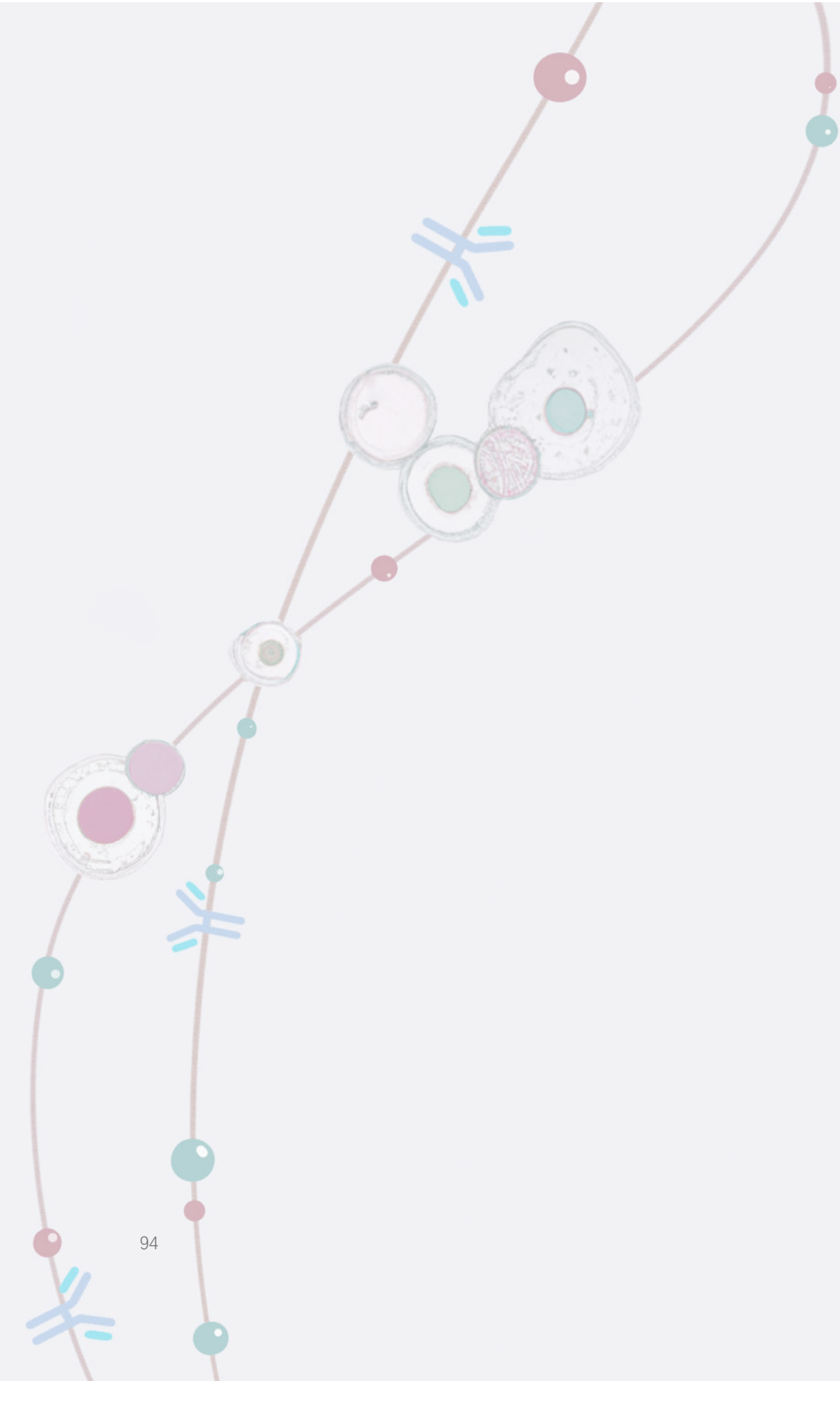
# Quantitative pharmacological modelling for optimizing treatment of sepsis

Liu, F.

## Section III

# Inflammation and biomarkers



## Chapter 4

# A system pharmacology Boolean network model for the TLR4-mediated inflammatory response in early sepsis

**Author**

Feiyan Liu

Linda B.S. Aulin

Sebastiaan S.A. Kossen

Julius Cathalina

Marlotte Bremmer

Amanda C. Foks

Piet H. van der Graaf

Matthijs Moerland

Johan G.C. van Hasselt

*J Pharmacokinet Pharmacodyn 2022 (49), 645-655.*

**Abstract**

Sepsis is a life-threatening condition driven by the dysregulation of the host immune response to an infection. The complex and interacting mechanisms underlying sepsis remain not fully understood. By integrating prior knowledge from literature using mathematical modelling techniques, we aimed to obtain deeper mechanistic insight into sepsis pathogenesis and to evaluate promising novel therapeutic targets, with a focus on Toll-like receptor 4 (TLR4)-mediated pathways. A Boolean network of regulatory relationships was developed for key immune components associated with sepsis pathogenesis after TLR4 activation. Perturbation analyses were conducted to identify therapeutic targets associated with organ dysfunction or antibacterial activity. The developed model consisted of 42 nodes and 183 interactions. Perturbation analyses suggest that over-expression of tumour necrosis factor alpha (TNF-α) or inhibition of soluble receptor sTNF-R, tissue factor (TF), and inflammatory cytokines (IFN-γ, IL-12) may lead to a reduced activation of organ dysfunction related endpoints. Over-expression of complement factor C3b and C5b led to an increase in the bacterial clearance related endpoint. We identified that combinatory blockade of IFN-γ and IL-10 may reduce the risk of organ dysfunction. Finally, we found that combining antibiotic treatment with IL-1β targeted therapy may have the potential to decrease thrombosis. In summary, we demonstrate how existing biological knowledge can be effectively integrated using Boolean network analysis for hypothesis generation of potential treatment strategies and characterization of biomarker responses associated with the early inflammatory response in sepsis.

