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Immune signal transduction in leishmaniasis from natural to artificial systems: Role of feedback loop insertion

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ABSTRACT

Background: Modulated immune signal (CD14–TLR and TNF) in leishmaniasis can be linked to EGFR pathway involved in wound healing, through crosstalk points. This signaling network can be further linked to a synthetic gene circuit acting as a positive feedback loop to elicit a synchronized intercellular communication among the immune cells which may contribute to a better understanding of signaling dynamics in leishmaniasis.
Methods: Network reconstruction with positive feedback loop, simulation (ODE 15s solver) and sensitivity analysis of CD14–TLR, TNF and EGFR was done in SimBiology (MATLAB 7.11.1). Cytoscape and adjacency matrix were used to calculate network topology. PCA was extracted by using sensitivity coefficient in MATLAB. Model reduction was done using time, flux and sensitivity score.
Results: Network has five crosstalk points: NIK, IκB–NFκB and MKK (4/7, 3/6, 1/2) which show high flux and sensitivity. PI3K in EGFR pathway shows high flux and sensitivity. PCA score was high for cytoplasmic ERK1/2, PI3K, Atk, STAT1/3 and nuclear JNK. Of the 125 parameters, 20% are crucial as deduced by model reduction.
Conclusions: EGFR can be linked to CD14–TLR and TNF through the MAPK crosstalk points. These pathways may be controlled through Ras and Raf that lie upstream of signaling components ERK ½ (c) and JNK (n) that have a high PCA score via a synthetic gene circuit for activating cell–cell communication to elicit an inflammatory response. Also a disease resolving effect may be achieved through PI3K in the EGFR pathway.
General significance: The reconstructed signaling network can be linked to a gene circuit with a positive feedback loop, for cell–cell communication resulting in synchronized response in the immune cell population, for disease resolving effect in leishmaniasis.

1. Introduction

Human body has an amazing protective immune system, which safeguards us from infections caused by bacteria, viruses, protozoans, parasites and fungi as well as from tumors growing within the body. The activation of different immune cells and molecular interaction among them will decide the extent of protective function of the immune system. The defense system comprises of an intricately woven network of signaling molecules within and between the interacting immune cells. These molecules and cells work in a concert ensuring appropriate response in resolving a diseased state. Past research efforts using powerful experimental approaches have identified a staggering number and variety of molecules participating in these immune functions. Among them are cytokines, cell surface receptors and adaptor proteins with individual properties, mediating cellular interactions to mount a precise immune response [1]. But the problem is not the sheer number of components participating in the immune response, it is the interactions between the molecular and cellular machinery that often makes the system unpredictable (nonlinear), and it becomes even more intriguing with the operation of positive–negative feedback and feedforward loops.

Mathematical and computational modeling in immunology is increasingly playing a role in data interpretation and attempts to extract general biological understanding, through a systems biology approach. Listing down of components participating in a signaling pathway can help engineer novel signaling pathways, by using repeated iteration of pretested domains, modules and motifs allowing the signaling proteins to be controlled in an increasingly predictable way. Thus integration of

Abbreviations: CD 14, cluster determinant 14; TLR, toll like receptor; TNF, tumor necrotic factor; EGFR, epidermal growth factor receptor; ODE, ordinary differential equation; PCA, principal component analysis; NIK, NFκB-inducing kinase; MKK, mitogen kinase kinases; PI3K, phosphatidylinositol 4-phosphate 3-kinase; ERK, extracellular regulated MAP kinase; Atk, agammaglobulinemia tyrosine kinase; STAT, signal transducer and activator of transcription; NFκB, nuclear factor kappa B; JNK, Jun NH2-terminal kinase; MAPK, mitogen activated protein kinases; IL, interleukins; APCs, antigen presenting cells; IFN, interferon; NO, nitric oxide; ROS, reactive oxygen species; Th1/2, T helper cells 1/2; PMN, poly morphonuclear neutrophil; LCF, leucocyte chemotactic factor; LPG, lipo phosphoglycan; IRAK, IL1 receptor associated kinases; SOCS, suppressor of cytokine synthesis; TNF, tumor necrotic factor; TRAF, TNF receptor associated factor; TAK, TGFβ activated kinase; TAB, TAK1 binding protein; PSA, parasite surface antigen; AMPs, antimicrobial (poly) peptides; PKC, protein kinase C; Ras, rat sarcoma; PP2A, protein phosphatase 2A; KEGG, Kyoto Encyclopedia of Genes and Genomes; INOH, Integrating Network Objects with Hierarchies; SBML, Systems Biology Markup Language; DAG, directed acyclic graph; FBA, flux balance analysis

