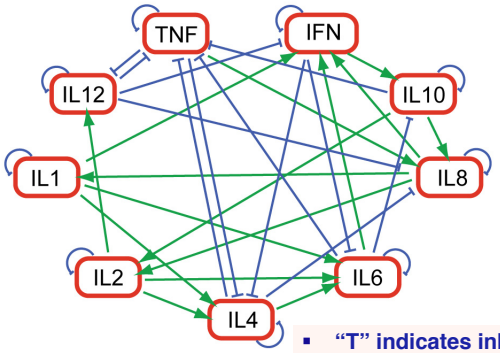
$$C_j = \frac{\sum_{i=1}^{9} \left| a_{2i,2j-1} \right| - \left| a_{2i,2i-1} \right|}{\left| a_{2i,2i-1} \right|} \times 100\,(\%), \quad j = 1,9$$

Effecter	Percent
TNF	78%
IFN	27%
IL10	111%
IL8	28%
IL6	151%
IL4	18%
IL2	27%
IL1	48%
IL12	39%

- Implications for control (i.e., treatment)

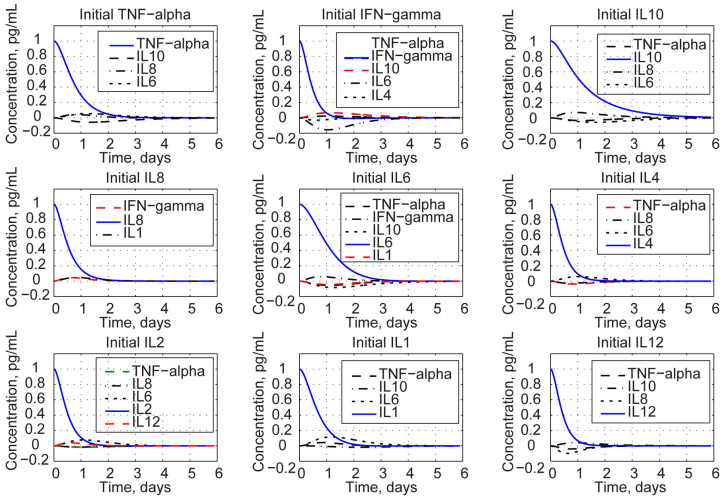
Most Significant Cytokine Interactions

(from concentration coupling matrix)



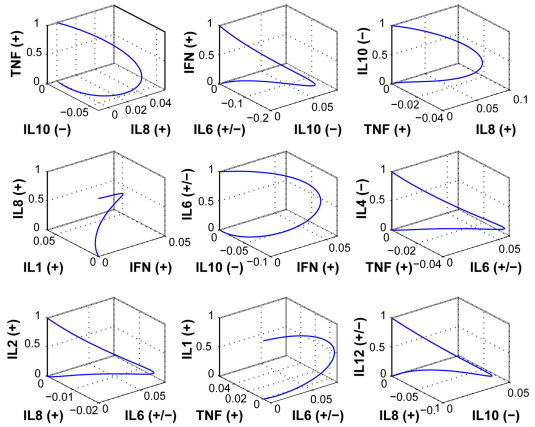
- "T" indicates inhibition
- "->" indicates excitation

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**

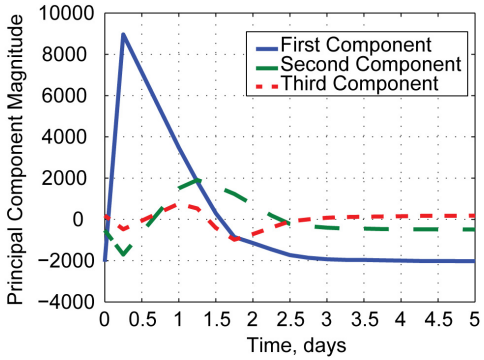
$$\mathbf{Z} = \mathbf{z}(t_k)\mathbf{z}^T(t_k)$$

