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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 similarly paromomycin and pentamidine also show 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

of the host. The induction of these natural negative regulators of TLR signaling dampens the generation of proinflammatory cytokines like TNF-α, IFN-γ and IL12, resulting in non-activation of the cell mediated immune responses [23,24]. These cytokines enhance the inflammatory response by acting as positive regulators, which are down regulated by the parasite action. As TNF fails to be expressed by the infected macrophages due to the abrogation of the TLR, the TNF pathway is not activated in them, which is needed for a Th1 response to alert the cells of the adaptive immune response for a cell mediated immunity [23].

Roupe et al. [25] have demonstrated the importance of EGFR signaling as an important inflammatory pathway, involved in wound healing in skin during infection. They found that injury generated prominent chemotactic activity toward neutrophils due to IL8 and antimicrobial (poly) peptides (AMPs) expression. In human skin, this injury-induced innate immune responses are mediated by activation of the epidermal growth factor receptor (EGFR), consequently, inhibition of the EGFR blocked both the chemotactic activity generated in injured skin and the expression of the majority of the AMPs. Conceptually, these data show that the growth factor response elicited by injury is important for the recruitment of neutrophils in skin wounds.

We hypothesize, that there could be a crucial crosstalk point between CD14–TLR, TNF and EGFR which can be points of transgene regulation using synthetic gene circuits acting as positive feedback loop for a disease resolving inflammatory response.

2. Materials and methods

2.1. Reconstruction of the signaling network

Signaling network reconstruction is the integration of data that describes the biochemical transformations that occur in a given network. It aids to gain deeper insight of interactions between the signaling proteins and explaining the complexity of biological system at higher level. Quantitative modeling of these interactions will play an important role in understanding fundamental intra- and inter-cellular processes [26]. Decomposition approach used in the study exploits the structure of a large network model to be broken into smaller components thereby solving the problem of parameter estimation associated with large network. This significantly improves the computational efficiency by reducing the dimensionality of the search space [27]. In this study signaling network was reconstructed by integrating the CD14–TLR, TNF and EGFR pathway. The interactome of the signaling cascade was assembled by extensive literature survey and from signaling databases like the KEGG, INOH Pathway Database (release 4.0), Pathway Interaction Database and Science Signaling Database. These databases have experimentally validated physical interactions among human proteins. The interactions between the signaling components were entered as elemental chemical reactions in MATLABs' SimBiology toolbox (7.11.1.866) (The MathWorks Inc.) which uses the Systems Biology Markup Language (SBML) machine language. The kinetic rate laws (mass action for association and dissociation reactions, Henri–Michaelis–Menten for phosphorylation/dephosphorylation/ubiquitination, Hills equation for gene expression) and initial concentrations (Supplementary Table S1 and S2) associated with the reactions were also defined. The reconstructed network was numerically simulated using Stiff Deterministic ODE15s solver (SimBiology toolbox) which generates the first order nonlinear ODEs (Supplementary Table S3) for each node, thus defining the mathematical structure of the model. Complex biological systems, such as the signaling network can be viewed as networks of chemical reactions that can be analyzed mathematically using ODEs, which is the most common simulation approach used in computational systems biology. ODE helps in determining time-dependent changes i.e. the time series data of the concentrations of the signaling proteins and protein complexes and thus the dynamics associated with it. The model was exported to Copasi (4.8.35) as a SBML file to generate the time series data.

2.2. Positive feedback loop insertion

It has been demonstrated that the presence of TNF in mice restores the ability to resolve the inflammatory lesion and enables an optimal control of parasite replication at the site of *Leishmania major* infection. Therefore the *in silico* network was analyzed with the introduction of a positive feedback loop, arising from the TNF pathway [23,28,29]. The synthesis of TNF from transcription factor NFκB was defined by the Hill's equation (Equation no. 43; Table S3) in the mathematical model. The introduction of the positive feedback loop in the *in silico* model will help indicate the crucial signaling protein for sustenance of the signal.