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synthetic biologic devices to a systems level understood biological process may help better gauge the kinetic and quantitative aspects of signaling dynamics [2].

This study exploits leishmaniasis as an infectious disease model system to show how approaches of systems biology can be useful in giving new insight in macrophage immune cell signaling by constructing *in silico* signaling network. Linking this signaling network to a logic based gene circuit via synthetic biology approach may activate and direct macrophages for triggering immune intercellular communication, for a disease resolving effect.

1.1. Immune response in leishmaniasis

Leishmaniasis is one of the most neglected tropical diseases of the world; 350 million people are considered to be at risk of contracting leishmaniasis, some 2 million new cases occur yearly, and it is endemic in 88 countries. Treatment options are few and with the available chemotherapeutics emergence of drug resistance is a serious concern [3]. It is one of the most diverse and complex of all vector borne diseases, caused by a kinetoplastid obligate intracellular protozoan parasite belonging to the genus *Leishmania*, causing self-healing cutaneous lesions to fatal visceral leishmaniasis. It has a distinct dimorphic life cycle: extracellular stage promastigotes multiply and develop within the digestive tract of sandfly, and intracellular amastigotes reside and multiply within the phagolysosomal vacuoles of mammalian phagocytes. When an infected sandfly bites a mammalian host, it injects metacyclic promastigotes into the skin where they are captured by phagocytic cells. Inside the phagocytes promastigotes metamorphose to amastigotes that multiply and are eventually released within the extracellular space, where they can be engulfed by another phagocytic cell or can be taken in the blood meal by a sandfly, to begin a new cycle of infection [4].

Current chemotherapeutics to treat leishmaniasis are the standard pentavalent antimonials (meglumine antimonate and sodium stibogluconate) that have been used as the first line of therapy for more than 60 years. Many drug repositioning efforts have shown that drugs like miltefosine (anti-cancer drug) and amphotericin B (fungicide) can be used effectively against *Leishmania*; similarly paromomycin and pentamidine are also shown with anti-leishmanial activity. New drug discovery strategies have led to the discovery of sitamaquine, 2-substituted quinolines, buparvaquone and derivatives, and 8-aminoquinolines as potential antileishmanial drugs and are in various stages of clinical trials in India and Kenya. Combination of chemotherapeutics (e.g. sodium stibogluconate and paromomycin) to combat *Leishmania* has been implemented, which is a standard practice in the treatment of infectious disease like tuberculosis, leprosy and malaria [5]. Though there has been a substantial improvement in the way leishmaniasis is treated, there still remains the problem of development of drug resistant strategies evolving in the parasite, which varies from species to species and host–parasite interactions. Also, many endeavors have been made in search for a vaccine for leishmaniasis and some have made it to the clinical trials like the second-generation vaccine LEISH-F1 +MPL-SE of Reed and coworkers, consisting of three recombinant *Leishmania* poly protein LEISH-F1 antigens (S. Reed, Personal communication, IDRI, Seattle, USA). Likewise, parasite surface antigen 2 (PSA-2) derived from leishmanial antigens has been tested; however, promising findings from animal models were overshadowed by mostly negative T cell responses in humans [6].

