IL-1 signalling through focal adhesion

Presenters: Q. Wang, D. Rajshankar, C. McCulloch
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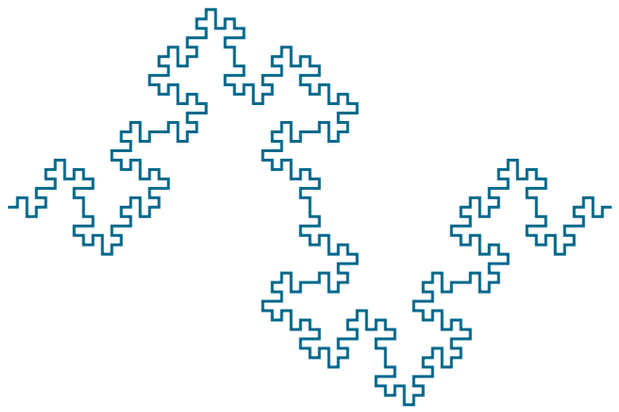
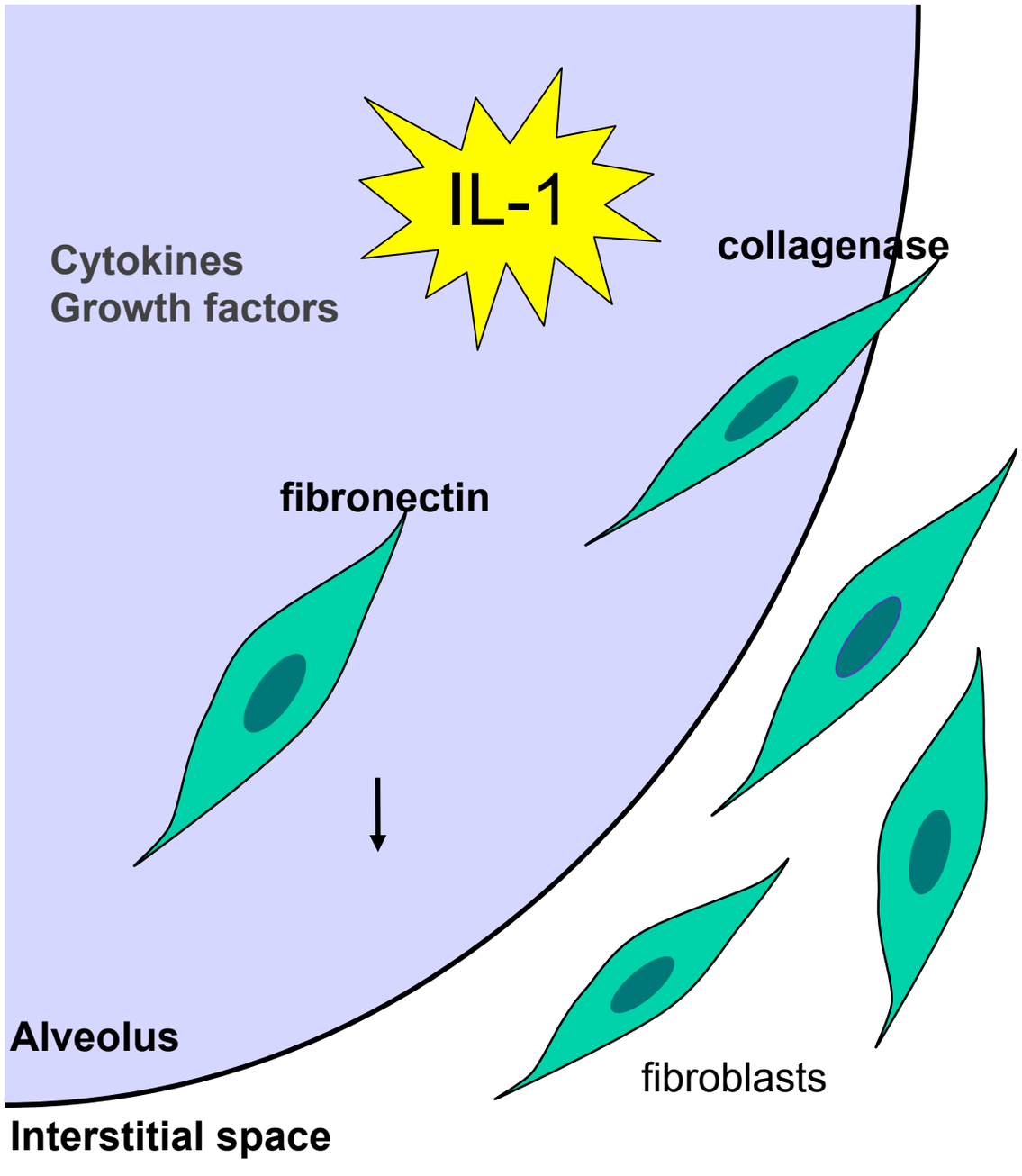


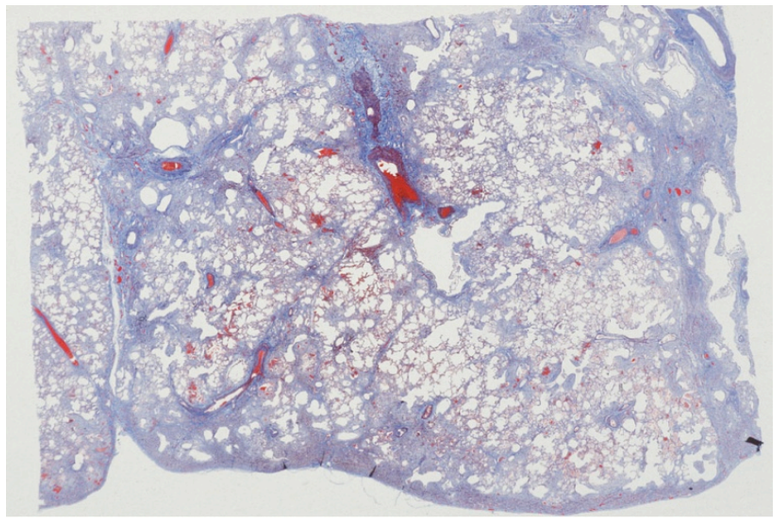
August 22, 2013

Reminder of Monday's presentation



Prolonged and recurrent inflammation

Fibrosis



From C. McCullough's talk

Reminder of Monday's presentation

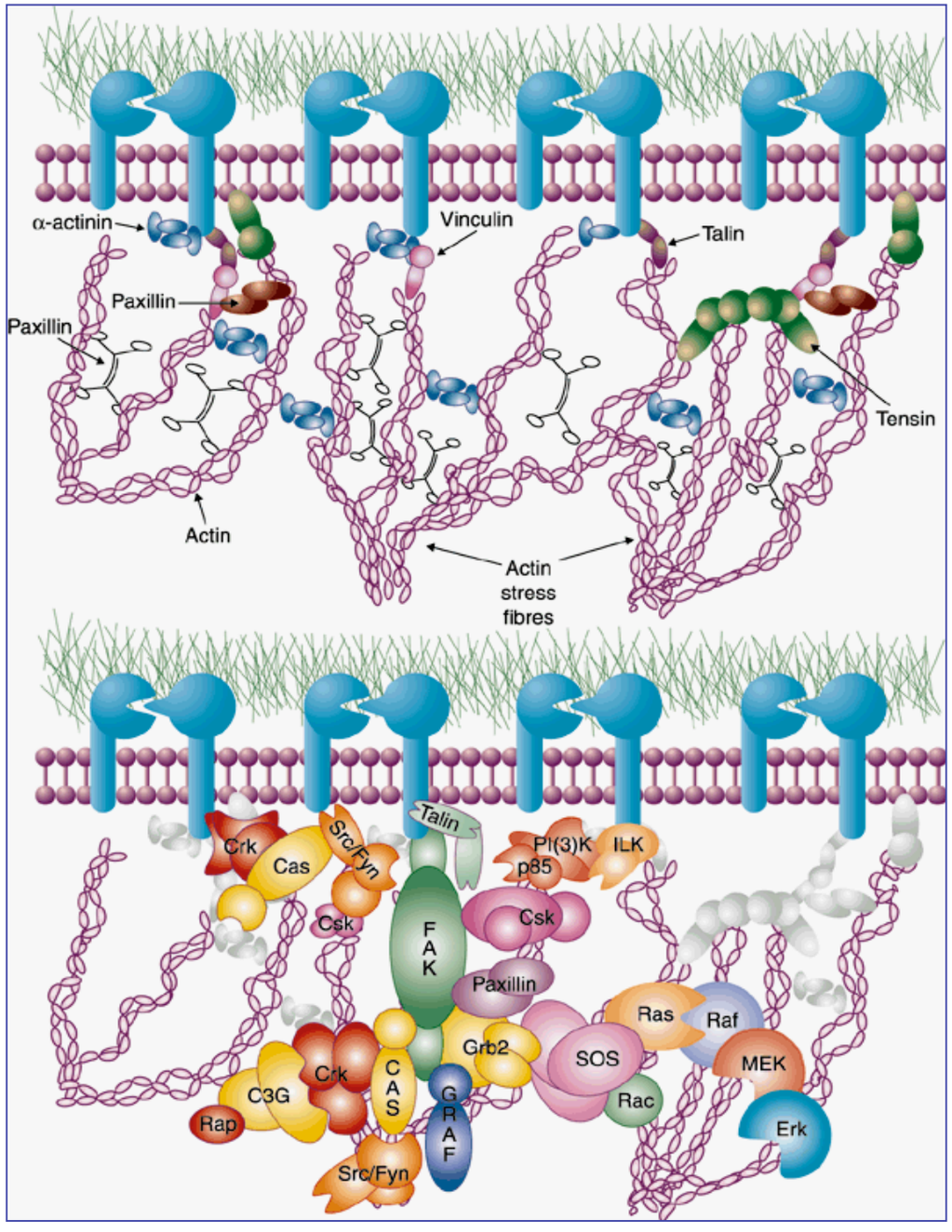
Focal Adhesions

Functions

- -cell adhesion
- -cell spreading
- -cell migration
- -cell signaling
- -Facilitate protein interactions