2.3. Parameter estimation

The equations defining the chemical transformation in the signaling cascade use variables as concentration of protein and mRNA (species involved in the reaction). Also these equations are dependent on the parameters like production and decay rate. Often these parameters are unknown, difficult (due to nonlinearity) and expensive to measure. In such situation parameter estimation is done i.e. the unknown parameters are determined indirectly using computational biology tools [30,31].

The parameters for reconstructed signaling network were manually trained within a range of parameter space which was further fine-tuned till the model was simulated to obtain a desired output with algorithmic data fitting due to lack of homogeneity in experimental measurements. This is an iterative process and is done till a reproducible graph depicting the biochemical reaction is obtained (Fig. 1).

2.4. Network validation

2.4.1. Network topology

It includes information about the general and specific properties of nodes, edges and modules within the network using graph theory. Cytoscape (2.8.2) was used to visualize the network topology in the form of a directed acyclic graph (DGA) and it was analyzed for network properties after simulated annealing the signaling network. Properties of nodes include degree (number of links for each node), node betweenness centrality (shortest paths that go through a node among all shortest paths between all possible pairs of nodes), and closeness centrality (average shortest path from one node to all other nodes). Properties of edges include edge betweenness centrality (number of shortest paths that go through an edge among all possible shortest paths between all the pairs of nodes) and edge directionality (indicates the upstream and downstream nodes connected by a particular link). Properties of networks include shortest path length (shortest path between all pairs of nodes), diameter and clustering coefficient (local density of interactions by measuring the connectivity of neighbors for each node averaged over the entire network) [32]. The shortest path length was computed using adjacency matrix (Fig. 2, Supplementary Table S4) [33].

2.4.2. Crosstalk points

The nonlinearity in a signaling network arises due to crosstalk between a signaling protein common to two or more pathways, which could be a crucial point of regulation and direction in response to a stimulus. In this study the network crosstalk points between CD14–TLR, TNF and EGFR was found considering the difference in the degree of the node in the network containing all considered pathways minus the maximum degree of this node in any one individual pathway. In this way a high network crosstalk value suggests that a node is a bifurcating node connecting two or more pathways [34].

2.4.3. Flux balance analysis (FBA)

FBA calculates the flow of metabolites through a metabolic network which aids in predicting the rate of production of a biologically important

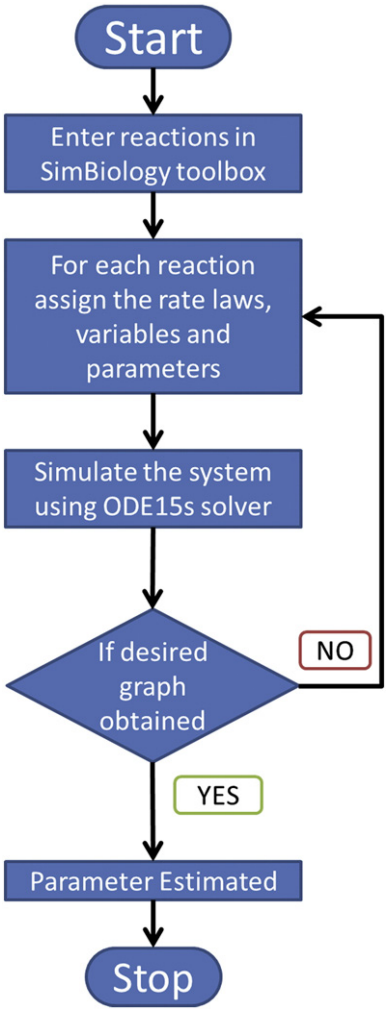

Fig. 1. Flow chart for parameter estimation.

metabolite. Though initially applied to only metabolic reactions, it is now increasingly being applied to signal transduction studies too [35]. In FBA, the mathematically represented signal transduction reactions are tabulated in the form of numerical matrix of the stoichiometric coefficients of each reaction (Supplementary Table S5). This matrix of stoichiometries enforces constraints on the flow of metabolites through the network. The flux balance constraint on the system ensures that the total amount of any compound being produced must be equal to the total amount being consumed at steady state (law of mass conservation). The imposed constraints define the space of allowable flux distributions of a system i.e. the rates at which every metabolite is consumed or produced by each reaction, which then defines the final phenotype of the system [36]. FBA was done using Copasi (4.8.35), a simulator for biochemical networks.