## Introduction

Sepsis is a complex syndrome with high morbidity and mortality associated with multi-organ dysfunction driven by the host inflammatory response to an infection. The initial inflammatory response is mainly activated by pattern recognition receptors, where Toll-like receptor 4 (TLR4) activation is one of the key receptors associated with Gram-negative bacterial infections commonly producing sepsis[1][2]. Organ dysfunction is a major cause of sepsis-associated mortality and morbidity, although the underlying mechanisms for these effects are only partly understood [3]. Besides treatment with antibiotics, very limited treatment options are currently available for sepsis. Considerable efforts in the past decades towards developing novel therapeutics against sepsis have failed during clinical trials [4][5]. The complexity of underlying immune system interactions in sepsis in relation to harmful effects on organ systems may be an important reason for these failures, warranting more holistic approaches.



A wealth of knowledge of isolated cellular and biochemical processes and their interactions associated with inflammation and sepsis is available in literature, but the utility of this is hampered by a lack of integration. To this end, the use of mechanistic mathematical modelling may help to integrate this knowledge in order to rationalize the design of treatment strategies, and the discovery of novel biomarkers that may be used to stratify patients and individualize therapies[6][7]. Indeed, quantitative ordinary differential equation models have been used extensively in systems biology and systems pharmacology for this purpose. However, a requirement for constructing such models is the availability of kinetic parameters, which are lacking for various sepsis and inflammation associated interactions and processes.

Boolean network (BN) models offer an attractive mathematical modelling strategy where inhibitory and stimulatory interactions that are commonly available in literature can be utilized, allowing a much more comprehensive integration of available biological knowledge. BN modelling approaches have been used previously to describe the behaviour of complex systems and to support identification of treatment targets[8][9]. Briefly, a BN model consists of nodes and edges. Nodes can have an active or inactive state and typically represent biological

components such as cells, mediatory molecules or genes[10]. Edges represent the interactions between the different nodes. The BN network is defined according to logic functions that determine the activation state of each node, which will also depend on the activation state of other nodes in the network. Within specific Boolean modelling tools, e.g. *SPIDDOR*[10], interactions between components can also be refined to cause specific activation, inhibition, and modulation of the nodes. Performing simulations with BNs can be used to identify stable states (known as attractors) of the system, which may be considered to correspond to phenotypes[11], thus providing insight into the probability of activation of endpoint nodes with clinical relevance. Comparing the attractors under different perturbations of nodes alone or in combination may be used to identify novel treatment strategies[12].

The aim of this study is to identify cellular or mediator-specific factors which modulate key clinically-relevant endpoints of sepsis, either as explanatory factors of inter-individual variation in treatment outcome, or, as target for potential mono- and combination treatment strategies. To this end, we developed a BN model for the TLR4-mediated host inflammatory response that plays an important role in the systemic inflammatory response in the early phase of sepsis.

## Methods

### *Model development*

An extensive literature search was performed in order to build the Boolean network model for TLR4-mediated sepsis. We collected experimental *in vitro* and *in vivo* data on activation or inhibitory events between key immune cells, intracellular signalling mediatory molecules such as inflammatory cytokines and membrane receptors, and sepsis pathogenesis endpoints including bacterial phagocytosis and thrombosis. The development of the initial version of the model was guided by several comprehensive reviews of the inflammatory response after TLR4 activation and sepsis, from where we systematically searched for each cell and/or mediator for all relevant additional interactions. The BN model was created by translating regulatory interactions between identified cells, receptors and molecules into Boolean functions: interactions of activation or inhibition between nodes were described as flexible combinations of Boolean operators *AND*, *OR* and

*NOT* in a mathematical expression. The network was visualized using Cytoscape (v3.8.2)[13].

***Model implementation***

The Boolean network analysis was performed in R (v 4.1.2) using the package SPIDDOR (v 1.0)[10], which has multiple essential functionalities for capturing the behaviour of immune responses. One of these functionalities is the introduction of time delays in the network interactions. Such delays are incorporated using threshold arguments (THR) that represent lag times for the initiation of node activation or inhibition. In addition, SPIDDOR allows for modulating the intensity of the activations and inhibitions of the network, by adding a duration for these interactions to occur[10]. In that sense, a regulator node could activate or inhibit the regulated node for only some time steps in the simulation.



To capture the stochasticity associated with biological systems, an asynchronous updating method was implemented for the simulations. This method assumes that only one node can be updated in a single time step and every node is equally likely to be updated[14]. In a BN simulation, each node is updated according to its Boolean function over the time steps, to either remain in, or switch to, one of the two possible states: 0 (inactive) or 1 (activated). The initial state of this BN is the onset of infection (i.e. *Infection* = 1, all other nodes = 0). The state sets of attractors for each simulated scenario, i.e. the percentage of activation (% activation) of each node in 100 repetitions, were used as readout.

***Simulation endpoints***

Simulation scenarios were evaluated based on two types of endpoints: the ability of the immune system to fight the infection, through endpoint nodes *Phagocytosis* and *membrane attack complex (MAC)*, and endpoints associated with organ damage, i.e. *Thrombosis* and *angiopoietin-2 (Ang2)*. These aspects could be indirectly represented by nodes in the Boolean network. The extent of activation of these endpoint nodes was used to assess the effect of perturbations of the network.

***Node activation analysis to explore inter-individual variation in clinical endpoints***

We studied how variation in immune cell nodes activation can explain differences in activation of selected endpoint nodes to better understand potential causes for variation in clinical outcomes between patients. To this end, we performed a sensitivity analysis by specifying the % activation of each immune cell node in the network from 0% to 100% activation. The sensitivity analysis allowed us to investigate the impact of immune components on clinical endpoint node activation. These analyses were implemented using the polymorphism functionality in SPIDDOR, which modifies the fractional activation patterns of nodes. For instance, when a polymorphism of 50% activity is introduced in a node, this node is only activated 50% of the times in which its regulator nodes are activated[10], therefore, decreasing the normal activity of the node by 50%.

