USING SYSTEMS BIOLOGY APPROACHES TO INVESTIGATE THERAPEUTIC MACROPHAGE POLARIZATION IN HUMAN DISEASES

[1]Chen Zhao*, [2]Thalyta X. Medeiros, [2]Brian H. Annex, [1]Aleksander S. Popel

[1]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA (*czhao22@jhmi.edu)

[2]Medical College of Georgia, Augusta University, Augusta, GA, USA

Introduction

Macrophages are a class of innate immune cells that play essential roles in the progression and resolution of inflammatory responses, which are key to a variety of major human diseases. The concept of differential macrophage polarization and phenotypes can be described in terms of the activation of different signaling pathways and transcription factors, together with the expression and secretion of a set of markers and cytokines[1,2]. The canonically activated macrophages (M1) and the alternatively activated macrophages (M2) represent the extremes of the total macrophage polarization spectrum, while in physiology and pathology most macrophages display "M1-like" or "M2-like" phenotypes. M1 (or M1-like) phenotypes are often induced by pro-inflammatory cytokines as well as certain pathogen- and damage-associated molecular patterns and are typically characterized by their antibacterial and antitumor functions, along with the high production of various pro-inflammatory cytokines as well as reactive nitrogen and oxygen species. On the other hand, cytokines such as IL-4/IL-13, IL-10 and TGF-β will contribute to M2 (or M2-like), anti-inflammatory phenotypes which are broadly involved in immunosuppression, angiogenesis, and tissue repair[2,3].

The continuum hypothesis which suggests a wide spectrum of macrophage activation and functional states, instead of the dichotomous M1-M2 notion, has been recently proposed to describe the dynamical shaping of macrophage polarization given the large number of signaling and regulatory pathways and the many sequential autocrine and induction mechanisms involved[4]. To address this complexity at the cell and tissue levels, systems biology modeling approaches which allow investigation of both the complex multi-modal signal transduction and cross-talks as well as the temporal expression of phenotypic cytokines, markers and cell-cell communications are key to the integrative understanding of macrophage polarization and functions in health and disease.

Methods

Here we develop the first set of mechanism-based, multi-pathway computational models (Fig.1) that quantitatively describe the integrated signal transduction and macrophage programming under various M1, M2 (IL-1β, TNFα, IFN-γ, IL-4, IL-10, VEGFA) and stress (hypoxia) stimulation at the cell-level. We use a two-step approach by first building a proof-of-concept (step-1) model with three representative pathways derived from the M1-M2 spectrum, and then we further expand the step-1 model with additional signaling mechanisms and pathways of high importance in human diseases (e.g. cancer, peripheral arterial disease) to collectively form a more advanced (step-2) model. In addition, we explore two multi-scale scenarios that would enable incorporation of our cell-level macrophage models into mechanistic disease models to computationally characterize disease progression at tissue and patient levels. The overall processes of model formulation, calibration, simulation and analyses are implemented using the MATLAB SimBiology Toolbox (MathWorks, Natick, MA). The stiff solver ode15s method provided in MATLAB is used for model simulations. Sensitivity analysis are carried out using the Partial Rank Correlation Coefficient (PRCC) method[5]; uncertainty quantifications are evaluated using bootstrapping.

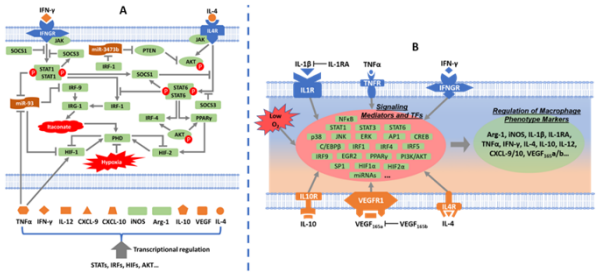

Figure 1 (top): Schematic overview of the (A) step-1 and (B) step-2 computational models that were developed to characterize macrophage polarization at the cell-level.