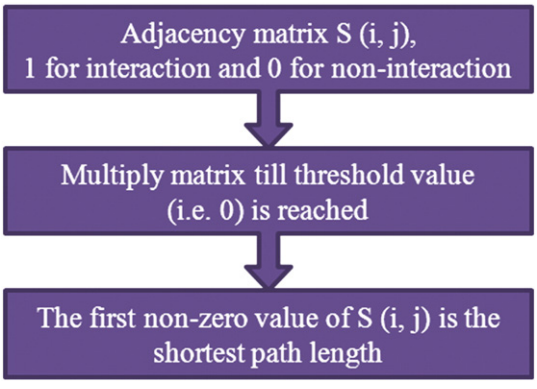

Fig. 2. Adjacency matrix.

2.4.4. Sensitivity analysis

Sensitivity analysis is used to determine how "sensitive" a model is to variables changes in the model, which helps study the uncertainties associated with the estimated parameters and thus build confidence in the model. It was calculated using the SimBiology toolbox, Sensitivity Analysis option which calculates the sensitivity coefficients by combining the original ODE system for a model with the auxiliary differential equations for the sensitivities. The additional equations are derivatives of the original equations with respect to parameters. SimBiology sensitivity analysis uses the "complex-step approximation" to calculate derivatives of reaction rates. It calculates the time-dependent sensitivities of all the species states with respect to species initial conditions and parameter values in the model. Thus, if a model has species x and two parameters y, and z, then the time-dependent sensitivities of x with respect to each parameter value can be represented as the time-dependent derivatives as Eq. (1)

$$\frac{dx}{dy}, \frac{dx}{dz} \tag{1}$$

where the numerator is the sensitivity output and the denominators are the sensitivity inputs to sensitivity analysis.

2.4.5. Principal component analysis (PCA)

PCA generates a new set of variables (principal component score), called principal components, from the multivariate data sets. Each principal component is a linear combination of the original variables. All the principal components are orthogonal to each other, so there is no redundant information. The principal components as a whole form an orthogonal basis for the space of the data. PCA was done in MATLAB using the function i.e. [score, coefficient] = princomp (A) where, "A" is the m*n matrix of sensitivity coefficients of each species in the signaling network. This command returns principal component coefficient and score of the sensitivity matrix, used to identify the principal component i.e. significant individual reactions in the signaling network.

2.4.6. Model reduction

Model reduction is a systematic method for eliminating reactions that do not contribute considerably to network output. In this study FBA and sensitivity analysis [37] and concentration at which the species shows the highest sensitivity was combined to reduce the dimensionality of the model. The concentrations were retrieved from the time series data. A reaction in the network can be deleted during model reduction if it has low sensitivity and also low flux, indicating its redundancy in the network.

3. Results

3.1. Network topology

The reconstructed signaling network has 43 interacting species (nodes) through 49 reactions (edges) governed by 82 kinetic laws (Fig. 3). It was analyzed for the network topology in Cytoscape after simulated annealing, and the network showed a clustering coefficient of 0.008 and network diameter 7. The shortest path and the average shortest path of the network is 10 and 35.6 respectively, which was calculated using the adjacency matrix. A low clustering coefficient indicates that the reconstructed network shows less interconnectivity,

