A transcriptional regulatory switch underlying B-cell terminal differentiation and its disruption by dioxin

Sudin Bhattacharya[1], Rory B. Conolly [2], Melvin E. Andersen[1] and Qiang Zhang[1]

[1]*Division of Computational Biology, The Hamner Institutes for Health Sciences , RTP, North Carolina 27709*

[2]*National Center for Computational Toxicology, U.S. Environmental Protection Agency, RTP, North Carolina 27711*

Introduction

- **Background**:
  - -The terminal differentiation of B lymphocytes into antibody-secreting plasma cells upon antigen stimulation is a crucial step in the humoral immune response.
  - -The mutually-repressive interactions among three key regulatory transcription factors underlying B to plasma cell differentiation, **Bcl-6**, **Blimp-1** and **Pax5**, give rise to double-negative feedback loops – a common design "motif" in the transcriptional regulatory networks underlying binary cell fate choice in the hematopoietic stem cell (HSC) lineage [1].
  - -The environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (**TCDD**) interferes with the immune response by suppressing the formation of antibody-secreting plasma cells.
- **Hypothesis**:
  - A bistable switch arising from the coupled double-negative feedback loops involving Bcl-6, Blimp-1 and Pax5 forms the basis of the B-cell to plasma cell differentiation program and its disruption by dioxin .

1

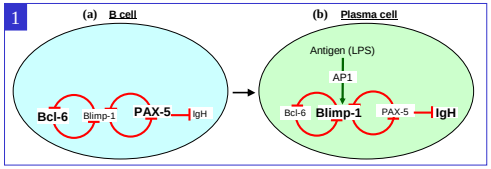

Fig 1. The state of the transcriptional regulatory network in **(a)** B cells and **(b)** plasma cells. When stimulated by the antigen LPS, the AP-1 protein triggers the differentiation switch by activating Blimp-1, which in turn leads to down-regulation of Pax5 and secretion of IgH (antibody).

2

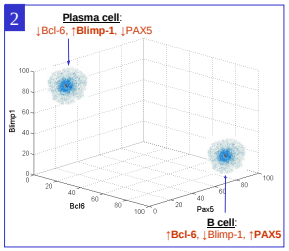

Fig 2. The two distinct phenotypic states comprise a "bistable" system.

Mature B cell:

↑**Bcl-6**, ↓Blimp-1, ↑**PAX5**, ↓IgM.

Plasma cell:

↓Bcl-6, ↑**Blimp-1**, ↓PAX5, ↑**IgM**.

Model Structure

3

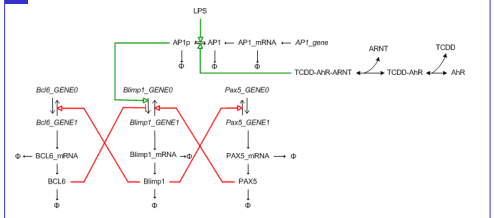

Fig 3. A graphical representation of our kinetic model. **Red** lines denote inhibitory effects of a transcription factor on a gene; **green** lines denote activating effects; bold black arrows denote binding interactions. Φ represents mRNA and protein degradation.

Results

4

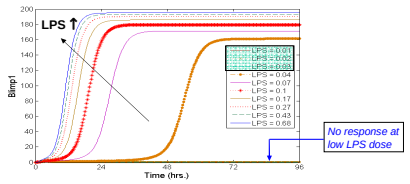

Fig 4. Time-course of Blimp-1 protein for various doses of antigen LPS, showing a sharp transition from the B-cell state (low Blimp-1) to the plasma-cell state (high Blimp-1).

5

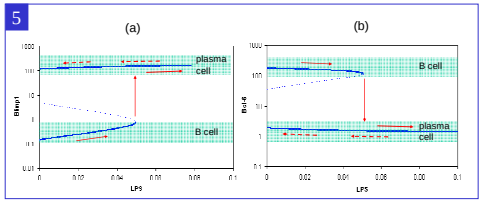

Fig 5. A threshold-driven **irreversible** switch: hysteresis and memory.

(a) Blimp-1 vs. LPS bifurcation diagram illustrating a threshold-dependent bistable switch. The solid blue lines represent stable steady states; the dashed line represents unstable steady states. The arrows show the direction of dosing, and the discontinuity at the switching threshold.

(b) Bcl-6 vs. LPS bifurcation diagram.

6

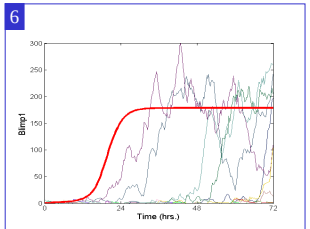

Fig 6. Deterministic vs. stochastic model.

In a **deterministic** model, the occurrence and timing of differentiation are uniquely determined by the LPS dose.

In **stochastic** simulations, fluctuations in the content of regulatory proteins impart a distributional characteristic to the occurrence and timing of differentiation.

7

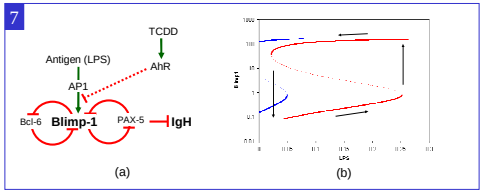

Fig 7. Modeling the disruption of B-cell differentiation by dioxin.

(a) TCDD inhibits the AP-1 dependent activation of Blimp-1, thus reducing the percentage of B cells differentiating to plasma cells.