Composition

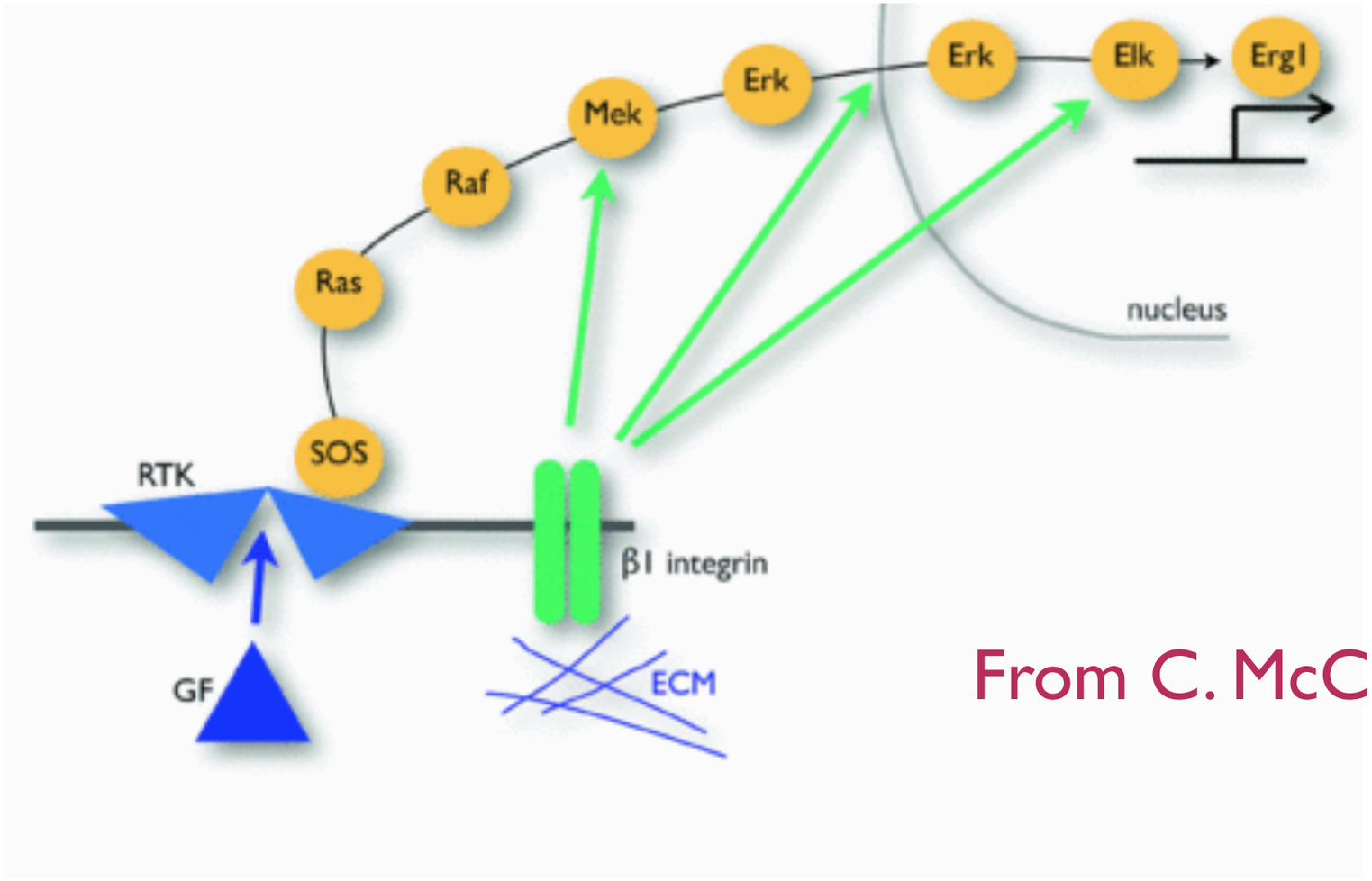
- -integrins
- -structural proteins
- -signaling complex
- -receptors ($IL\text{-}1R_1$)



From C. McCullough's talk

Reminder of Monday's presentation

Concept → **Integrin adhesions organize IL-1 receptor machinery, providing a regulatory locus for cellular responses in inflammatory lesions**



From C. McCullough's talk

Interleukin-1 (IL-1)

- IL-1 help communication and regulating repair processes and repair of injury
- Recurring inflammation if recovery goes wrong
- IL-1 generates signals which promote inflammation
- Stimulates other molecules, enzymes which break down extracellular matrix
- Generates MMPS, ROS, and other factors
- Antagonists (interfere with IL-1) - anakinra interferes with how IL-1 binds to receptors

Focal adhesion

- Fibroblasts stick to collagen substrate
- Contain signalling factors
- In the presence of IL-1 binding to receptors on Fibroblasts, biochemical cascade occurs
- End product of cascade are collagen-cutting factors (MMPs)s

Questions from Chris's presentation

- What are the important molecules in these adhesions?
- Which proteins are most likely to regulate signalling?
- Inhibiting which proteins with drugs is also least likely to interfere with normal cell functioning?