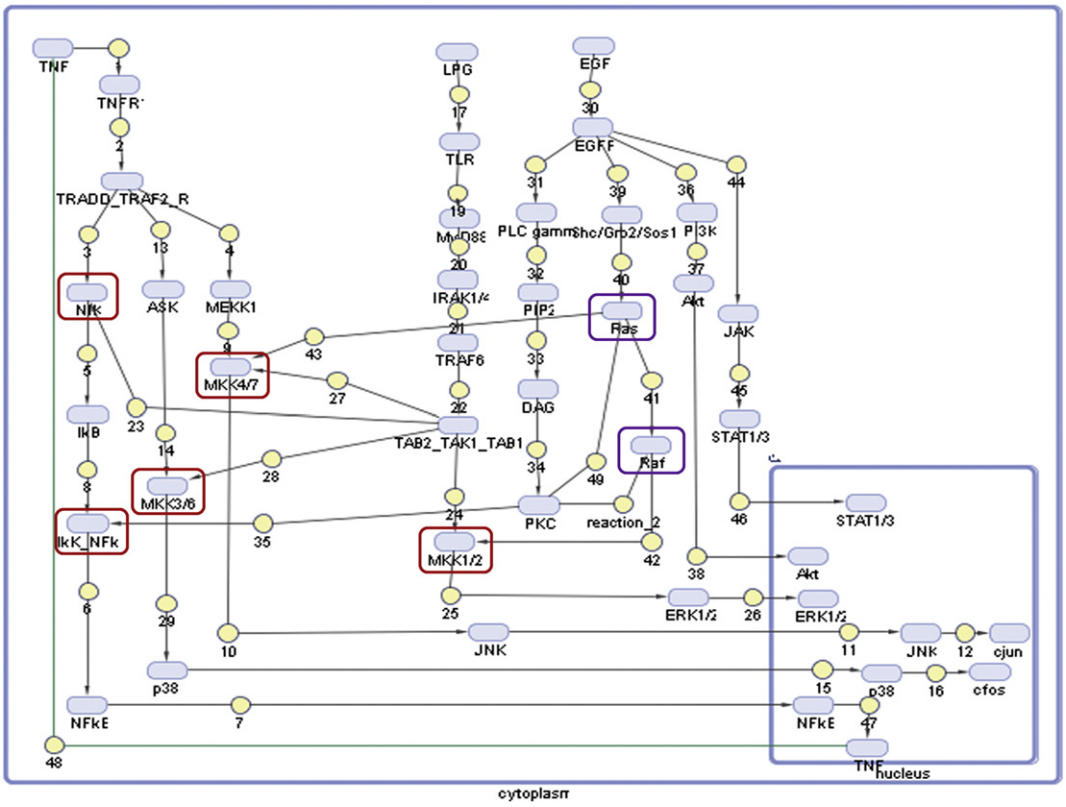

Fig. 3. Graphical representation of reconstructed signaling network comprising of TNF, TLR and EGF (crosstalk points, probable points of transgene intervention, positive feedback loop).

which helps in transmission of the signal in one direction. Also the shortest path and network diameter suggest that the time taken for converting an input to an output by the system will be less. The positive feedback loop inserted in the system is in the form of TNF (green loop in Fig. 3) an output from the CD14–TLR pathway which is negatively modulated by the parasite induced negative modulators.

3.2. Crosstalk points

Five crosstalk points were identified in the network; they are cytoplasmic NIK, IκB–NFκB, MKK4/7, MKK3/6 and MKK1/2. Of these signaling components MKK4/7 showed the highest network crosstalk value of 2 degree, while the others have a value of 1 degree.