- **Singular-Value Decomposition of Z produces the Principal Components**

$$\mathbf{y}(t_k) = \mathbf{C}\mathbf{z}(t_k), \quad k = 0, k_f$$

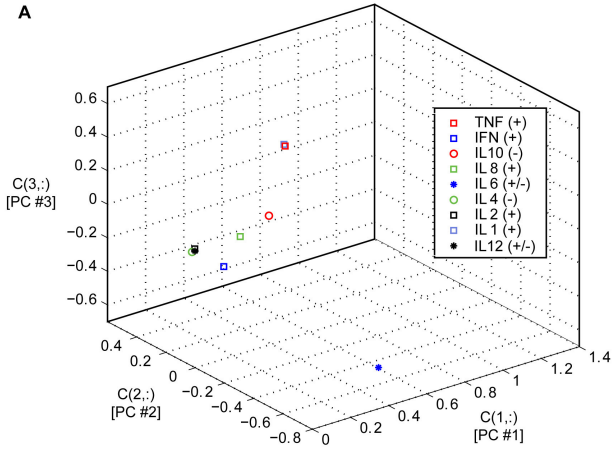
- **Principal components identify similarity but not causality**

Shapes of Three Most Significant Principal Components



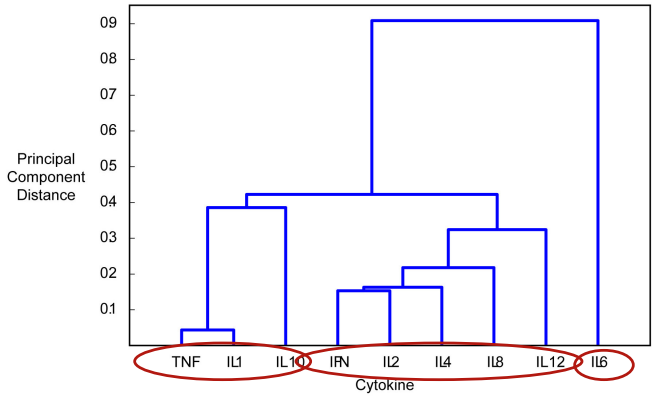
99% explanation of measured wave shapes in 1st 3 components

Coefficients of the 1st Three Principal Components



Dendrogram Identifies Three Cytokine Clusters By Distance

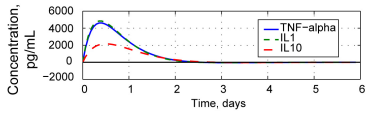
- **Principal Component Analysis identifies similarity in wave forms without regard to causality**



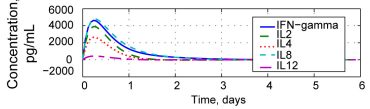
Modeled Responses for Three Cytokine Clusters