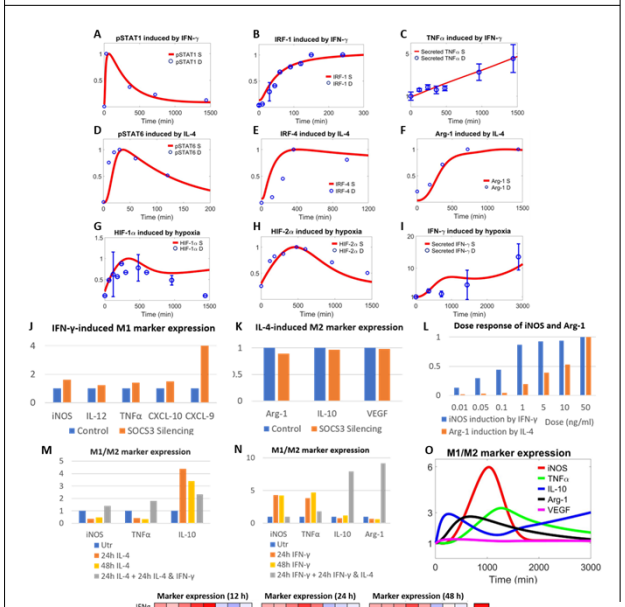

Figure 2 (left). Calibration of the step-1 model and the simulated expression profiles of macrophage functional markers in response to different treatments. (A-I) A subset of the experimental data used for step-1 model calibration (A-C: IFN-γ pathway; D-F: IL-4 pathway; G-I: hypoxia pathway) and the comparisons with corresponding model simulations ('S'–simulation, 'D'–data, all value are normalized). The complete set of data used in model calibration is described in [6]. (J-K) Relative expression of M1 and M2 markers upon SOCS3 silencing (values are normalized to control). (L) Dose response relationships of iNOS (to IFN-γ) and Arg-1 (to IL-4). Values are normalized to maximum levels. (M-N) Relative expression of M1 and M2 markers under different treatment schedules of IFN-γ and IL-4 ('Utr'-untreated, values are normalized to untreated levels). (O) Relative marker expression profiles (normalized to time 0) in response to IFN-γ plus IL-4 simultaneously. (P) Relative expression of macrophage markers and (Q) activation of major transcription factors in response to different treatment combinations at different timepoints (values are normalized to time 0 levels and log2 transformed).