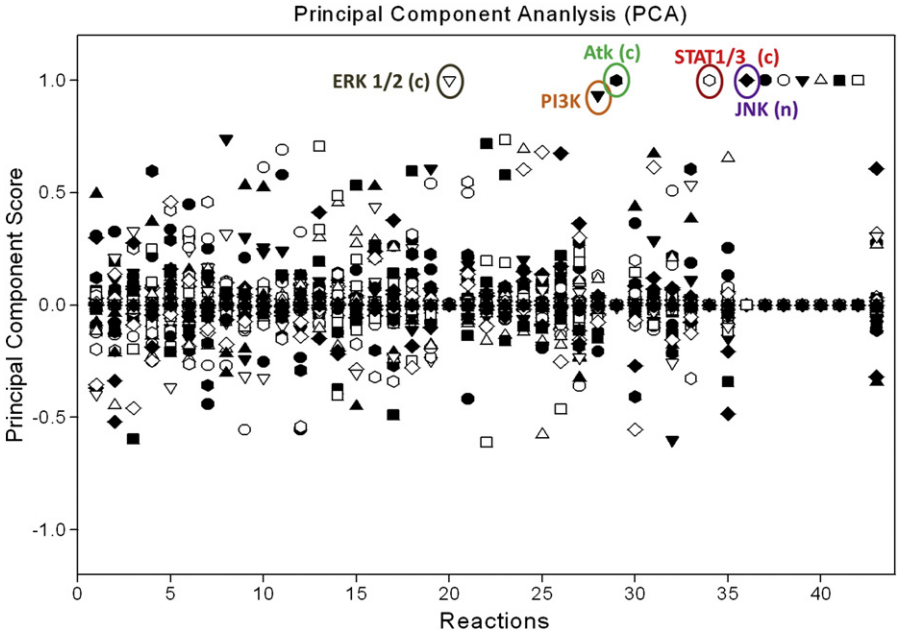

Fig. 4. Principal component analysis (PCA) of the network with positive feedback loop showing the signaling proteins with a high principal component score (positive scale is a high PCA score while negative scale is a low PCA score).

3.3. PCA

The PCA analysis of the sensitivity coefficients (multivariate data set) extracts those variables that may have a profound effect on the output of the system i.e. a positive scale is a high PCA score while negative scale is a low PCA score. For the reconstructed signaling network, the PCA (Fig. 4) shows that cytoplasmic species ERK1/2, PI3K, Akt and STAT1/3; and nuclear species JNK has a high PCA score i.e. any change in the parameters associated with these species may change the output of the system.

3.4. Flux and sensitivity analysis

A comparison of flux (nmoles/s) and sensitivity coefficients of the crosstalk points and the components showing high PCA (Fig. 5) was done. It may be noted that cytoplasmic species ERK1/2, Akt, and STAT1/3 and nuclear species JNK with high PCA score, show no sensitivity associated with them i.e. any change in the parameters related with these species may not affect the outcome. But considering sensitivity and PCA, parameters associated to two species that lie downstream of the crosstalk points (ERK1/2 downstream of MKK1/2, JNK downstream of MKK4/7) and Akt downstream of PI3K that shows high sensitivity as well as PCA score, should not be over looked and can be considered for further interventions.

3.5. Model reduction

We came across five crosstalk points in the network; they are cytoplasmic NIK, IκB–NFκB, MKK4/7 and MKK3/6, MKK1/2. On positive feedback loop introduction in the network, the crosstalk signaling components show high flux (nmoles/s) and sensitivity, also PI3K belonging to the EGFR pathway shows high flux, sensitivity and PCA score in the network. Model reduction serves as an important step to get an insight into the signaling pathways and eliminate several unimportant parameters for which the experimental estimation seems to be unfeasible. It was done, taking into account the flux (nmoles/s), sensitivity and concentration (nmoles) (Supplementary Table S6) of the signaling components and it suggests that of all the 125 parameters (32 reaction velocities, 49 rate constants and 1 exponent from Table S1; 43 initial concentrations from Table S2) from the proposed model analyzed, 80% of the parameters can be rejected without significant changes on the model prediction. Further 20% parameters (Supplementary Table S7) were focused to have significant influence on the systems, although the overall sensitivity is low. The 3D mesh plot (Fig. 6) shows the reaction parameters that show a significant effect on the model with a higher value i.e. in the range from 1 to 1.5. Although the metabolically structured model was affected, the dynamics of the network are generally well represented. It can also be emphasized by comparing the simulation results of the original and reduced model that the species trajectories did not change considerably with time.

Our model reduction preserves the modularity in terms of input–output behavior of each of the modules as they are using detailed analysis and simulations. In a nutshell, the approach laid maintains the network structure, retains the steady states, and keeps track of the errors introduced in the reduction but does not allow the system to switch on its dynamics to the fast transient state.

4. Discussion

The negatively modulated pathways can be controlled through Ras and Raf that lie upstream of the crosstalk points MKK3/6, MKK4/7 and IκK–NFκB via a synthetic gene circuit for activating cell–cell communication to elicit an inflammatory response. Also a disease resolving effect