***Perturbation analysis to identify novel mono- and combination treatment targets***

Mediatory molecules such as pro-inflammatory cytokines TNF-α and IL-1 are commonly investigated as therapeutic targets in drug development for sepsis[5]. For this analysis, we evaluated the potential of targeting each individual mediatory molecule that was included in the final network. Perturbations were performed via knocking-out or over-expressing a certain mediator node, either at infection onset or at a later stage of the infection until the activation of all nodes in attractors would not change over time steps. We then repeated this analysis where we modulate two nodes at the same time to study the effect of a combination treatment. Here, either we targeted to mediator nodes, or we combined modulation of a mediator with inhibition of the bacterial node to mimic antibiotic treatment. The resulting endpoints activations of attractors were compared to their activations without perturbation. An efficacy cut-off of 20% for the relative activation change between perturbed and non-perturbed scenarios was used to identify promising therapeutic targets.

## Results

***Boolean network development***

A Boolean network (**Fig 4.1**) associated with early phase TLR4-mediated sepsis was informed by data extracted from 108 publications (**Table S4.1)**. The developed BN consisted of 42 nodes and 183 interactions. The underlying Boolean functions are

defined further in **Table 4.1** and **Table S4.1**. The developed network describes several different mechanisms underlying the disease progression of sepsis, including the regulation of immune cells, endothelial cells, complement and coagulation cascades, which contribute to bacterial clearance but may also lead to activation of harmful effects associated with organ damage.

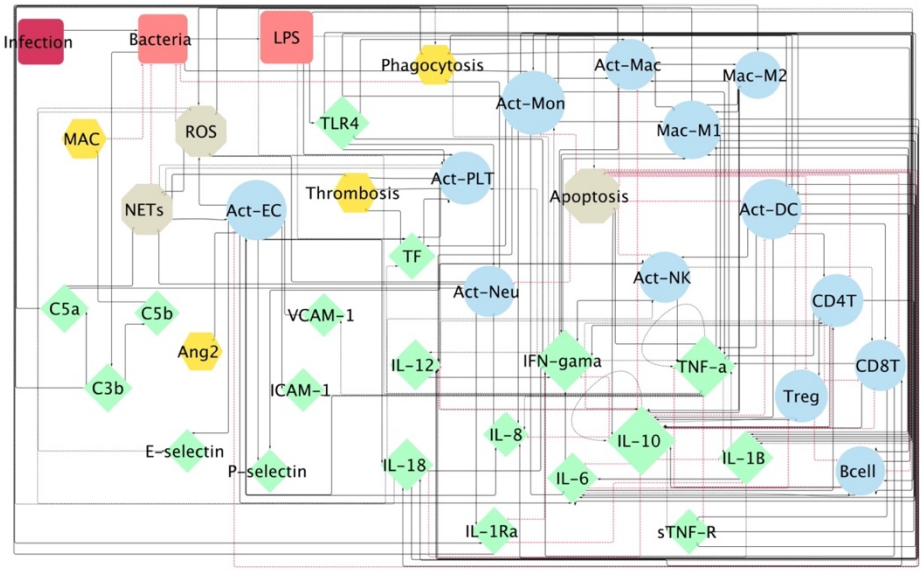



**Fig 4.1 Boolean network model structure representation for the TLR4 activation in early sepsis.** Shapes and colors represent different node types, including 3 pathogen related nodes (in red), 13 host cell nodes (in blue), 19 mediator nodes (in green), 4 selected outcome nodes (in yellow) and 3 other nodes (in grey). Size of nodes represents the number of interactions related to a certain node, with the bigger size indicating more interactions. Lines represent the regulations where black solid lines for activation, black dashed lines for positive modulation including auto-secretion and red dashed lines for inhibition.

The network used modulations to account for changes in expression, auto-secretion and feedback relationships in a more refined manner (**Fig 4.1**). Threshold parameters (see Methods) were applied to account and differentiate biological time delays for different events, including the clearance of bacteria (*B_CL*), early and late phagocytosis (*Phag_E* and *Phag_L*), cell apoptosis (*Apop*), production of tissue factor (*T_TF*), formation of membrane attack complex (*T_MAC*) and the release of anti-inflammatory cytokines (*Anti_inflam*). Thresholds were set to two time steps

to represent the binding and functioning steps, while the threshold relating to early phagocytosis was set to one since it occurs earlier than the phagocytosis caused by other immune cells. The threshold of anti-inflammatory cytokines production, mainly IL-10, was set to three due to an additional required signal transduction for the cytokine synthesis[15].