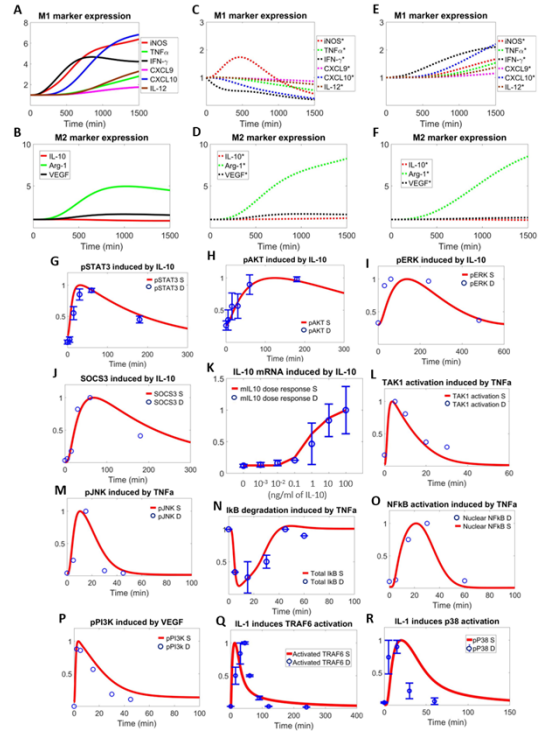

Figure 3 (top). Step-1 model sensitivity analysis identifies strategies of therapeutic macrophage repolarization; calibration of the step-2 model. Time course expression of various M1 and M2 markers under (A-B) hypoxia (2% O2) only, (C-D) hypoxia and IFN-γ inhibition, and (E-F) hypoxia and HIF-1α inhibition as predicted by the step-1 model. Marker expression levels are normalized to their respective t = 0 values (normoxia, unstimulated). (G-R) A subset of the experimental data used for step-2 model calibration (G-K: IL-10 induces the activation of several transcriptional regulators and also its own production; L-O: TNFα induces the activation of several kinases such as TAK1 and JNK which leads to subsequent IκB degradation and NFκB activation in the nucleus; P: VEGF activates PI3K through VEGFR1 signaling; Q-R: IL-1β activates downstream signaling) and the comparisons with corresponding model simulations ('S'–simulation, 'D'–data, all value are normalized).

Results and Next Steps

The step-1 model (Fig.1A) consisting of three major pathways was formulated to reproduce experimental time-course observations relating to different macrophage phenotype perturbations (e.g. 70+ datasets), and it has suggested novel insights regarding the hierarchical and temporal control of M1-M2 features through an integrative analysis of direct cytokine signaling, hypoxic response, transcriptional/post-transcriptional regulation, and autocrine feedbacks (Figs.2 and 3)[6]. Using the step-1 model as a basis, we are currently finalizing our step-2 model (Fig.1B) which includes 4 additional pathways and is calibrated against 150+ sets of quantitative experimental data derived from macrophages (Fig.3), plus original data obtained specifically for this project from our collaborators at Augusta University. For next steps, we have also started the formulation of a tissue-level model of ischemia that considers endothelium-muscle-macrophage communications (Fig.4A), as well as the integration of macrophage signaling as a module into a QSP platform of immuno-oncology developed by our lab[7] (Fig.4B).

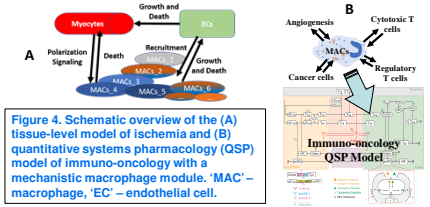

Figure 4. Schematic overview of the (A) tissue-level model of ischemia and (B) quantitative systems pharmacology (QSP) model of immuno-oncology with a mechanistic macrophage module. 'MAC' – macrophage, 'EC' – endothelial cell.

Summary

Our computational models are calibrated extensively against experimental data, and by using these models we mechanistically elucidated a number of signature feedbacks behind the M1-M2 antagonism and investigated the dynamical shaping of macrophage phenotypes spanning the M1-M2 spectrum. Model sensitivity analysis also revealed key molecular nodes and interactions as targets with potential therapeutic values for the pathophysiology of peripheral arterial disease and cancer. In addition, we are also in the process of designing and implementing computational strategies that further incorporate our cell-level macrophage models into mechanistic tissue-level models of ischemia as well as patient-level immuno-oncology simulation platforms. In summary, through simulations that dynamically capture the signal integration and phenotypic marker expression in the differential macrophage polarization responses, we believe that our data-driven models can provide an important computational basis toward a more quantitative and network-centric understanding of the complex physiology and versatile functions of macrophages in human diseases.

Acknowledgements

This work is supported by NIH grants R01HL101200, R01HL141325, and American Heart Association Grant #19PRE34380815.

References

1. Wang N. et al. (2014) Front Immunol. 5:614.
2. Sica A, Mantovani A. (2012) J Clin Invest. 122(3):787-95.
3. Martinez FO, Gordon S. (2014) F1000Prime Rep. 6:13.
4. Xue J. et al. (2014) Immunity. 40(2):274-88.
5. Marino S. et al. (2008) J Theor Biol. 254(1):178–96.
6. Zhao C. et al. (2019) PLoS Comput Biol. 15(11):e1007468
7. Wang H. et al. (2020) Front Bioeng Biotechnol. 8(141)