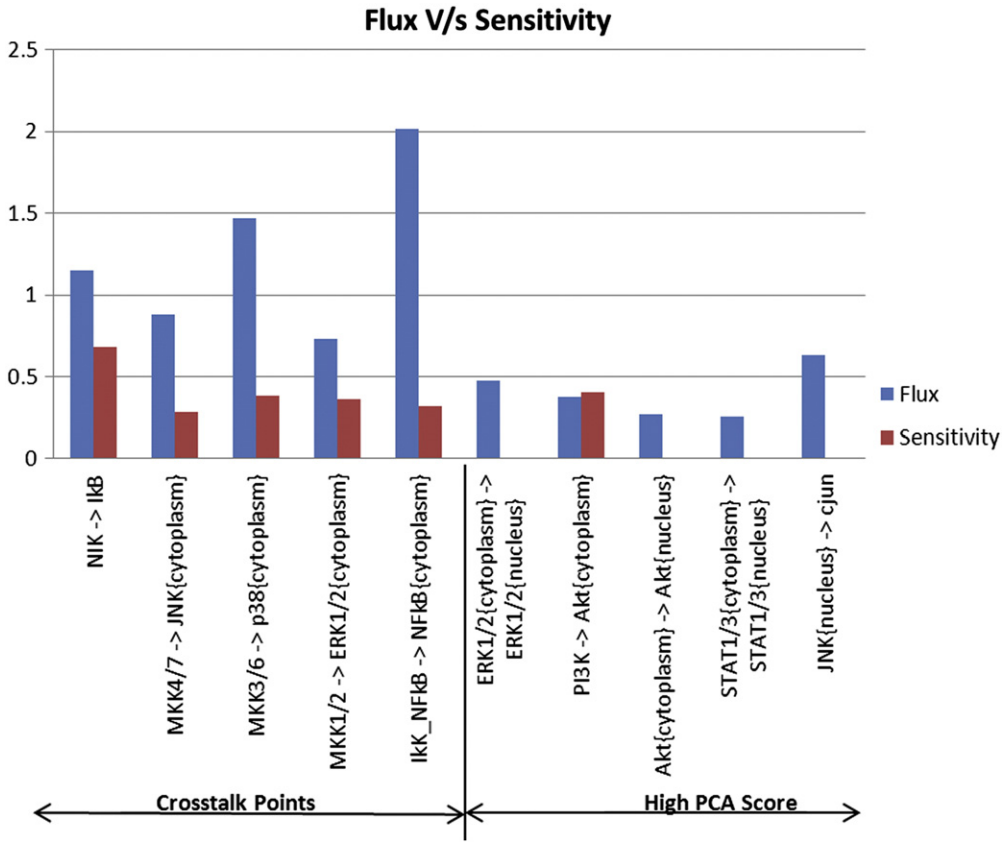

Fig. 5. Flux V/s Sensitivity of the signaling components at the crosstalk point and those having high PCA score.