- **Groupings suggested by dendrogram identify similar responses**

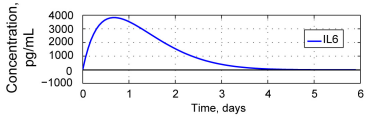
Group A



Group B

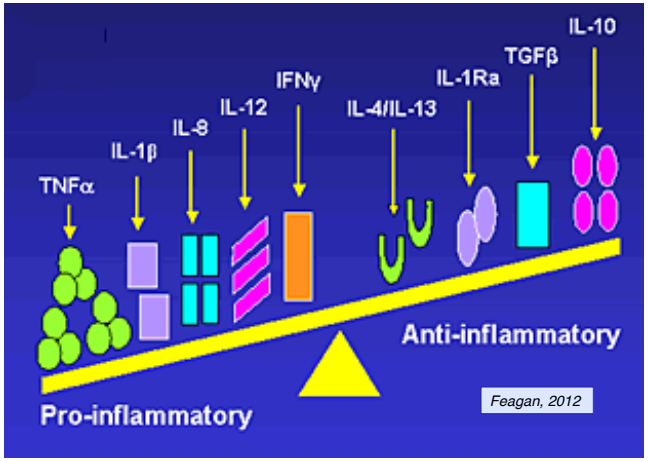


Group C



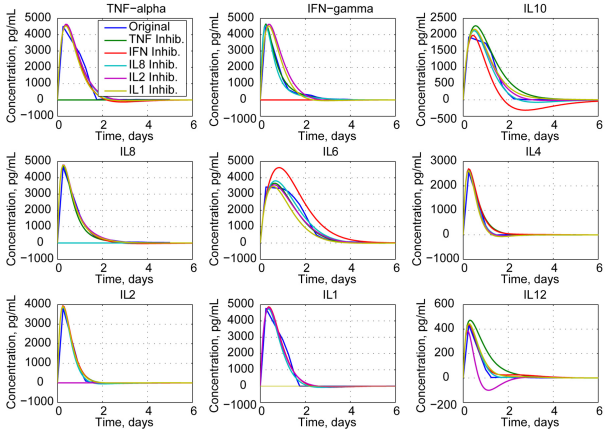
- **Consistent with trends suggested by Tisoncik et al, 2012**

Pro- and Anti-Inflammatory Cytokines

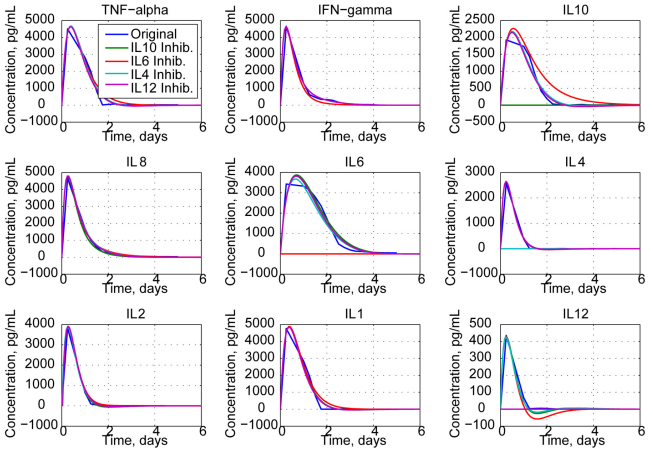


Effects of Inhibiting Pro-Inflammatory Cytokines

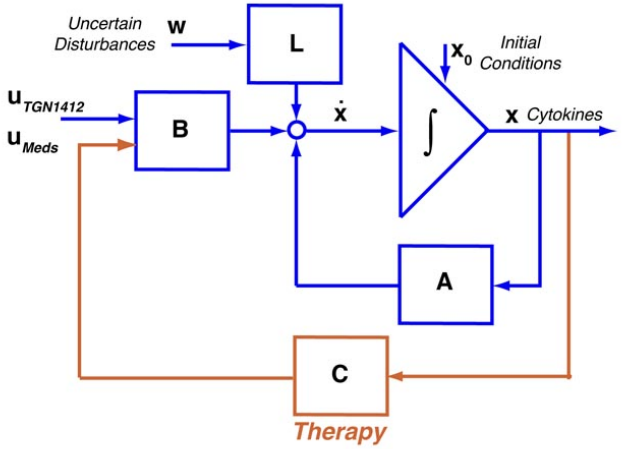
- **Respective rows of A set to zero**
- **Remaining cytokine responses computed as before**

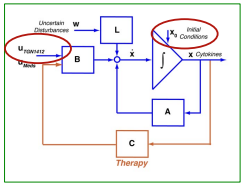


Effects of Inhibiting Anti- and Mixed Inflammatory Cytokines



Overview of Linear Dynamic Model with External Forcing





Linear Dynamic Model with External Forcing

- **Model with TGN1412 effect subsumed in initial condition**

$$\dot{\mathbf{x}}(t) = \mathbf{A}_{estimated}\mathbf{x}(t) \triangleq (\mathbf{A} + \mathbf{B}_2\mathbf{C})\mathbf{x}(t), \quad \mathbf{x}(0) \text{ estimated}$$