Problem statement and modeling goals

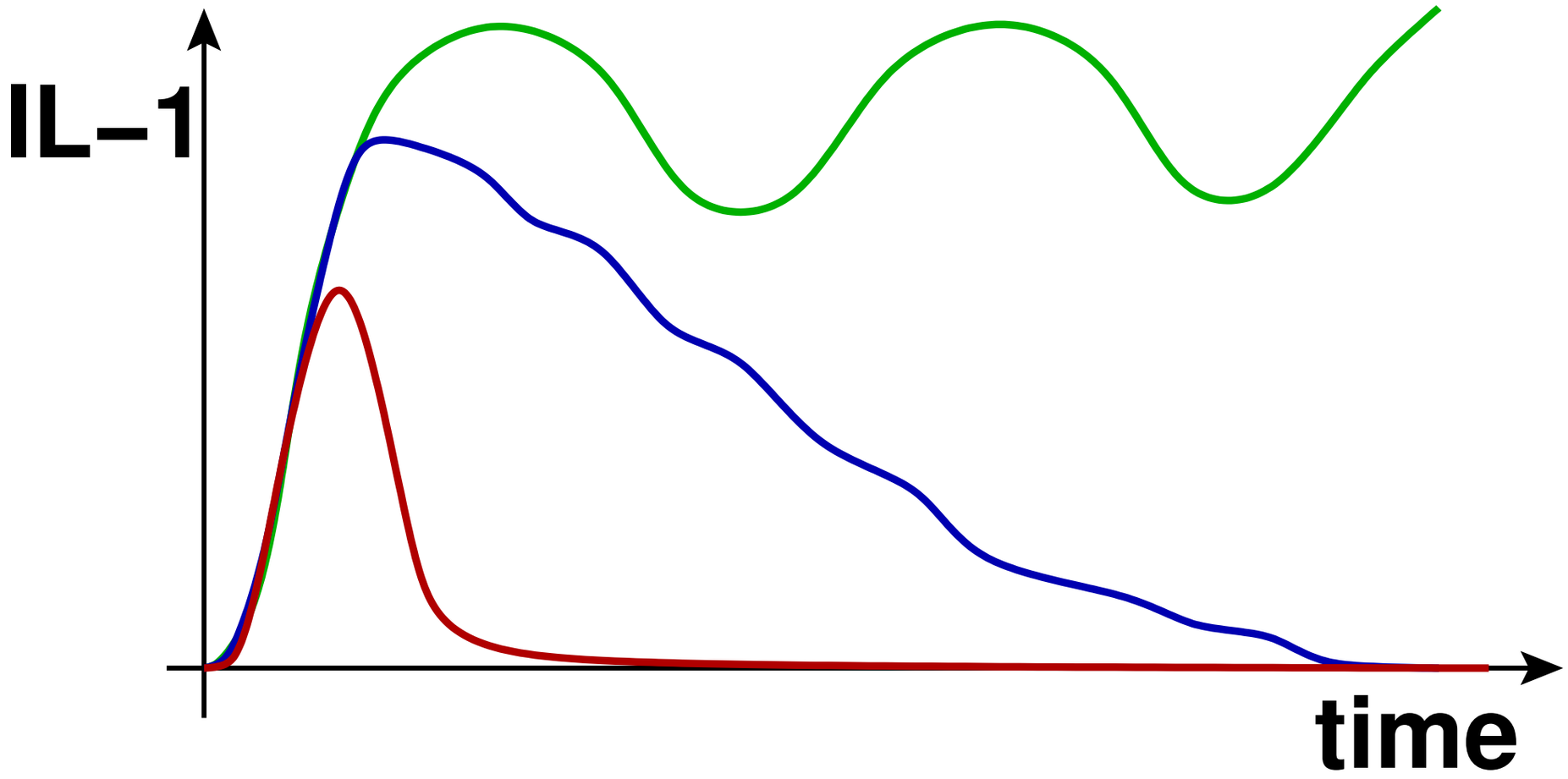
Problem statement

- What is the behaviour of the sites in terms of MMPs and bone loss which would lead to 'aggressive' bone loss?
- What parameters would lead to this behaviour?
- Are there predictive statements we can make, i.e., based on base levels of factors in a patient?

Goals.

- Identify key mechanisms involved in IL-1 levels
- Identify quantifiable differences between normal (physiological) and abnormal (pathological) response to inflammatory stimulus, in vivo, and peridontal connective tissues
- Identify important scales

Physiological v/s Pathological response



Physiological v/s Pathological response

Clinically, pathological response to inflammation is characterised via functional and structural changes to connective tissue

Physiological response to inflammatory event

- An initial spike in IL1 rapidly decreases to normal levels
- Induced change in MMP level tracks the IL1 and bone density does not change at all.

Pathological but *'Stable'* response to inflammatory event

- Reduction of IL1 is on a slower time scale, with oscillatory behaviour
- Bone density decreases, but then stabilizes at $\approx 70\%$
- Fibroblast numbers decrease as collagen degrades, but then eventually stabilizes

Physiological v/s Pathological response, contd.

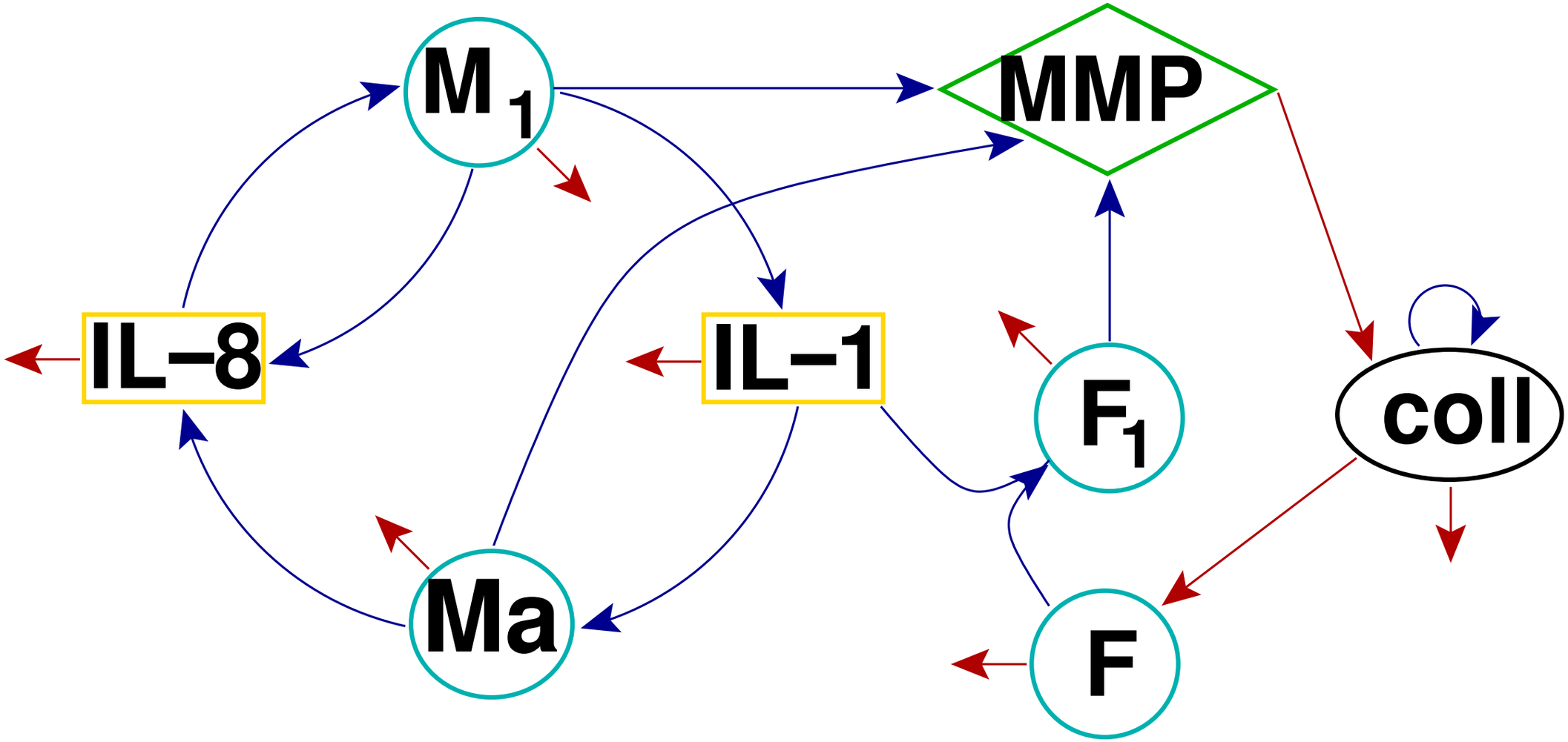
Pathological *'aggressive'* response to inflammatory event

- Mean IL-1 and MMP levels do not decrease from peak levels. Concentrations do oscillate
- Bone density decreases, potentially to 0.
- Fibroblast density also decreases, potentially to 0.

Background and modelling goals

- IL-1: Interleukin-1
- IL-8: Interleukin-8
- MMP: active MMP
- coll: collagen matrix

- F=Fibroblast
- F1: Fibroblast with receptor-bound IL-1
- Ma: Mast cells
- M1: Macrophages



$$\frac{d}{dt}IL1 = -k_1 IL1 - k_2 IL1\, F + k_3 M_1, \quad IL1 = \text{IL1 conc.}$$

$$\frac{d}{dt}IL8 = -k_4 IL8 + k_5 M_{ast} + k_6 M_1 \quad IL8 = \text{IL8 conc.}$$

$$\frac{d}{dt}M_1 = k_8 IL8 - k_9 M_1, \quad M1 = \text{macrophages}$$

$$\frac{d}{dt}M_{ast} = k_{10} IL1 - k + 11 M_{ast}, \quad M_{ast} = \text{mast cells}$$

$$\frac{d}{dt}F = -\underbrace{k_{12} H(IL1)}\, F - k_{13}(\bar{y} - y) \quad F - \tilde{k_2} IL\, F, \text{F=Fibroblasts}$$

$$\frac{d}{dt}F1 = -\underbrace{k_{12} H(IL1)}\, F - k_{13}(\bar{y} - y) \quad F1 - \tilde{k_2} IL\, F1,$$

F1= Fibroblasts with IL-1

$$\frac{d}{dt}MMP = k_{14} F1 - k_{15} MMP + k_{17} M_1, \quad MMP = \text{ MMP}$$

$$\frac{d}{dt}y = -k_{18} MMP + k_{21} - k_{22} y, \text{y=collagen}$$

Initial conditions

- $IL1(0) = 0.$
- $IL8(0) = \bar{IL8}$
- $MMP(0) \approx 0$
- $y(0) = 1$
- $M_{ast}(0) = \bar{M_{ast}}$
- $F(0) = \bar{F}$ where $\bar{F} \approx 10^4 =$ basal number of fibroblasts
- $M_1(0) = \bar{M_1}$

Important scalings

- *Bone density* decreases on the order of months, collagen fibres may degrade faster.
- F cytotoxin-driven death occurs on the order of minutes
- $IL-8$ response is on the order of days
- In the absence of any inflammation, collagen levels saturate to $\bar{y} = \frac{k21}{k22} = 1$.