Therefore, keeping these views in mind there is an urgent need to look beyond chemotherapeutics, and bring about a radical change in the way leishmaniasis is treated and managed, not only to overcome the serious problem of drug resistance but also to lower the toxicity effects. One such line of attack could be using strategies of synthetic biology through a systems based approach, and engineer new modular systems with predicted behavior, through simple methods of genetic recombination giving rise to an artificial system that has evolved from the natural system. Synthetic gene circuits have been developed and applied to easy-to-manipulate bacterial [7], viral [8] and lower eukaryotes like yeast [9] systems and applying these principles to the more complex and evolved mammalian cells is gradually becoming possible as assorted heterologous transcription control systems are being assembled and reprogrammed [10,11]. In mammalian cells, several transgene control mechanisms have been developed as assorted heterologous transcription control systems are being assembled and characterized; synthetic circuits can be applied to mammalian systems like the tetracycline responsive [12], biotin-responsive elements [13], arginine-responsive [14], or phloretin-responsive [15].

Similarly, a synthetic gene circuit can be designed considering the murine experimental leishmaniasis model which suggests that protective immunity against the parasite is dependent on the development of a Th1 cell mediated immune response which is characterized by production of IL12 by APCs, IFN-γ by CD4+ T cells and nitric oxide (NO) and reactive oxygen species (ROS) by macrophages to eliminate the intracellular parasites. Whereas disease progression is thought to result from the induction of Th2 responses, characterized by release of IL4 or IL10 by APC instead of IL12, leading to the priming of IL4 producing CD4+ T cells and resulting in alternatively-activated macrophages, these become a favorable niche for the safe intracellular survival for the parasite leading to the development of symptoms associated with *Leishmania* infection. Thus in leishmaniasis, there exists a fine balance between the Th1 (protective)/Th2 (susceptible) response, which ultimately decides the fate of the disease [16]. The Th1/Th2 paradigm is thought to occur in leishmaniasis due to the interaction of parasite's proteins with the host immune signaling mechanisms, tipping the scale towards a Th2 response. During infection with promastigotes polymorphonuclear neutrophils (PMNs) are recruited to the site of inoculation in response to the chemokines produced by the infected tissue and leucocyte chemotactic factor (LCF) by the parasite. The parasites are phagocytosed by the PMNs, and they release IL8 amplifying the migration of PMNs to the site of infection. After ingestion, *Leishmania* survives intracellularly in the PMNs and delays the spontaneous apoptosis of PMNs, during which they release monocyte-attractant chemokine MIP-1b recruiting monocytes to the site of infection who ingest the Trojan horses i.e. the apoptotic PMNs that harbor viable parasites. Uptake of apoptotic PMN silences the antimicrobial functions of macrophages and the parasites survive and multiply in them, thus gaining entry in its host macrophage by using neutrophils as an intermediate carrier [17].

1.2. Immune signaling pathways modulated by Leishmania

Though *Leishmania* is recognized by its pathogen associated molecules like lipophosphoglycan (LPG) by toll like receptor (TLR)s [18], it has the ability to counteract TLR detection by interfering with TLR signaling and silencing the immune cell activation rendering them refractory to subsequent TLR stimulation. LPG on binding to TLR2 induces the expression and activation of the serine/threonine phosphatase PP2A that acts on TLR cytoplasmic adaptor proteins like IRAK-1, MAPKs, and IκB causing their inactivation leading to tolerance. The induction of PP2A requires p38 and NF-κB, which are the downstream effector MAPKs, in the TLR signaling [19]. Similar to induction of PP2A, Baldwin et al. [20] have shown that LPG can induce the expression of suppressors of the cytokine signaling (SOCS-1 and SOCS-3) family proteins. Srivastav et al. [21] have shown that the TLR2 mediated pathway is modulated by the parasite through the inhibition of the IκK–NFκB and suppression of IL12 and TNF-α production, by inducing the deubiquitinating enzyme A20. It acts by inhibiting the association of TRAF6 with TAK–TAB complex and thus impairing the recruitment of TRAF6 in TLR2 signaling. LPG also blocks the production of NO and ROS by binding to the regulatory domain of PKC [22]. These evidences suggest that *Leishmania* exploits host PP2A, SOCS and A20 to inhibit the TLR2 mediated proinflammatory gene expression and escapes the immune responses

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