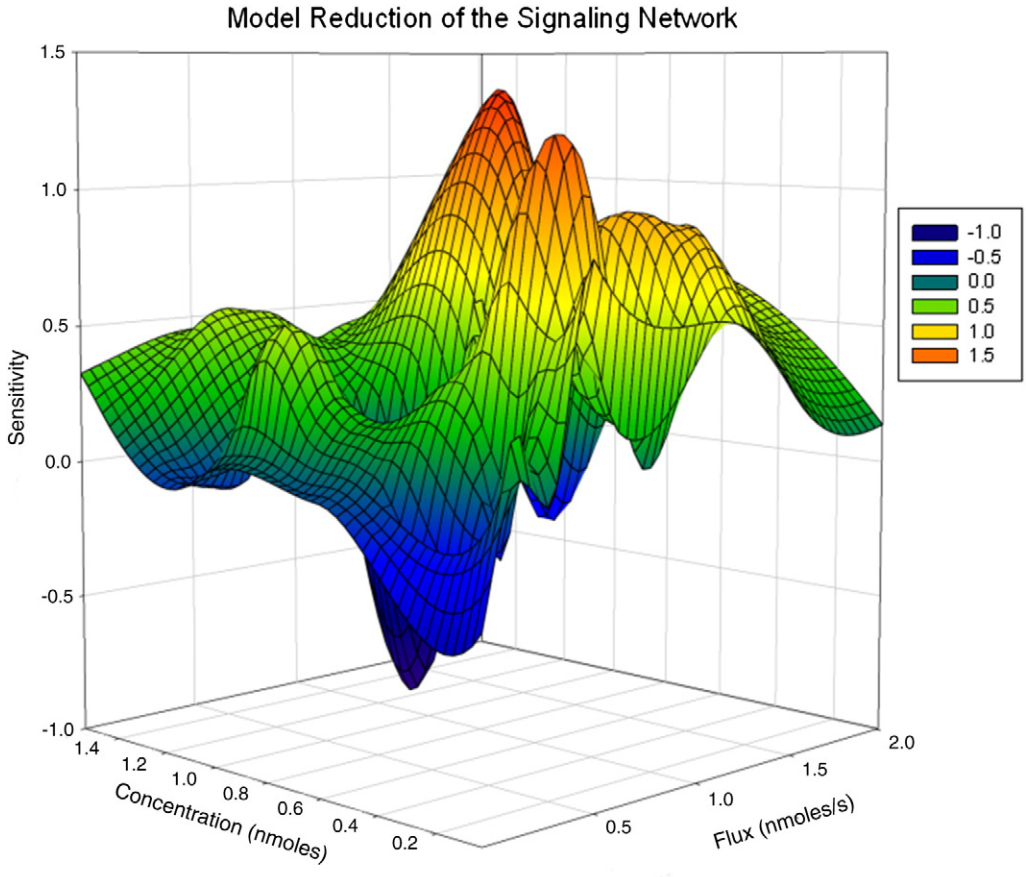

Fig. 6. 3D mesh plot for model reduction shows the relation between flux, sensitivity and concentrations of each of reactions in the reconstructed signaling pathway. Higher value i.e. ranging from 1 to 1.5 is the reaction parameters that show a significant effect on the model.

may be achieved through PI3K in the EGFR pathway. In leishmaniasis, anti-inflammatory cytokines like IL10, 4 and TGFβ are produced, which can be used as an input signal for triggering the synthetic gene circuit (Fig. 7). To the best of our knowledge, this is the first attempt to construct a mathematical model incorporating the modulated macrophage immune signaling in leishmaniasis and EGFR, through major MAPKs crosstalk points. From this point onwards we propose, that this reconstructed signaling model can be combined with a synthetic gene circuit (gene signaling circuit), incorporating a positive feedback loop for activation of EGFR during *Leishmania* infection, which may help rewire the negatively regulated CD14 and TNF, through the crosstalk points, lying down stream of the modulated signaling proteins in these pathways. A myriad of repressors, promoters, activators and enzymes may be considered as modules for designing the synthetic circuit targeting the immune signal response in leishmaniasis with the insertion of feedback loop mechanism. This may allow the shutoff of the immune evading strategies and enable us to design a biomaterial for application in biomedical application. We also aim to achieve a concerted biological behavior in a population of immune cells that is synchronized through intercellular communication as shown by Matsuda et al. [38] who used synthetic biology approach to understand the signaling dynamics and assign function to network motifs using relatively simple genetic circuit, to propagate a signal across a cell population if certain conditions are met: specifically — sufficient amplification of the signal and proper matching of promoter strengths. Thus, the introduction of synthetic gene circuit with a positive feedback loop linked to a cellular process such as macrophage immune signaling could provide a precise fine-tuning of transgene expression under a diseased condition. The design of a gene circuit could use an output from one process as an input signal to achieve tunable artificial cell–cell communication. This synthetic cellular circuitry behavior response can be observed through a systems biology approach to be proved *in vitro* and *in vivo* conditions in mammalian cells to attain simplicity and tunability at different stages of infectivity in leishmaniasis with time delay response in the circuitry.

5. Conclusion

This artificial cell-to-cell communication system inspired by the natural regulatory mechanism in mammalian macrophages during an infection could serve as a versatile tool for regulated gene expression through gene regulatory networks. Since, macrophages are highly flexible and adaptive cells of the immune system and are sensitive to activation by direct sensing of pathogen during an infection, using them as a possible therapeutic intervention system is an attractive proposition. Computational modeling of signaling pathways in the macrophages and regulating them through key players will help us understand the molecular, genetic and proteomic function of these protein components in relation to inflammation in infectious disease. Moreover, the computational tool offers us an advantage of analyzing the signaling cascade in terms of its stability and robust performance.