Some dynamics occur on a time scale of $\frac{1}{\text{bioavailability of interleukins}}$

Nondimensional model: $t \longrightarrow \frac{1}{k_1} t'$. Drop primes.

$$
\begin{aligned}
\frac{d}{dt} IL1 &= -IL1 - \frac{k_2 F_c}{k_1} IL\,F + M_1 \\
\frac{d}{dt} IL8 &= -IL8 + k_5 M_{ast} + k_6 M_1 \\
\frac{d}{dt} M_1 &= \frac{1}{k_1 P_1} IL8 - \frac{k_9}{k_1} M_1 \\
\frac{d}{dt} M_{ast} &= \frac{k_{10} k_3}{k_1^2} IL1 - \frac{k_{11}}{k_1} M_{ast} \\
k_1 \frac{d}{dt} F &= -k_{12} H(IL1 \times IL1_c) F - k_{13}(1-y) F - \tilde{k_2} IL_c IL\,F, \\
k_1 \frac{d}{dt} F1 &= -k_{12} H(IL1 \times IL1_c) F1 - k_{13}(1-y) F1 + \tilde{k_2} IL_c IL\,F1 \\
\frac{d}{dt} MMP &= \frac{k_{14} F_c}{M1_c} F1 - \frac{k_{15}}{k_1} MMP + M_1 \\
k_1 \frac{d}{dt} y &= -\frac{k_{18} k_{17}}{k_1} M1_c MMP + k_{21}(1-y)
\end{aligned}
$$

Stability of the two fixed points

Uninflamed equilibrium:

Collagen $= 1$, Fibroblast $\#$ arbitrary, all other species 0

Linear stability: all real eigenvalues (so far...)

- Zero eigenvalue - in 'F' direction
- Other eigenvalues - negative with baseline parameters

STABLE

Un-biological equilibrium:

Negative amts of collagen and fibroblasts!

Linear stability:

- Zero eigenvalue - in 'F' direction
- Other eigenvalues - some positive real/ complex with positive real part

UNSTABLE

Reduced dynamics with scaled model: 1

Coupling dynamics of $IL-1$ and M_{ast}, we get

$$\ddot{M}_{ast}+\left(\frac{k_{11}+k_1+k_2F_cF}{k_1}\right)\dot{M}_{ast}+\frac{k_{11}}{k_1}\left(\frac{k_1+k_2F_cF}{k_1}\right)M_{ast}=\frac{k_1^2}{k_{10}k_3}M_1$$

$k_{11}\approx 0$.

Damping of Mast cells (which produce IL-1) is on time scale set by: $k_1+k_2F_cF$

Biological interpretation:

How fast the inflammatory response is cleared depends on:

- the number of fibroblasts initially present at the site,
- the removal/binding rates of Interleukin-I.

Reduced dynamics with scaled model: 2

Coupling dynamics of $IL-8$ with macrophages $M1$, we get

$$\ddot{M}_1 + \left(\frac{k_9 + k_1}{k_1}\right)\dot{M}_1 + \left(\frac{k_9 - k_6/P_1}{k_1}\right)M_1 = \frac{k_5}{k_1 P_1} M_{ast}$$

$P_1 = O(1)$.
Damping of M_1 depends on a time scale $\approx \frac{k_9+k_1}{\sqrt{k_1}}$.

Biological interpretation:

How fast the inflammatory response is cleared depends on:

- the removal rates of macrophages.
- the removal rate of IL-1

Reduced dynamics with scaled model: 3

Combining fibroblast and fibroblast+IL-1 (both are adherent) populations, we get

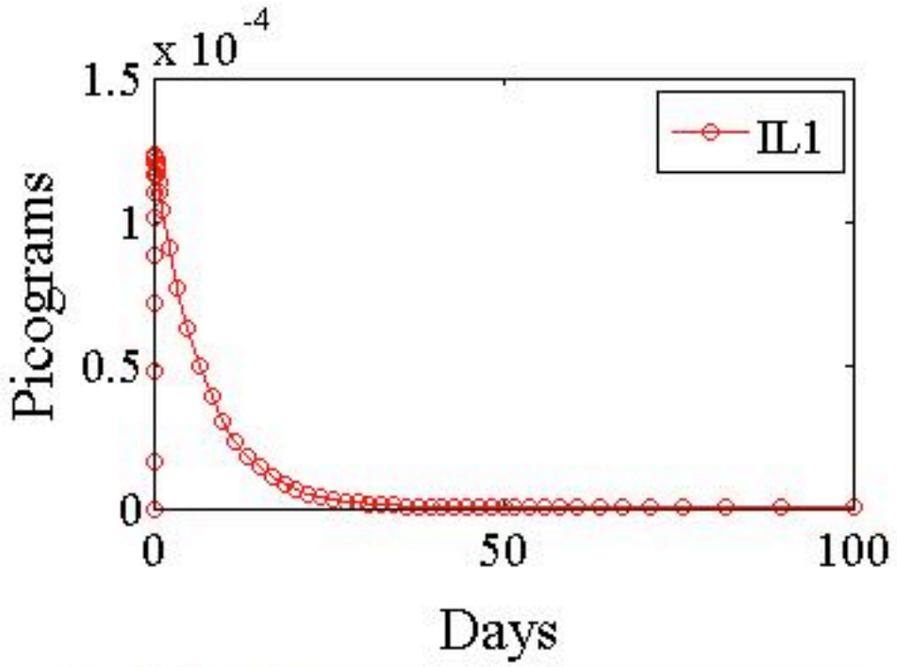
$$k_1 \frac{d}{dt}(F_1 + F) = -k_{12} H(IL_c \times IL)(F_1 + F) - \underbrace{k_{13}(1-y)(F1+F)}_{\text{death due to matrix loss}}$$

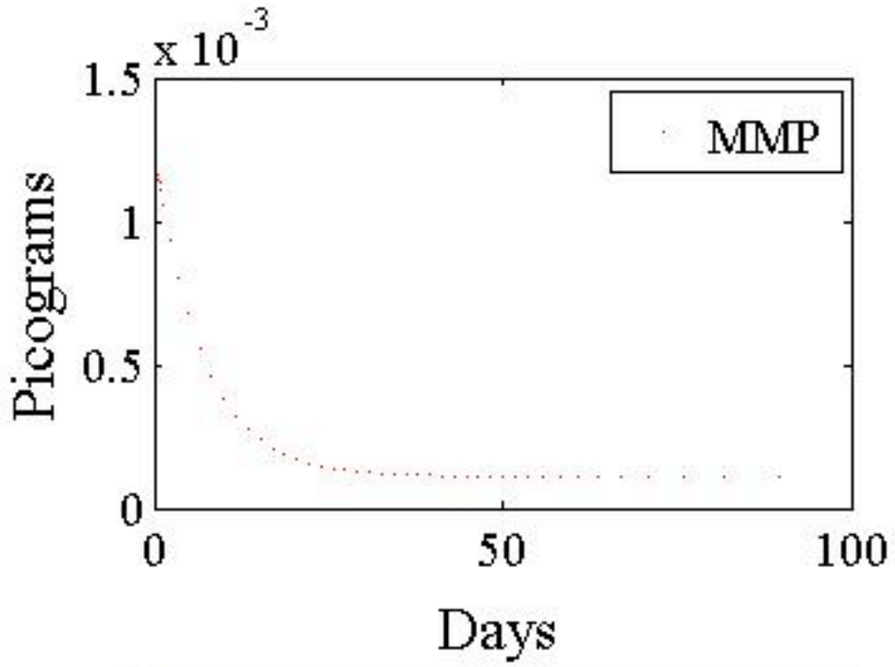
Biological interpretation:

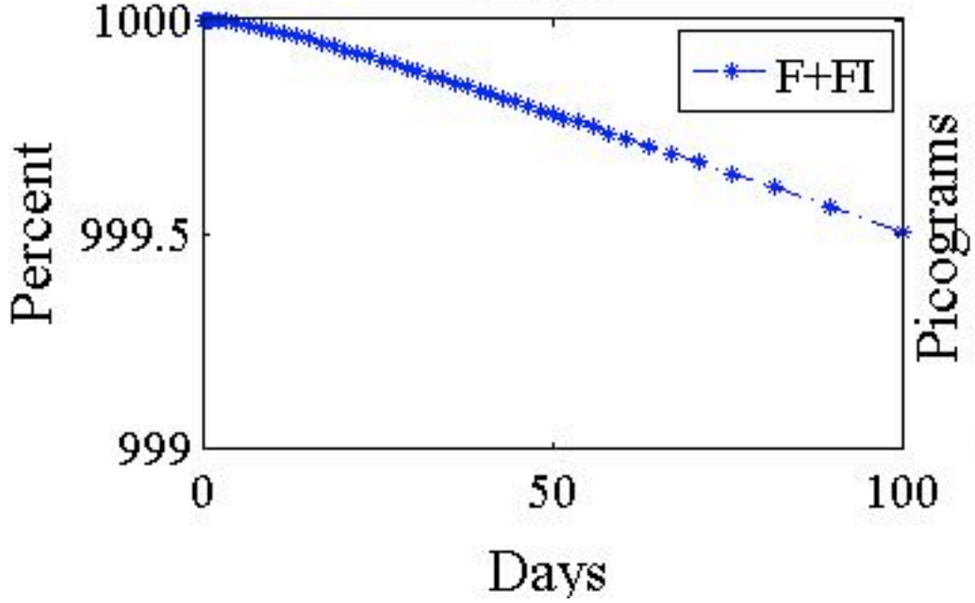
Fibroblasts in the system after inflammatory stimulus