**Table 4.1** Boolean functions of the developed model for TLR4-mediated early sepsis.

| ***Nodes*** | **Boolean functions[1]** |
|---|---|
| *Infection* | Infection = Infection |
| *Bacteria* | Bacteria = Infection &! (Bacteria & (THR_MAC[B_CL][2] \| THR_Phagocytosis[B_CL] \| THR_ROS[B_CL] \| THR_NETs[B_CL])) |
| *LPS* | LPS = Bacteria |
| *TLR4* | TLR4 = LPS \| (TLR4 & IFN-gamma) |
| *Act-Mon* | Act-Mon = (TLR4 \| IL-1B \| (Act-Mon & IFN-gamma)) &! (Act-Mon & (Apoptosis &! IFN-gamma)) |
| *Act-Mac* | Act-Mac = (TLR4 \| Act-Mon \| C5a \| (Act-Mac & (TNF-a \| IFN-gamma)) \| (IFN-gamma & TNF-a) \| (Act-Mon & IL-1B)) &! (Act-Mac & (IL-10 \| (Apoptosis &! IFN-gamma))) |
| *Mac-M1* | Mac-M1 = (Act-Mac & (TNF-a \| IFN-gamma)) \| (Act-Mon & IL-1B) \| (Mac-M2 & (TNF-a \| IFN-gamma \| IL-1B)) |
| *Mac-M2* | Mac-M2 = (Act-Mac & (IL-10 \| IL-1Ra)) \| (Mac-M1 & (IL-10 \| IL-1Ra)) |
| *Act-DC* | Act-DC = (TLR4 \| Act-Mon) &! (Act-DC & (Treg \| IL-10 \| Apoptosis)) |
| *Act-Neu* | Act-Neu = (TLR4 \| C5a \| IL-8) &! (Act-Neu & Apoptosis) |
| *Act-NK* | Act-NK = (IL-12 \| Act-DC \| (Act-NK & (IL-12 & IL-18))) &! (Act-NK & Apoptosis) |
| *Act-EC* | Act-EC = (TNF-a \| NETs) &! (Act-EC & Apoptosis) |
| *Phagocytosis* | Phagocytosis = (THR_C3b[Phag_L] & THR_Bacteria[Phag_L]) \| THR_Act-Mon[Phag_L] \| THR_Act-Mac[Phag_L] \| THR_Act-Neu[Phag_E] \| THR_Act-DC[Phag_L] \| (Phagocytosis & IFN-gamma) \| (Phagocytosis & IL-18) |
| *Apoptosis* | Apoptosis = THR_TNF-a[Apop] \| THR_Bcell[Apop] \| (Apoptosis & LPS) |
| *ICAM-1* | ICAM-1 = Act-EC \| (ICAM-1 &TNF-a) |

| | |
|---|---|
| *VCAM-1* | VCAM-1 = Act-EC \| (VCAM-1 & TNF-a) |
| *E-selectin* | E-selectin = Act-EC \| (E-selectin & ROS) |
| *P-selectin* | P-selectin = Act-EC \| Act-PLT |
| *NETs* | NETs = Act-Neu \| ROS \| (Act-Neu & C5a) \| (NETs & Act-PLT) |
| *Act-PLT* | Act-PLT = LPS \| TLR4 \| TF \| Thrombosis \| (Act-PLT & (NETs \| IFN-gamma)) |
| *TF* | TF = THR_Act-Mon[T_TF] \| (TF & (THR_Act-EC[T_TF] & (TNF-a \| LPS))) \| (TF & (THR_Act-Mon[T_TF] & (TNF-a \| LPS))) |
| *Thrombosis* | Thrombosis = NETs & (TF & Act-PLT) |
| *C3b* | C3b = Bacteria |
| *C5a* | C5a = C3b |
| *C5b* | C5b = C3b |
| *MAC* | MAC = THR_C5b[T_MAC] |
| *ROS* | ROS = Act-Neu \| (Act-Mac \| Mac-M1) \| Act-EC \| (ROS & (TNF-a \| IL-18)) |
| *Ang2* | Ang2 = Act-EC |
| *TNF-a* | TNF-a = (Mac-M1 \| Act-Mon \| Act-NK \| Act-DC \| CD4T \| CD8T \| (TNF-a & (IFN-gamma \| Act-Mac \| ROS )) \| (IL-1B & Act-EC)) &! (TNF-a & ((IL-10 &! IFN-gamma) \| sTNF-R)) |
| *IL-1B* | IL-1B = (Act-Mon \| Mac-M1 \| (IL-1B & (TNF-a \| Act-Mon \| Act-PLT))) &! (IL-1B & (IL-10 \| IL-1Ra)) |
| *IFN-gamma* | IFN-gamma = (Act-NK \| (IFN-gamma & (Act-DC & IL-12)) \| (Mac-M1 & (IL-12 & IL-18)) \| ((CD4T \| CD8T) & (IL-12 \| (IL-12 & IL-18))) \| (IFN-gamma & (CD4T & IL-6))) &! (IFN-gamma & IL-10) |
| *IL-6* | IL-6 = ((Act-Mon & IL-1B) \| Mac-M1 \| Act-EC \| Act-DC \| Bcell) &! (IL-6 & IL-10) |
| *IL-8* | IL-8 = (Act-Mon \| Mac-M1 \| Act-EC \| (IL-8 & TNF-a)) &! (IL-8 & IL-10) |
| *IL-12* | IL-12 = (Act-Mon \| Mac-M1 \| Act-DC \| (IL-12 & (Act-NK \| IFN-gamma \| IL-1B))) &! (IL-12 & IL-10) |
| *IL-18* | IL-18 = (Mac-M1 \| Act-DC \| Act-EC) &! (IL-18 & IL-10) |
| *IL-10* | IL-10 = (THR_Mac-M2[Anti_inflam] \| THR_Act-DC[Anti_inflam] \| (THR_CD4T[Anti_inflam] \| THR_CD8T[Anti_inflam] \| |

| | |
|---|---|
| | THR_Treg[Anti_inflam] \| THR_Bcell[Anti_inflam]) \| (IL-10 &(Act-DC \| IL-12)) \| THR_Apoptosis[Anti_inflam]) &! (IL-10 & IFN-gamma) |
| *sTNF-R* | sTNF-R = THR_Act-Mon[Anti_inflam] \| THR_CD4T[Anti_inflam] \| THR_CD8T[Anti_inflam] \| (sTNF-R & IL-10) |
| *IL-1Ra* | IL-1Ra = (THR_Act-Neu[Anti_inflam] \| THR_Act-Mon[Anti_inflam]) &! (IL-1Ra & IFN-gamma) |
| *CD4T* | CD4T = (Act-DC \| IL-6 \| (CD4T & (IL-12 \| IFN-gamma))) &! (CD4T & (IL-10 \| Treg \| Apoptosis)) |
| *CD8T* | CD8T = (Act-DC \| IL-18 \| (CD8T & Act-NK)) &! (CD8T & (Treg \| IL-10 \| Apoptosis)) |
| *Treg* | Treg = (CD4T \| (Treg & IL-10)) &! (Treg & (IL-6 \| Apoptosis)) |
| *Bcell* | Bcell = (TLR4 \| (Act-DC & LPS) \| (Bcell & IL-6)) &! (Bcell & (Treg \| IL-10 \| Apoptosis)) |