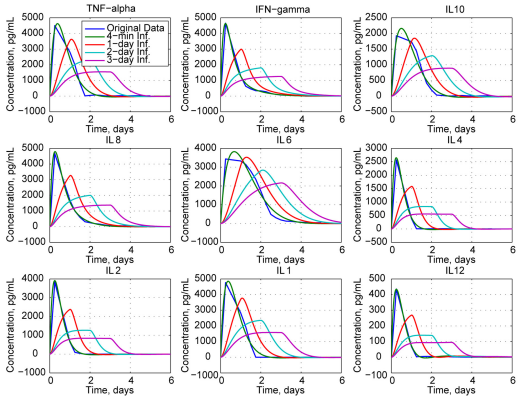
- **Model with TGN1412 effect as constant input for short period**

$$\dot{\mathbf{x}}(t) = \mathbf{A}_{estimated}\mathbf{x}(t) + \mathbf{B}_1\mathbf{u}_{TGN1412}(t), \quad \mathbf{x}(0) = \mathbf{0}$$

$$\mathbf{B}_1 = (2880[mg/d]/8[mg])\mathbf{x}(0) = 360\mathbf{x}(0)$$

$\mathbf{B}_2$ & $\mathbf{C}$ indeterminate without additional information

Estimated Effects of TGN1412 Infusion Duration



- **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**

Evaluation of Uncertainty on Cytokine Response

Mean Value Vector

$$\bar{\mathbf{x}}(t) \triangleq E[\mathbf{x}(t)] = \int_{-\infty}^{\infty} \mathbf{x}\, \mathrm{pr}(\mathbf{x})\, d\mathbf{x}$$

Covariance Matrix

$$\mathbf{P}(t) \triangleq E\left\{[\mathbf{x}(t) - \bar{\mathbf{x}}(t)][\mathbf{x}(t) - \bar{\mathbf{x}}(t)]^T\right\}$$

$$= \int_{-\infty}^{\infty} [\mathbf{x} - \bar{\mathbf{x}}][\mathbf{x} - \bar{\mathbf{x}}]^T\, \mathrm{pr}(\mathbf{x})\, d\mathbf{x}$$

Square roots of diagonal elements are cytokine standard deviations

Evolution of the Mean State Vector

Continuous-Time Model

$$E\left[\dot{\mathbf{x}}(t)\right] = E\left[\mathbf{A}\bar{\mathbf{x}}(t)\right] = \mathbf{A}E\left[\mathbf{x}(t)\right]$$

$$\triangleq \dot{\bar{\mathbf{x}}}(t) = \mathbf{A}\bar{\mathbf{x}}(t) \qquad E\left[\mathbf{x}(t)\right] = \bar{\mathbf{x}}(0) \text{ given}$$

Discrete-Time Model

$$\bar{\mathbf{x}}(t_{k+1}) = e^{\mathbf{A}\Delta t}\bar{\mathbf{x}}(t_k) = \boldsymbol{\Phi}(\Delta t)\bar{\mathbf{x}}(t_k)$$

Propagation of the State Covariance Matrix from Initial Condition

$$\mathbf{P}(0) \text{ given}$$

$$E\left\{\left[\mathbf{x}(t_{k+1}) - \bar{\mathbf{x}}(t_{k+1})\right]\left[\mathbf{x}(t_{k+1}) - \bar{\mathbf{x}}(t_{k+1})\right]^T\right\}$$

$$= \boldsymbol{\Phi}(\Delta t)E\left\{\left[\mathbf{x}(t_k) - \bar{\mathbf{x}}(t_k)\right]\left[\mathbf{x}(t_k) - \bar{\mathbf{x}}(t_k)\right]^T\right\}\boldsymbol{\Phi}^T(\Delta t)$$

$$\triangleq \mathbf{P}(t_{k+1}) = \boldsymbol{\Phi}(\Delta t)\mathbf{P}(t_k)\boldsymbol{\Phi}^T(\Delta t)$$