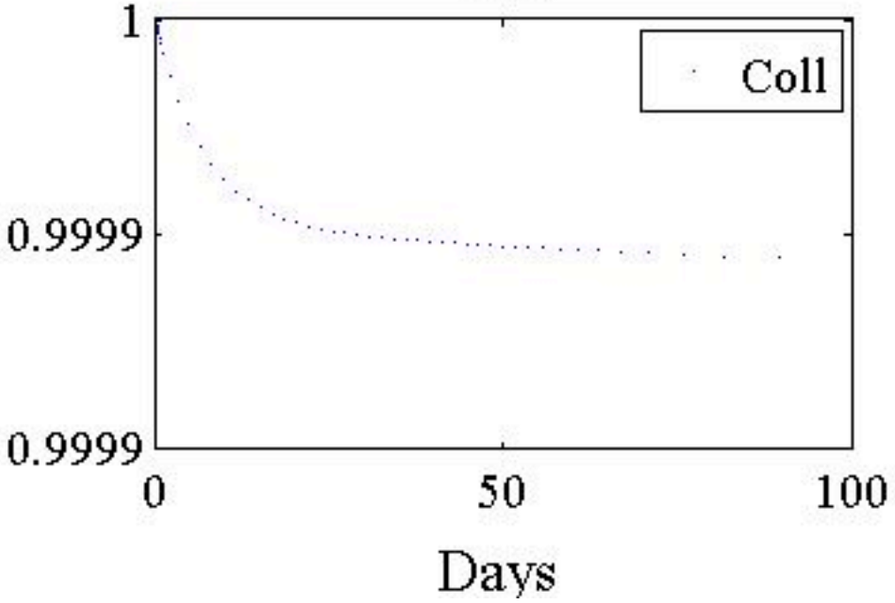
- decay to some constant level in physiological ($IL \to 0, y \approx 1$) situations
- can decay to very low levels in aggressive pathological situations (IL$\to 0, y \approx 0$)