[1]Boolean functions were mathematical expressions with different nodes and flexible combinations of logic operators AND (&), OR (|) and NOT (!), where "&" and "|" mainly represented for different activation mode and "!" for inhibition. For example, the Boolean function for node bacteria, "Bacteria = Infection &! (Bacteria & (THR_MAC[B_CL]** | THR_Phagocytosis[B_CL] | THR_ROS[B_CL] | THR_NETs[B_CL]))", means bacteria appears upon infection while either membrane attack complex or host cell phagocytosis or reactive oxygen species or neutrophil extracellular traps works to clear bacteria with certain time delays. Definitions of all nodes and related regulatory interactions were shown in supplemental **table S4.1**.
[2]Threshold arguments were shown in [ ] referring to time delay, where for bacterial clearance (B_CL), late phagocytosis (Phag_L), apoptosis (Apop), membrane attack complex (T_MAC) and tissue factor (T_TF) the thresholds were set as 2, for early phagocytosis (Phag_E) and anti-inflammatory markers (Anti_inflam) thresholds were set as 1 and 3, respectively.

### *Node activation analysis to explore inter-individual variation in clinical endpoints*

We performed a sensitivity analysis to evaluated the impact of node activation alterations on innate and adaptive immune cell nodes as well as activated endothelial cells, by performing simulations where we decreased the activation of these nodes a 10% in each simulation and then compared the effect caused on the endpoints with the state of these endpoints on attractors with no alteration (100% activation). As a result, we identified three cell nodes whose activation situation had considerable effect on the selected endpoints: (1) activated endothelial cells (*Act-EC*) on angiopoietin-2 (*Ang2*), (2) activated monocytes (*Act-Mon*) on

thrombosis (*Thrombosis*), and (3) activated platelets (*Act-PLT*) on thrombosis (*Thrombosis*) (**Fig 4.2)**.

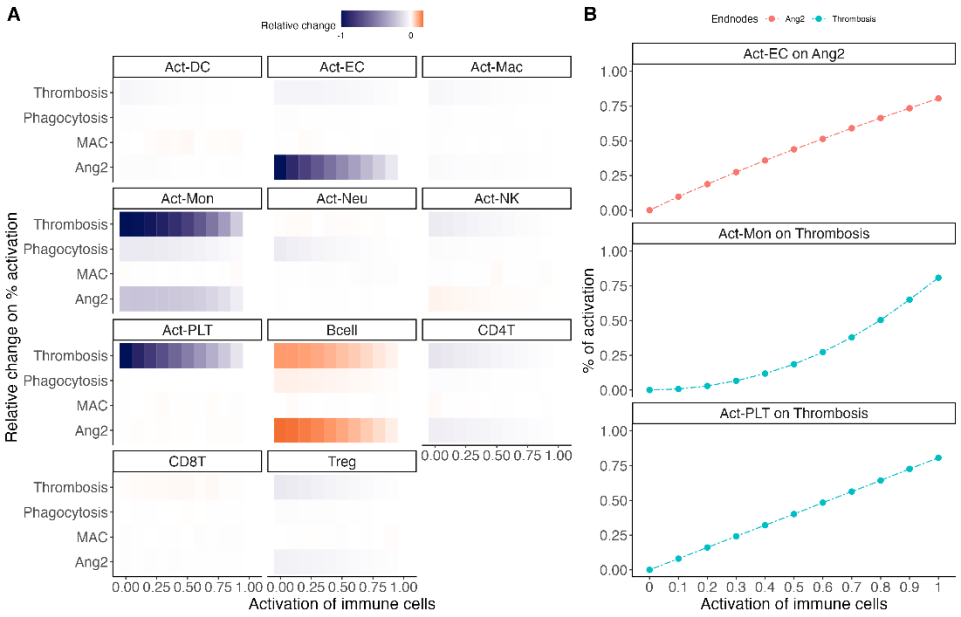



**Fig 4.2 Sensitivity analysis of the effect of immune cells activation on four selected endpoints.** The heatmap (A) showed the effect of decreased activation of different immune cells, ranging from 0% (deactivated) to 100% (normal activation), on four endpoints compared with their normal activation pattern (100% activation), colors of the heatmap represented the negative, neutral and positive relative changes of endpoints % activation on attractors with blue, white and orange, respectively; The scatter plot (B) with lines showed three identified effects of immune cells on selected endpoints: of activated endothelial cells (*Act-EC*) on angiopoietin-2 (*Ang2*), and activation variations of activated monocytes (*Act-Mon*) and activated platelets (*Act-PLT*) on thrombosis (*Thrombosis*). Effect of B cells (*Bcell*) activation on *Thrombosis* and *Ang-2* were not identified due to the small relative changes of % activation of endpoints on attractors.

The activation level of endothelial cells was positively correlated with angiopoietin-2 activity, with higher activation of *Act-EC* as initial state leading to a higher activation of *Ang2* on attractors. This finding is in line with previous studies, where activated endothelial cells have been shown to release more angiopoietin-2 into circulation during inflammation compared to non-inflammatory condition[16]. The risk of thrombosis, i.e., activation of the *Thrombosis* node in our network, was correlated with increasing monocyte and platelet activation. These two cell types

play key roles in thrombosis as monocytes are the direct production source of tissue factor (TF)[17], while tissue factor and platelet activation form the very foundation of thrombotic events. The increased activation of platelets could partly explain the increased risk of thrombosis and thromboembolism seen in the elderly patients[18].