- **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** $\mathbf{P}(0) = \mathbf{0}$
- **Focus on exogenous effects**

$$\mathbf{P}(t_{k+1}) = \boldsymbol{\Phi}(\Delta t)\mathbf{P}(t_k)\boldsymbol{\Phi}^T(\Delta t) + \mathbf{W}(t_k)$$

where

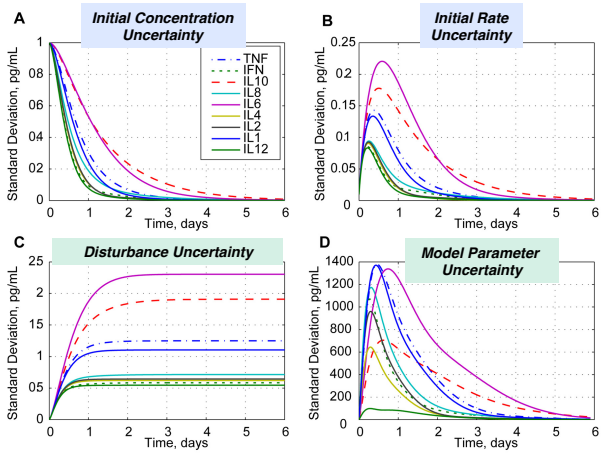
$$\mathbf{W}(t_k) = \mathbf{L}(t_k)\mathbf{W}_D\mathbf{L}^T(t_k)\Delta t$$

$\mathbf{W}_D$: Covariance matrix of exogenous disturbance

$\mathbf{L}(t_k)$: Disturbance-effect matrix for continuous model

$\Delta t = 0.01$ days for calculation

Effects of Uncertainty on Cytokine Concentration Standard Deviation



Cellular-Cytokine Associations

(from the literature)

Mixed

	Group A			Group B					Group C
	TNF-α	IL1	IL10	IFN-γ	IL2	IL4	IL8	IL12	IL6
Innate System									
Monocyte	S	S	S	R	R		R		S, R
Macrophage	S, R	S, R	S, R	R		R	S, R	S	S
Dendritic Cell	S, R	S	S, R	S, R	S	R	S	S	
Mast Cell	S	S, R	S	S	S	S	S, R		S
Neutrophil	S, R	S, R	R			S	S, R	S	S, R
Eosinophil	S	S, R	S	S		S, R		S	S
Basophil	S	S, R		R		S			S
NK	S, R	S, R	S, R	S	S, R	S, R		R	
Adaptive System									
B	R	S, R	S, R	S, R	R	S, R		S, R	S, R
Th1	S, R	S, R	S, R	S, R	S, R	S, R		S, R	S, R
Th2	S, R	S, R	S, R	S, R	S, R	S, R		S, R	S, R
CTL	S, R	S, R	S, R	S, R	S, R	S		S, R	S, R
Other									
Fibroblast	S, R	S, R		R			S		S
Epithelial Cell	S	S	S	R			S, R		S, R
Endothelial Cell	S, R	S, R		R			S, R		S, R
Smooth Muscle	S, R	S, R		R		S	S		S, R
Adipose Tissue	S, R	S							S, R

Cell 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

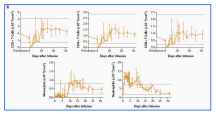
Discussion

Analytical Results

- Cytokine coupling effects are well-portrayed by the linear model
- 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