Healthy response





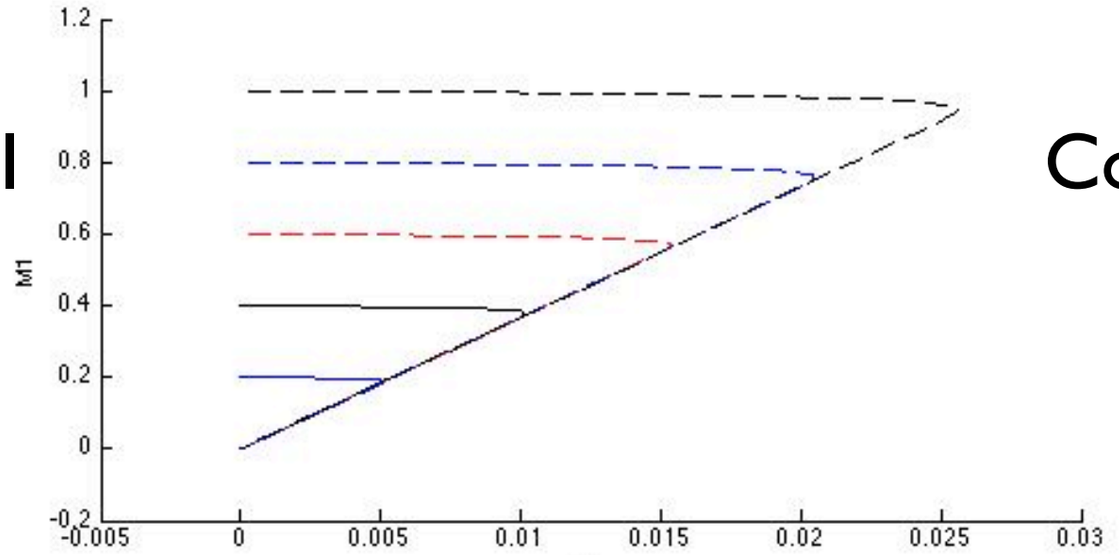




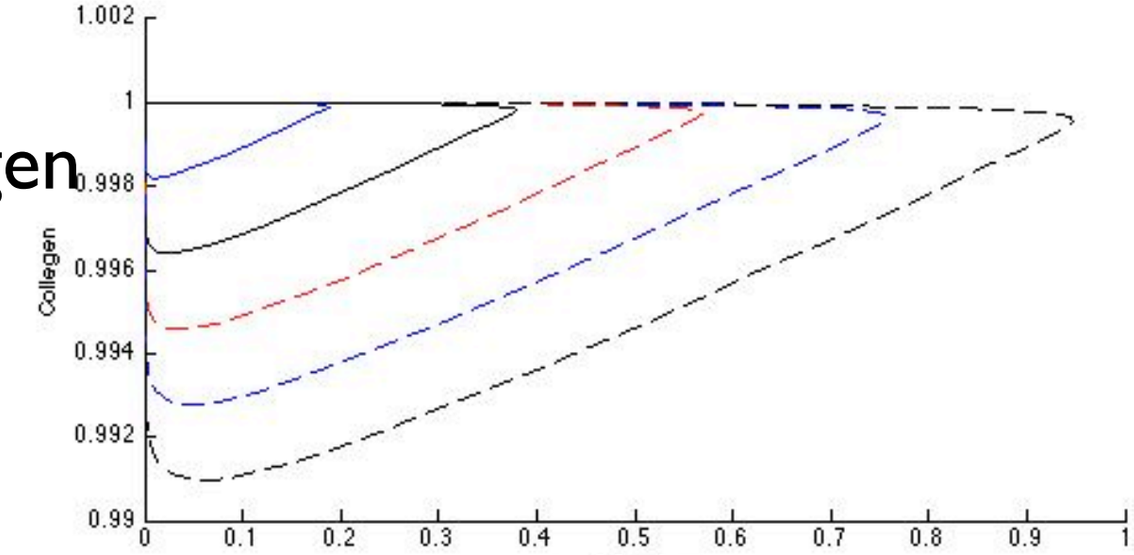
For Chris Breward

Phase portraits, nondimensionalized system

M1



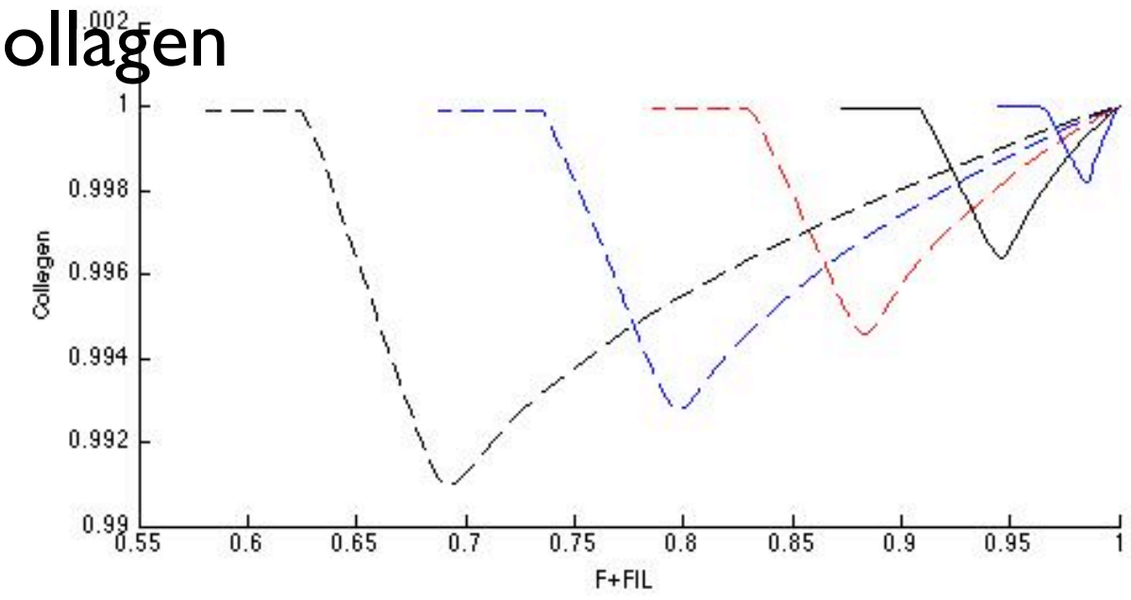
Collagen



IL-8

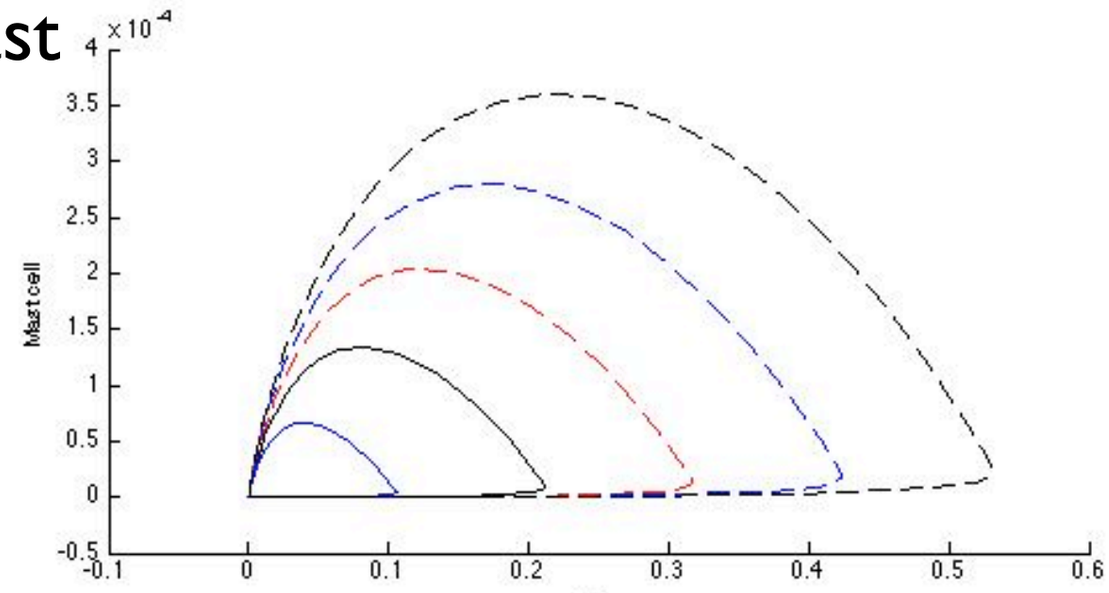
MMP

Collagen



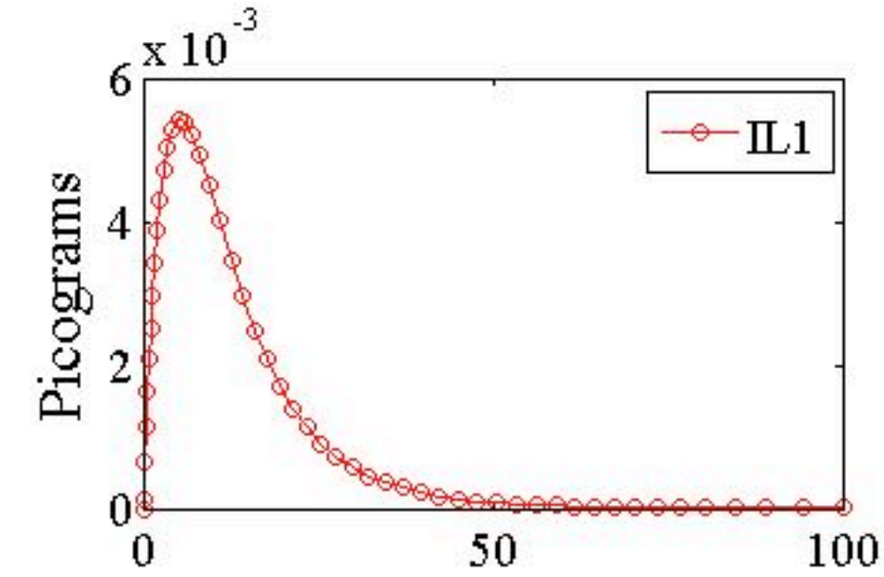
Total Fibroblasts

Mast

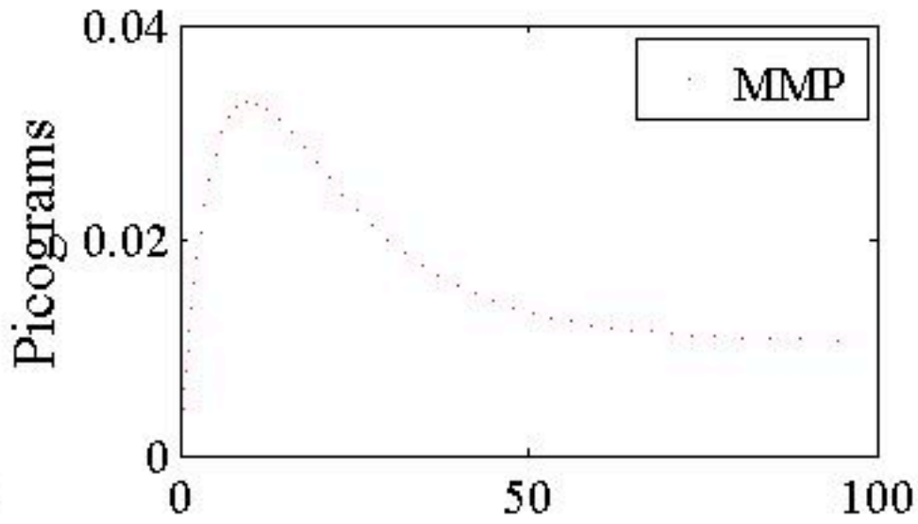


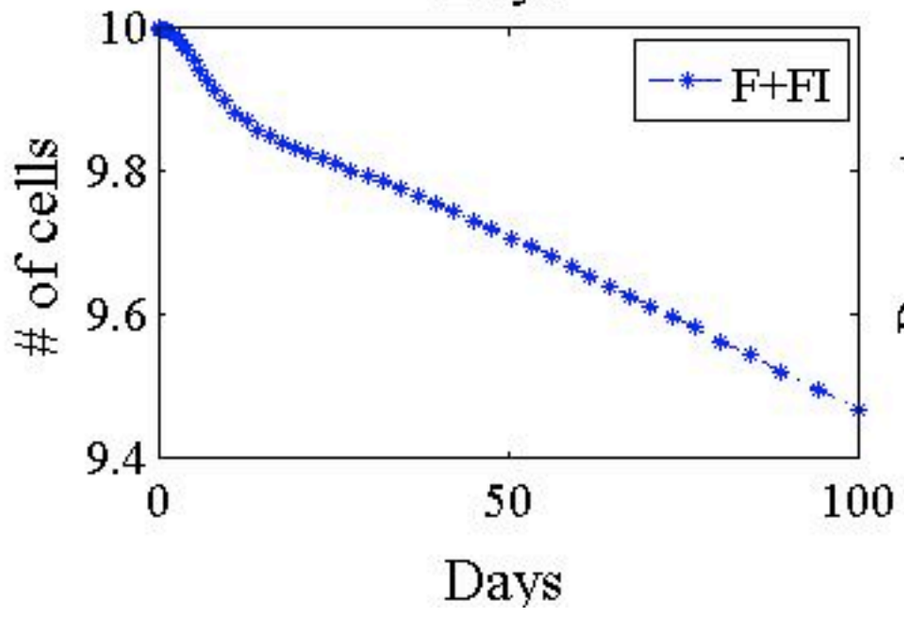
IL-8

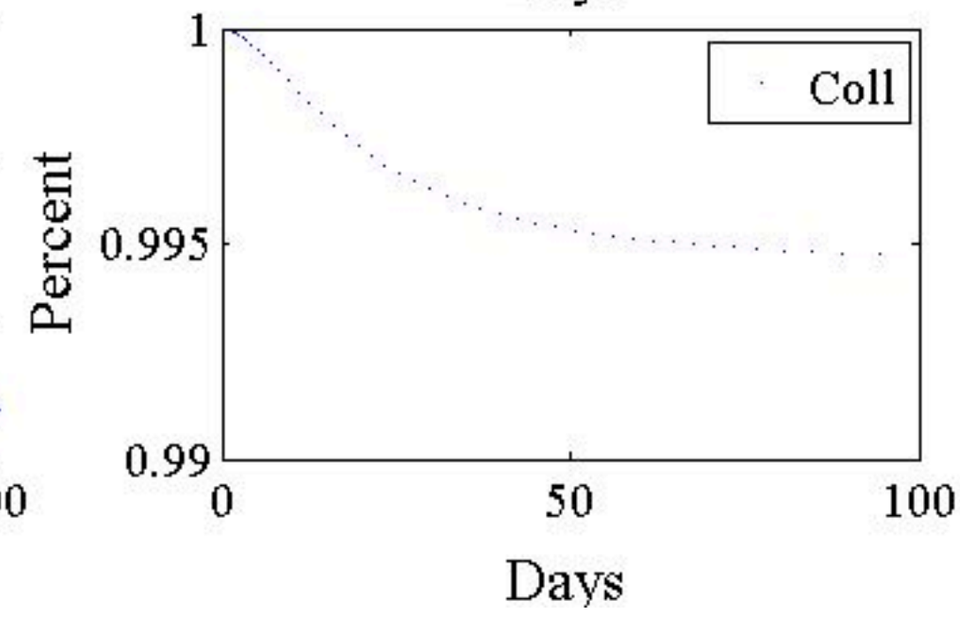
Decrease
k1 or k2 F



Pathological
response (chronic)