(b) Blimp-1 vs. LPS bifurcation diagrams for TCDD = 0 (**blue**) and TCDD = 0.3 (**red**). The effect of TCDD on the bistable switch is manifested as a higher "on" threshold for the LPS dose. TCDD may also render the switch **reversible**.

8

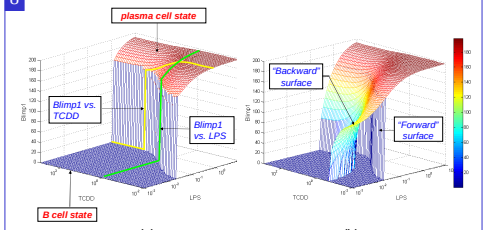

Fig 8. Dose-response surfaces (*deterministic*)**.**

(a) "Forward" dose-response surface generated from the **deterministic** model by starting simulation from B-cell state, with typical Blimp-1 vs. LPS and Blimp-1 vs. TCDD dose-response curves shown.

(b) "Backward" dose-response surface obtained by starting simulation from plasma-cell state, superimposed on the forward surface.

9

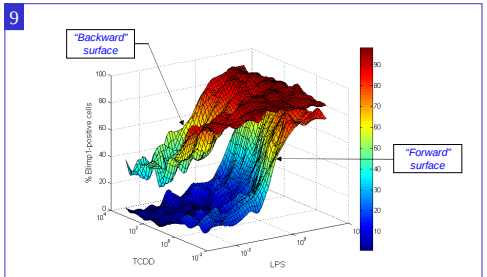

Fig 9. Dose-response surfaces (*stochastic*)**.**

"Forward" and **"backward"** dose-response surfaces generated from the **stochastic** model by starting simulation from B-cell state and plasma-cell state respectively.

10

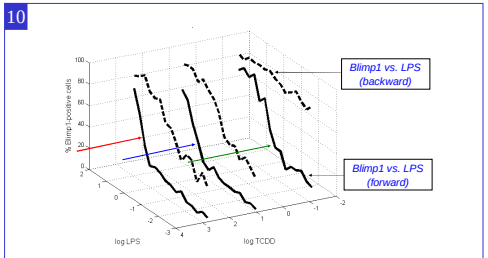

Fig 10. TCDD ***raises*** the LPS dose required to cause a given percentage of B cells to differentiate to plasma cells.

11

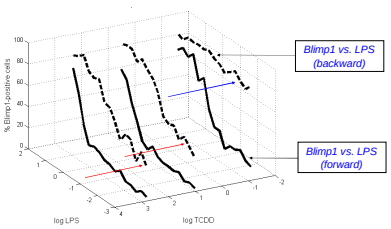

Fig 11. When LPS is removed:

(a) With no or low TCDD, plasma cells remain as plasma cells (**blue** arrow).

(b) At high TCDD doses, plasma cells may ***de-differentiate*** to B cells (**red** arrows).

Immunotoxicological Implications:

- Our model is consistent with the observation that TCDD inhibits the antibody-forming cell response
  - *This behavior, depending on the dose-response, could have health implications in suppressing **initiation** of humoral immune response.*
- Additionally, the model predicts that TCDD may cause some of the antibody-forming plasma cells to "de-differentiate" into B cells as the LPS dose is reduced
  - *This would imply that TCDD disrupts the **maintenance** of the humoral response by causing long-lived plasma cells to de-differentiate to B cells.*

Evidence for induced "de-differentiation" of plasma cells into B cells:

- Fujita et al. *Cell*, 2004 [2]
  - Exogenous expression of Bcl-6 and associated protein MTA3 in ***malignant*** plasma cells leads to:
    - repression of plasma cell-specific transcripts
    - reactivation of the B cell transcriptional program
    - expression of B lymphocyte cell surface markers
    - reprogramming of cell fate.
  - *Might there be a similar effect in "normal" plasma cells on dioxin treatment?*

Conclusions

1. The architecture of the B-cell transcriptional regulatory network consists of **coupled mutually-repressive feedback loops** involving the three transcription factors Bcl-6, Blimp-1 and Pax5. This structure forms the basis of an **irreversible bistable switch** directing the B-cell to plasma cell differentiation process – i.e., the switch remains on after the activating stimulus (antigen) is removed.
2. Using a kinetic model and bifurcation analysis, we suggest that TCDD may regulate the proportion of B-cells that differentiate into plasma cells by **raising** the **threshold** dose of antigen lipopolysaccharide (LPS) required to trigger the bistable switch in individual cells.
3. The model also indicates that TCDD could cause some plasma cells to **de-differentiate** to a B-cell state.
4. Stochastic modeling of gene expression, which allows cell-to-cell differences in the content of regulatory proteins, introduces distributional characteristics to the timing and probability of differentiation among a population of B-cells. This cell-to-cell variability is likely to be a key determinant of dose-response and sensitivity of a population of cells to differentiation.

References

1. Orkin, S. H. and L. I. Zon (**2008**). "Hematopoiesis: An evolving paradigm for stem cell biology." Cell 132(4): 631-644.
2. Fujita, N., D. L. Jaye, et al. (**2004**). "MTA3 and the Mi-2/NuRD complex regulate cell fate during B lymphocyte differentiation." Cell 119(1): 75-86.

This work was supported by the NIEHS Superfund grant P42ES04911.
This work is not a statement of official policy of the U.S. EPA.