***Perturbation analysis to identify novel mono- and combination treatment targets***

We compared the relative change (Eq. 4.1) in % activation of endpoints between scenarios with different perturbation initiation times. In this analysis, we found that the relative changes were similar over perturbation initiation time in both singular and combination perturbation analysis. The result may indicate that variation in timing of the perturbation does not lead to relevant differences on the % activation on attractors of our selected endpoints (**Fig S4.1A-B**).

$$Relative\ change = \frac{\%\ activation\ under\ perturbation - \%\ activation\ without\ perturbation}{\%\ activation\ without\ perturbation} \quad \text{(Eq. 4.1)}$$

We identified a set of potential mono-therapeutic targets that were associated with a decreased activation of *Ang2* and *Thrombosis* and/or to increase *MAC* (**Fig 4.3A**). Two targets (*sTNF-R* and *TNF-a)* were identified for *Ang2*, six targets (*IL-12*, *sTNF-R*, *IFN-gama*, *TNF-a* and *TF*) were selected for *Thrombosis*, and two targets (*C3b* and *C5b*) were selected for *MAC*. No single perturbation displayed an impact on *Phagocytosis* based on our evaluation criteria. Furthermore, we found that either over-expressing tumour necrosis factor alpha (TNF-α) or blocking soluble TNF receptor (sTNF-R) could lead to a reduction of both of the organ dysfunction endpoints (*Ang2* and *Thrombosis*). Blocking TLR4, TF or inflammatory cytokines interferon (IFN)-γ or interleukin (IL)-12 could reduce the risk of thrombosis but showed no beneficial effect on activation of *Ang2*. For the bacterial clearance related endpoint *MAC*, the over-expression of complement component C3b and C5b showed to increase its average long-term activation. This is in line with the well-established role of C5b as an essential composition of membrane attack complex (MAC) and that the cleavage of C5 to C5b requires C3b[19]. Although the interaction between the complement system and MAC is not an unexpected finding, it adds towards building confidence in the model predictions.

We identified a total of six multi-target treatment strategies that showed potential benefit (**Fig 4.3B**) in which one combination, by blocking IFN-γ and IL-10 together, could reduce both the risk of thrombosis and vessel leakage which is represented by activation of node *Ang2* based on our network. Another combination shown to decrease the activation of *Ang2* was blocking cytokines IL-10 and IL-12 together. Three of all the other four combinations to decrease the activation of *Thrombosis* included targeting TLR4, while the last one relied on the simultaneous blocking of IL-1β and IL-18. For therapy directed towards improving bacteria clearance by increasing *MAC* or *Phagocytosis*, no effective combinations were identified.

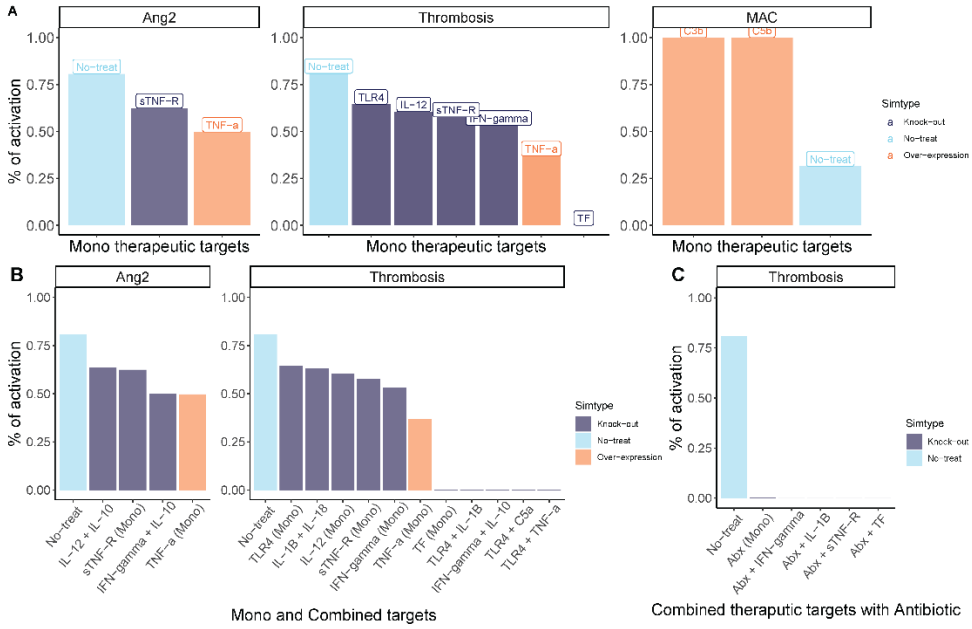



**Fig 4.3 Therapeutic targets identified through perturbation analyses.** The bar plots A represented the effect of selected mono-therapeutic targets on endpoints; B represented the effect of both mono and combine-therapeutic targets on their corresponding endpoints; C represented the effect of antibiotic and/or combined therapeutic target with antibiotic on endpoint thrombosis. Colors of the bar plots represented the no perturbation, knocking out and over expression with blue, dark blue and orange, respectively.

When combining an immune targeting therapy with antibiotic treatment, where the antibiotic has a rapid and direct effect on bacterial clearance, the time of initiation of treatment is of importance. The effect of clearing bacteria on our

selected endpoints differed over time and showed to be most beneficial during the early stage of infection (i.e. before 4 time steps, **Fig S4.2**). This finding adds to the evidence of rapid initiation of antibiotic therapy improves outcomes in septic patients[20]. Although the use of antibiotics as mono-therapy showed a reduction of *Thrombosis* activation by more than 20%, our perturbation analysis suggests that there are still potential beneficial options of combining antibiotics with a *Thrombosis* focused therapy.