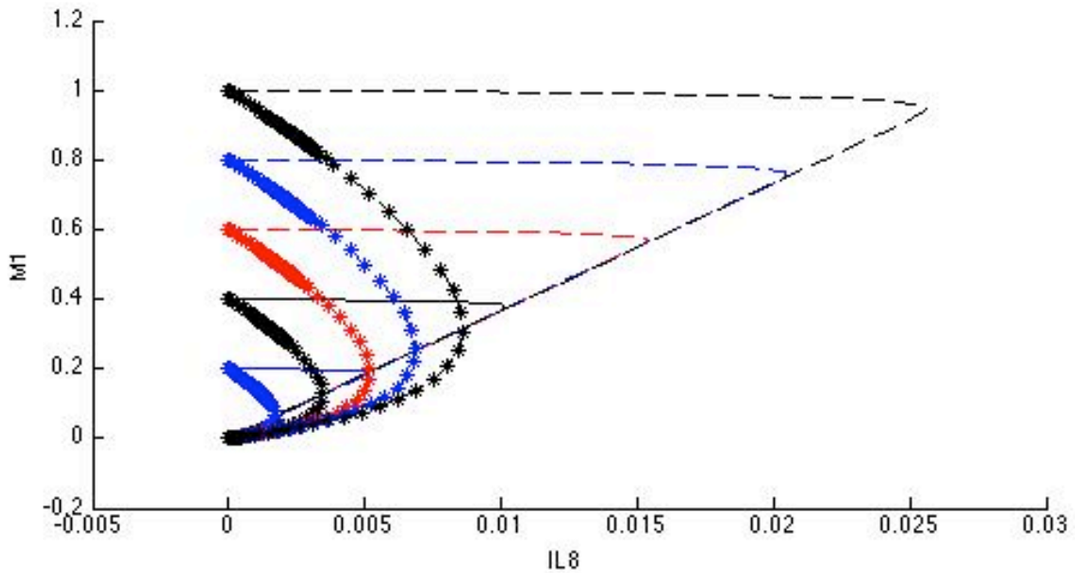


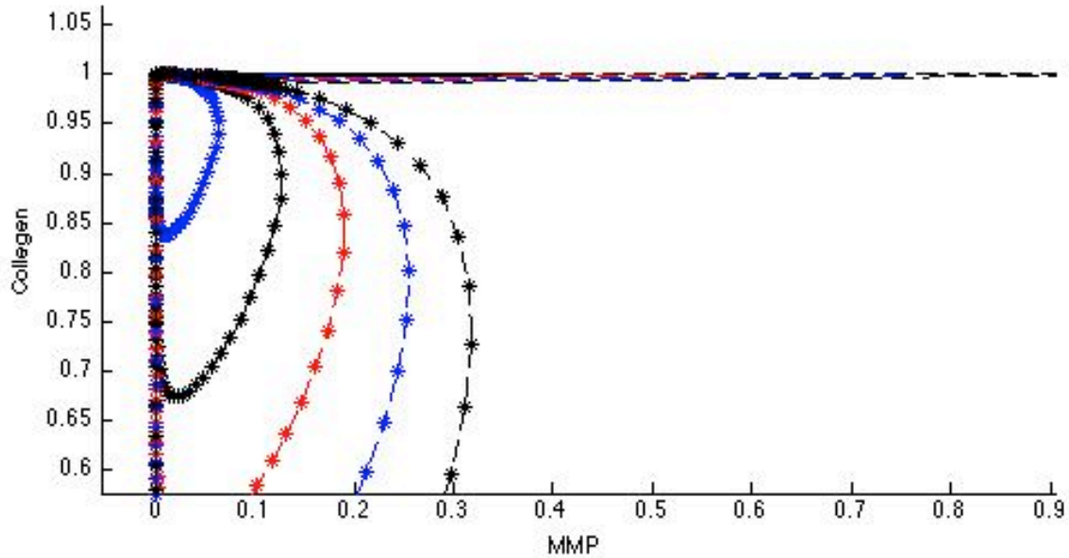


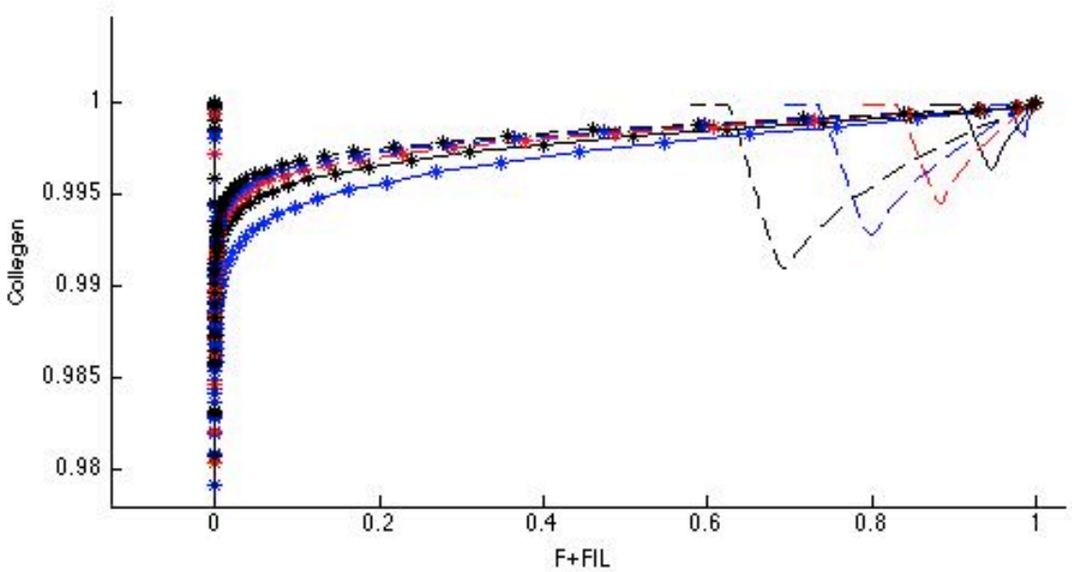


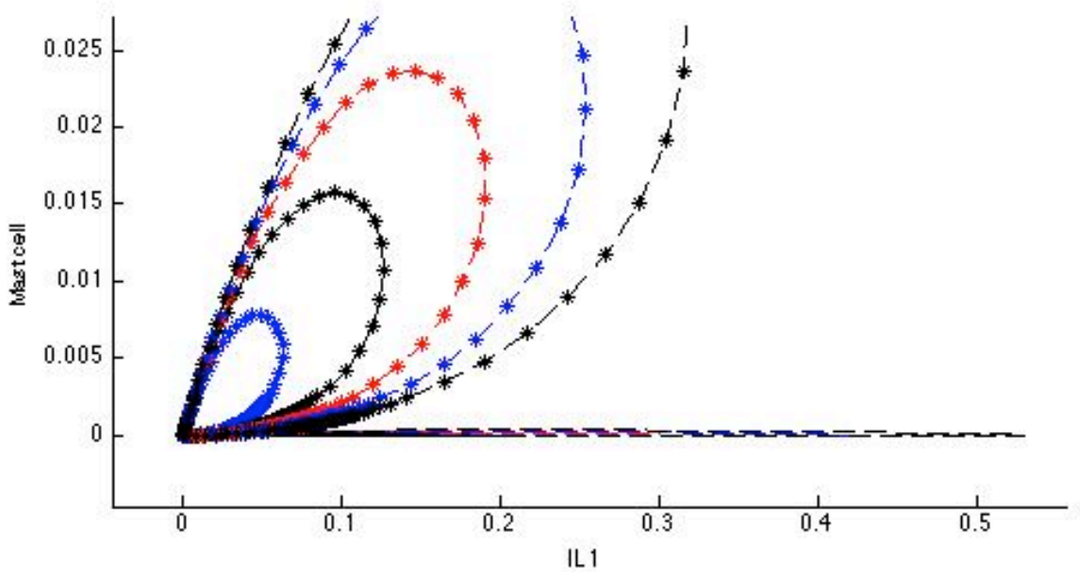
For Chris Breward

Phase portraits:
healthy response (dashes),
chronic response (stars)