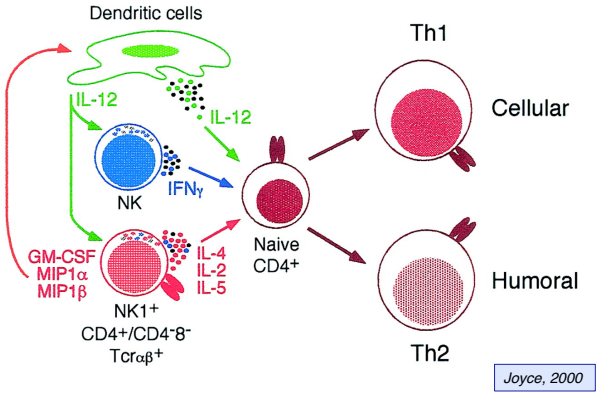
- Dynamic modeling of temporal data provides new insights into cytokine response
- Early, synchronized measurements are important
  - Know the start time for stimulus and immune response
  - Make closely spaced measurements during the first 48 hr of response
- Practical value in linear modeling
- 2nd-order 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

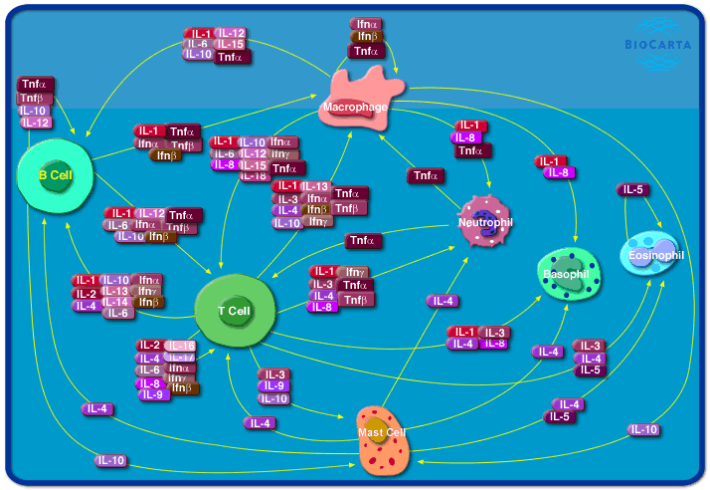
- Hao H. Yiu, currently Staff Engineer, Integra Life Sciences
- Andrea L. Graham, Assistant Professor, Ecology and Evolutionary Biology, Princeton University