Overall, we identified four therapeutic targets that could be beneficial to target in combination with antibiotic therapy to decrease the activation of *Thrombosis* (**Fig 4.3C),** in which three of them were already identified in mono-therapy evaluation, i.e. IFN-γ, sTNF-R or TF, but blocking them could almost deactivate *Thrombosis* when combined with antibiotics. Another identified target, pro-inflammatory cytokine IL-1β, was not identified in mono-therapy but appeared in combined mediators specific therapies**.** For node *Ang2,* no combination showed a benefit to decrease its activation. Predictably, no increased immune regulated antibacterial effect could be identified due to the rapid bacterial eradication mediated by the antibiotics.

## Discussion

A novel Boolean network model was developed, which leveraged prior knowledge of immune response-related processes for the TLR4-mediated host response associated with early phase sepsis. The developed network incorporated key immune cells and mediatory molecules, as well as key clinical endpoint nodes to assess inter-individual variability and treatment interventions. By using a simulation approach, we identified several potential targets showing promise of improving bacterial clearance and/or reducing the possibility of organ dysfunction. The identified mediators might constitute potential therapeutic targets for treatment of sepsis and could be considered in further clinical studies.

The long-term behaviour, i.e. attractors, of this developed network showed to be stable according to the overall single perturbation analysis, where either knocking-out or over-expression of most nodes did not trigger considerable changes on the activation of the rest nodes on attractor (**Fig S4.3**). This stabilization could be a result of the complex interactions within the network, which might

explain in part the failures of many clinical trials investing treatments against sepsis. Recently, selective or non-selective targeting of endogenous mediator molecules have been investigated as strategies to modify the systemic inflammatory response, such as blocking TNF-α and IL-1β[4][5]. However, none of these agents showed significant improvement on septic survival rate. These results are comparable to our single node perturbations in which knocking-out TNF-α mainly lead to decreasing cell apoptosis while knocking-out IL-1β showed no big influence on other nodes.

We utilized a Boolean network as a tool to screen promising treatment targets for sepsis based on endpoints related to bacteria clearance (*Phagocytosis* and *MAC*) and vessel leakage and multi-organs dysfunction (*Ang2* and *Thrombosis*). When evaluating mono-target therapies, we found over-expressing TNF-α, instead of blocking it, was associated with a decreased activation of *Ang2* and *Thrombosis*, which can be related to decreased organ dysfunctions. This finding is inconsistent with previous clinical studies where TNF-α was blocked but have not shown a significantly improved survival rate in sepsis patients[5]. Additionally, treatments blocking either TF or IFN-γ were identified to reduce *Thrombosis* in our analysis. These targets have also been studied in clinical trials, but so far no clinical effect has been identified[4]. One reason for these inconsistent results might be the differences in selected endpoints. Clinical trials for sepsis mainly use mortality as the primary endpoint, while we used four surrogate endpoints.



Our simulations suggest a decrease in activation of *Ang2* after over-expressing TNF-α. In contrast, a previous *in vitro* study suggested TNF-α can induce both angiopoietin-2 mRNA expression and protein levels in human umbilical vein endothelial cells[21] at 2 hours after TNF-α exposure. Importantly, the positive interaction between angiopoietin-2 and TNF-α is in fact included in our model, with activated endothelial cells as intermediate node (**Table 4.1**). However, unlike the in vitro experiment involving a single cell type, our Boolean model also incorporates other relevant interaction events derived from other additional experiments, thereby illustrating the value of deriving expected outcomes which are the results of multiple cellular interaction events.

The effect of TNF-α on thrombosis remains inconclusive. Previous studies suggested either an antithrombotic activity through the stimulation of nitric oxide[22] or a prothrombotic effect via acting on TNF-α receptor subtype 2[23]. Recently, a *in vivo* study in mice showed a positive regulation of TNF-α/TNF receptor p55 singling axis in the resolution of venous thrombus[24]. In our simulations, long term over-expression of TNF-α was likely to decrease the activation of node *Thrombosis*, which might be a result of its beneficial role in thrombus resolution as indicated in the animal study. Worth noting are the inevitable inter-species differences when using animal models to mimic pathophysiological features in humans[25].

For multi-target treatment strategies, the combination of blocking IFN-γ and IL-10 was identified as a potential treatment to decrease the risk of organ dysfunction, via reducing activation of both *Ang2* and *Thrombosis*. Cytokine IFN-γ functions as a positive modulator of activated platelets[26], which plays a crucial role in the process of thrombosis. Although IL-10 shows an inhibitory effect on the production of most pro-inflammatory cytokines, increased IL-10 blood levels has been associated with the development of organ failure in septic shock[27]. Nevertheless, since IFN-γ and IL-10 are negative modulators of each other, few studies have addressed the co-operative action of these combination, while Yoshiki *et al* found simultaneous treatment with IL-10 and IFN-γ can significantly suppress the function of murine bone marrow-derived dendritic cells[28]. Due to the complexity of regulatory interaction between cytokines, the blockage of IFN-γ and IL-10 together could potentially reduce the risk of organ dysfunction.

When treating with antibiotics in the very early phase of infection, all nodes in this network remained inactive or returned to baseline immediately (**Fig S4.2**). This behaviour is in line with the clinical recommendation of administering antibiotics as early as possible for adults with possible septic shock or a high likelihood for sepsis[2]. A delayed start of antibiotic therapy, simulated by removing bacteria after 4 time steps, showed to be ineffective in inhibiting the initiation of the immune cascade reaction, which can be seen from the unchanged activation on attractors (**Fig S4.4**). This phenomenon may explain the failures of clinical trial focusing on anti-endotoxin agents[5], where neither human antiserum

to endotoxin nor monoclonal IgM antibodies that inactivates endotoxin could significantly improve survival in sepsis.