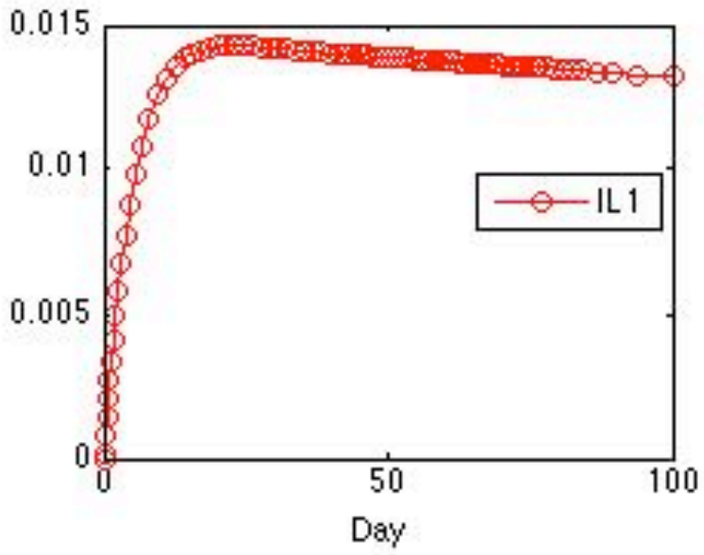


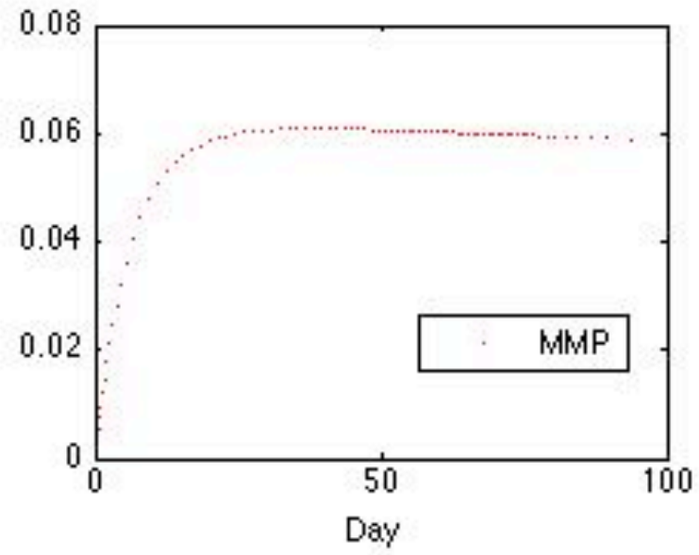


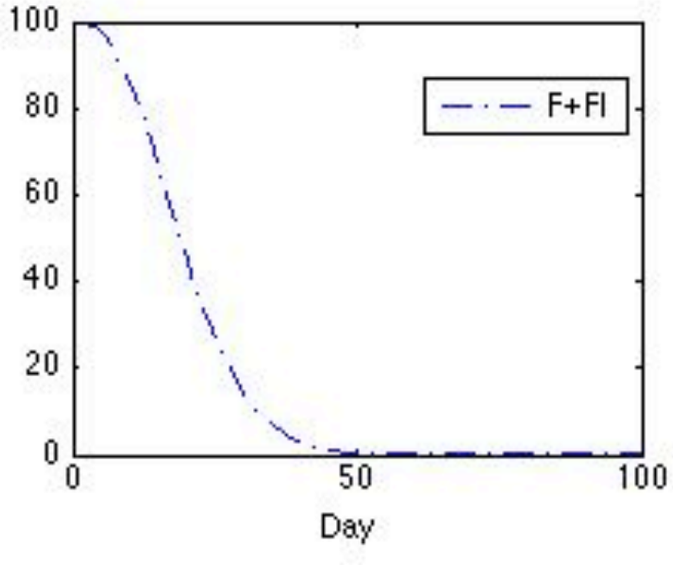


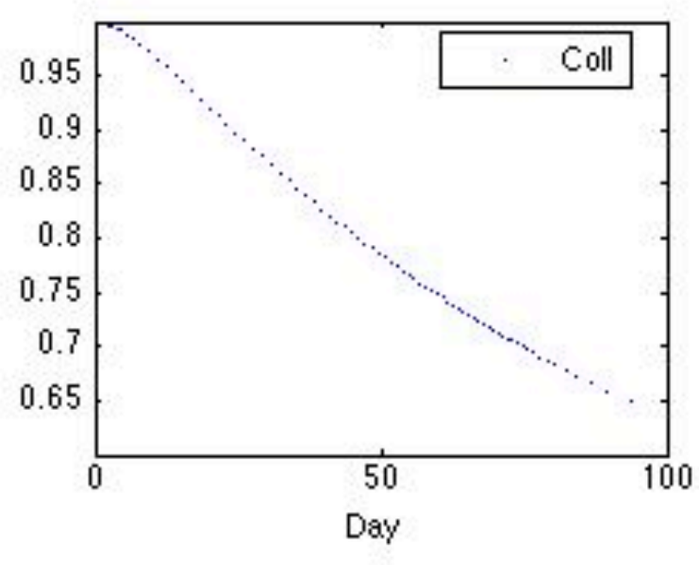


Aggressive inflammation? No oscillations, though...





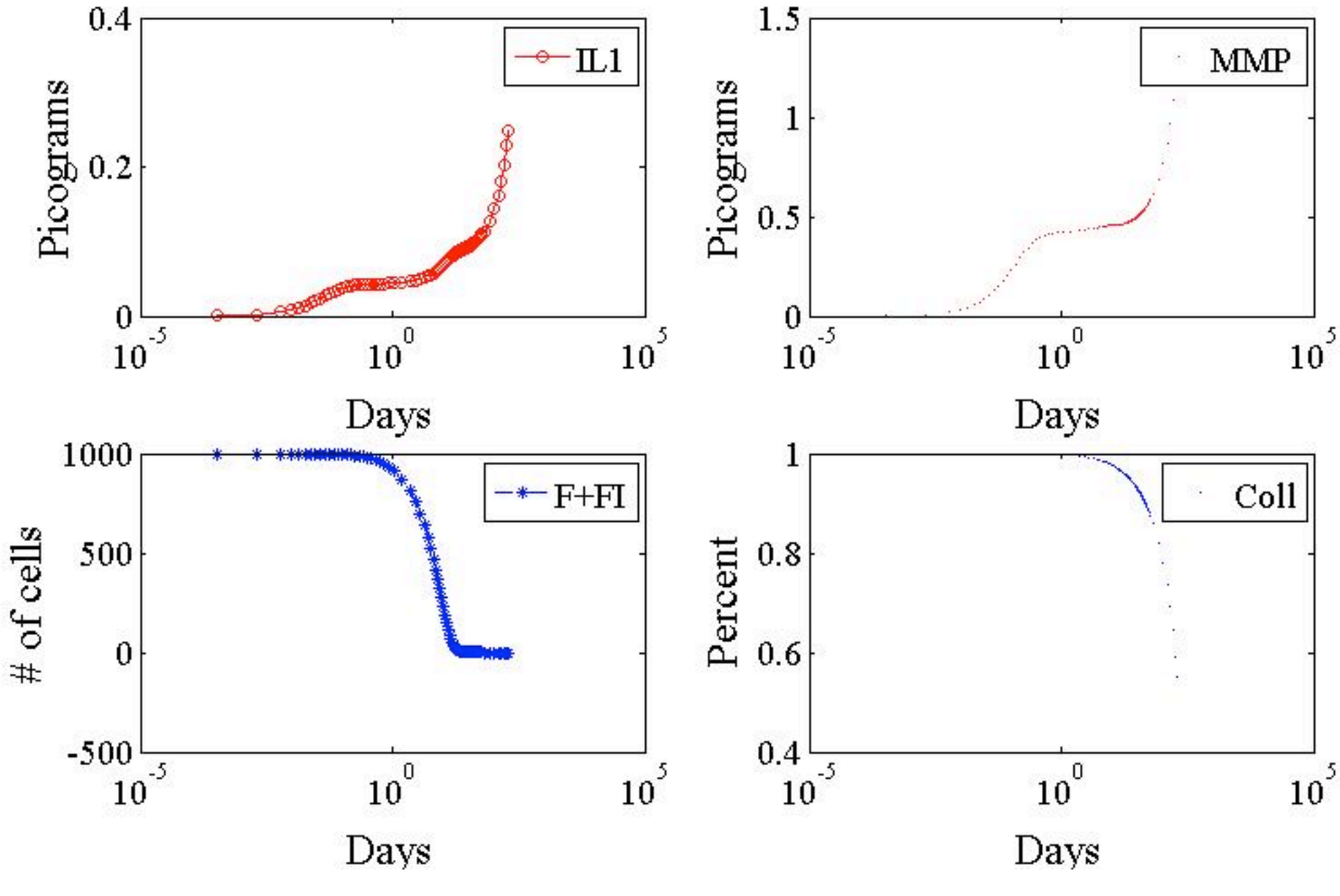




What we did

- Have initial model which explains physiological and 'steady' pathological response to inflammation
- Numerical and asymptotic results suggest possible mechanisms to shift between such states
- Have a testing ground for future biological hypotheses

Example: Can pathological responses be caused by long-lived macrophages?



Future work

The model can be refined in several directions.

- As the collagen levels become constant, fibroblast loss should stop. Currently have no biologically-motivated mechanism in the model for this.
- Still unable to capture the 'aggressive' pathological behaviour (specifically, oscillatory behaviour) with this model.
- We currently capture *qualitative* behaviour, not *quantitative* behaviour. We need a more careful look at experimental data!