Supplemental Material

Cellular Secretion of and Regulation by Cytokines

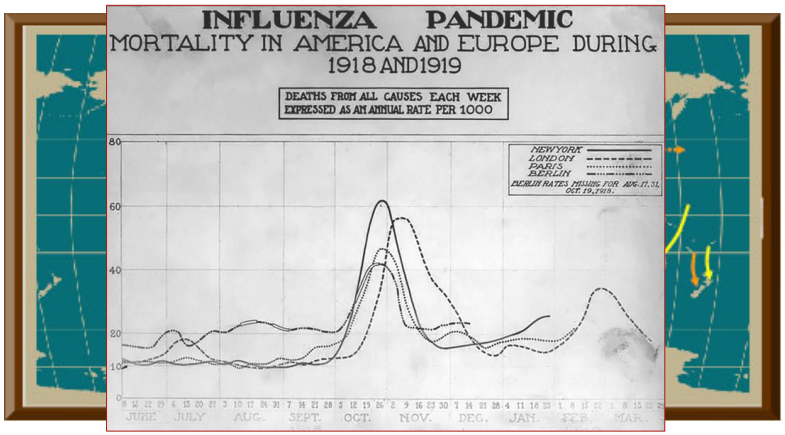


Immune Cell-Cytokine Associations



Signaling pathways derived from diverse experiments

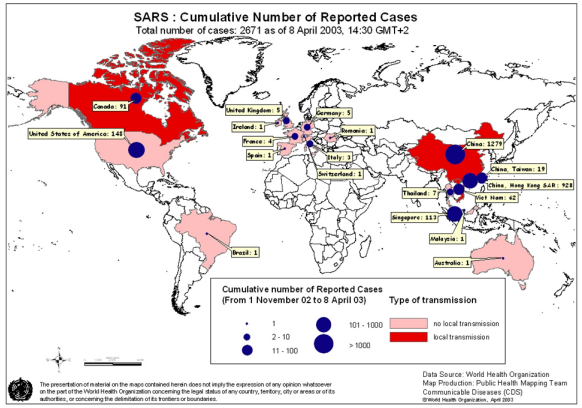
Spanish Flu Pandemic of 1918

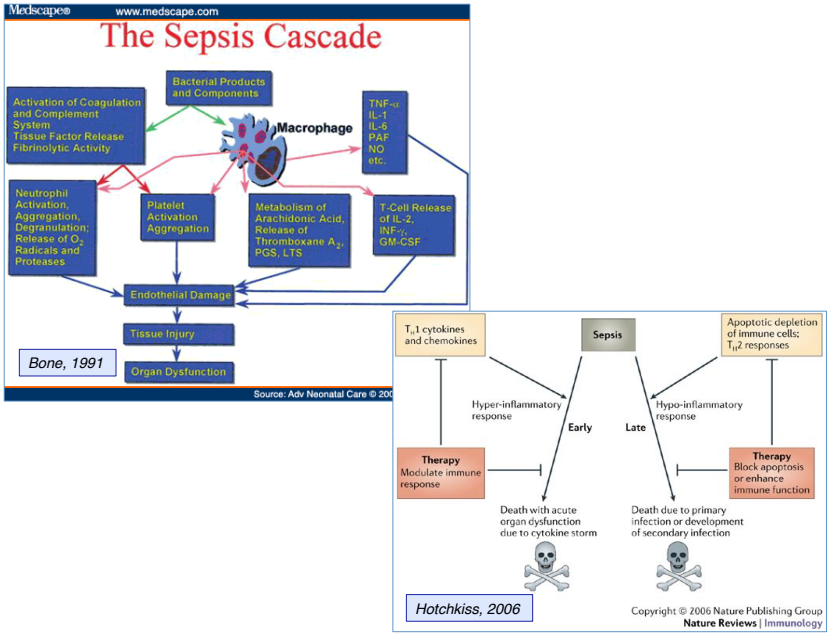


- **500 million cases worldwide**
- **50 to 130 million died**

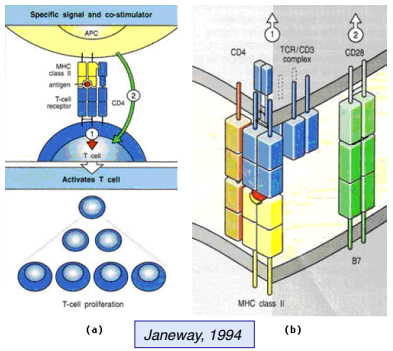
Severe Acute Respiratory Syndrome (SARS) Epidemic of 2003

- 8,422 cases worldwide
- 10.9% mortality



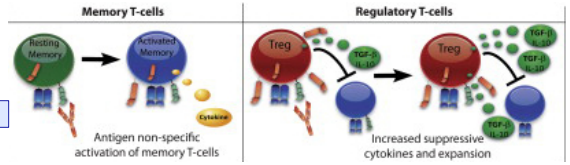


T-Cell Activation



Janeway, 1994

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



Curran, 2012

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 2nd-Order Model

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

$$\left(\lambda_i \mathbf{I} - \mathbf{A}\right)\mathbf{e}_i = 0, \quad i = 1,n$$

Eigenvectors

$$\mathbf{e}_1 = \alpha\begin{bmatrix} 1 \\ \lambda_1 \end{bmatrix}; \quad \mathbf{e}_2 = \alpha\begin{bmatrix} 1 \\ \lambda_2 \end{bmatrix}$$