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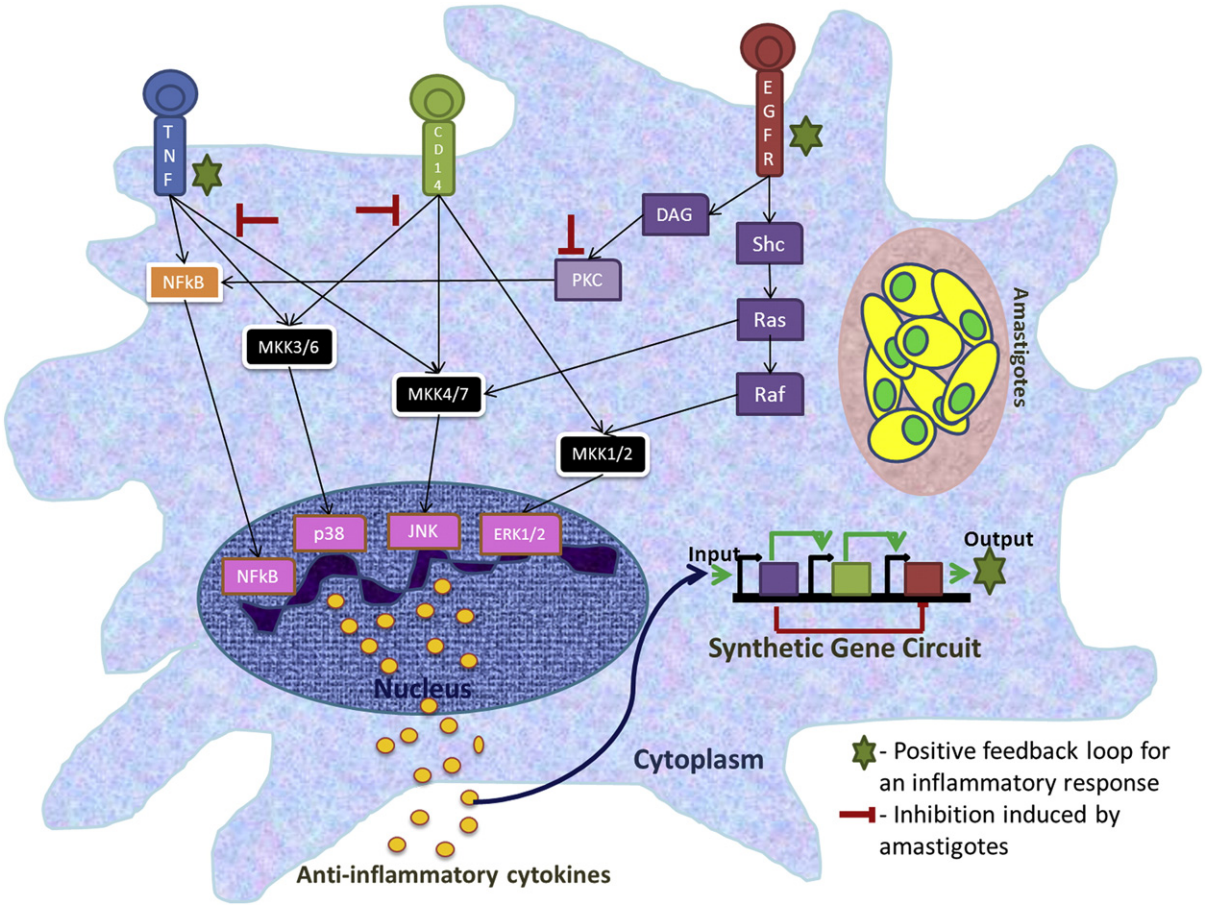

Fig. 7. Proposed experimental model: Modulation of the immune signaling by the leishmanial parasite leads to the production of anti-inflammatory cytokines which can act as inducers for activating the positive feedback loop, such that there is activation of the EGFR (through Ras, Raf and PI3K) and the TNF pathway for immune cell–cell interaction leading to an inflammatory, disease resolving outcome.

This model was deposited in BioModels Database [39] and assigned the identifier MODEL1308080000.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagen.2013.08.018.

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