Local thrombosis contributes to the initial defence against bacterial invasion in mammals[29]. We find that combination therapies with delayed initiation of antibiotic therapy, such as antibiotic treatment combined with IL-1β blockade, may show beneficial effects, decreasing *Thrombosis* node activation. These results are in line with a previous study where an increase in IL-1β mRNA expression in patients who suffered thrombotic episodes compared with healthy age-matched controls[30] was observed. Another clinical study showed the anti-inflammatory therapy targeting IL-1β pathway led to a significantly lower rate of recurrent cardiovascular events than placebo[31]. These data indicate that IL-1β might be a relevant therapeutic target, although treatment of inhibiting IL-1β alone did not show sufficient decrease of *Thrombosis* activation in our analysis.



Interleukins have been of recent interest as potential treatment in sepsis due to their contribution to thrombosis and their potential therapeutic effect in animal models[32], including pro-inflammatory IL-6[33] and anti-inflammatory IL-10[34]. However, a population-based study suggested that an altered inflammatory profile of these interleukins is more likely to be associated with a result rather than an increased risk of venous thrombosis[35]. IL-12 was another identified target in our simulations. However, a previous study concluded that IL-12 can activate both coagulation and fibrinolysis in patients with renal cell carcinoma[36]. The potential of these inflammatory targets thus still need to be evaluated in well-controlled clinical studies.

Antibiotic treatment was mimicked by setting the node *Bacteria* to 0% activation in our simulation. The dynamic pattern over time steps of other nodes varies after deactivating *Bacteria* (**Fig S4.2**), in which the simulated activation of complement factors, i.e., *C3*, *C5a* and *C5b*, as well the complex *MAC* returned to baseline immediately. This consistency indicates the potential of complement factors as biomarkers for monitoring antibiotic treatment efficacy in early sepsis. Indeed, a recent prospective study evaluated complement levels in bacteremia patients, and hypothesized the measurement of C3, C4 and C9 levels may help stratify gram-negative bacteremia patients at increased risk for mortality[37].

Activation of complement system is a key event in the pathogenesis of sepsis[38], adapting crucial complement factors as biomarkers might be of prognostic value, when their sensitivity and specificity were carefully evaluated.

Although the use of a Boolean network approach can support developing understanding the behaviour of complex systems, especially in the lack of quantitative data, the approach is associated with inherent limitations. The time steps in a Boolean network are not related to real time. Thus, simulation results cannot be directly linked to time-concentration data, such as specific biomarker peak times, which further complicates model validation using clinical data. The attractors of mono perturbations on our BN were compared with previous experimental results under certain intervention, revealing some similarities between our simulations and *in vivo* animal studies. However, human studies with comparable endpoints are still required to validate both of the identified mono and multi therapeutic targets.

The development of the Boolean network model in this study was guided by including key biological processes previously identified as key consensus mechanisms associated with TLR4-activation and early sepsis. We systematically searched the literature to identify interaction partners between involved cell types, receptors and their ligands to populate a complete network. Nonetheless, the developed Boolean network model may need further revision and additions depending on new findings and specific objectives for applying this model. With respect to (clinical) endpoint nodes we have selected biological events which may closely relate to key clinical events in the disease pathology of sepsis. Yet, it is important to recognize this model does not directly predicts clinical outcomes, which also complicates the comparison of our results to existing clinical trials. These two shortcomings could be overcome by gradually by extending this network with a higher number and clinically related nodes.

In conclusion, the developed Boolean network model for TLR4-mediated host immune response in early phase of sepsis exemplifies the value of using Boolean networks to increase the knowledge of complex biological systems, and constitutes a relevant strategy to deepen our understanding of systemic inflammatory diseases,

analyse the influences of immune cells diversity among patient groups, and identify potential therapeutic targets for sepsis.

## References


1. Pop-Began V, Păunescu V, Grigorean V, et al (2014) Molecular mechanisms in the pathogenesis of sepsis. J Med Life
2. Evans L, Rhodes A, Alhazzani W, et al (2021) Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med.
3. Vincent JL, Nelson DR, Williams MD (2011) Is worsening multiple organ failure the cause of death in patients with severe sepsis? In: Critical Care Medicine
4. Marshall JC (2014) Why have clinical trials in sepsis failed? Trends Mol. Med.
5. Seam N, Suffredini AF (2007) Mechanisms of sepsis and insights from clinical trials. Drug Discov. Today Dis. Mech.
6. Saleh MAA, Van De Garde EMW, Van Hasselt JGC (2019) Host-response biomarkers for the diagnosis of bacterial respiratory tract infections. Clin. Chem. Lab. Med.
7. Aulin LBS, de Lange DW, Saleh MAA, et al (2021) Biomarker-Guided Individualization of Antibiotic Therapy. Clin Pharmacol Ther.
8. Bloomingdale P, Nguyen VA, Niu J, Mager DE (2018) Boolean network modeling in systems pharmacology. J Pharmacokinet Pharmacodyn.
9. Montagud A, Béal J, Tobalina L, et al (2022) Patient-specific Boolean models of signalling networks guide personalised treatments. Elife 11:1–81.
10. Irurzun-Arana I, Pastor JM, Trocóniz IF, Gómez-Mantilla JD (2017) Advanced Boolean modeling of biological networks applied to systems pharmacology. Bioinformatics.
11. Hopfensitz M, Müssel C, Maucher M, Kestler HA (2013) Attractors in Boolean networks: A tutorial. Comput Stat.
12. Schwab JD, Kühlwein SD, Ikonomi N, et al (2020) Concepts in Boolean network modeling: What do they all mean? Comput. Struct. Biotechnol. J.
13. Shannon P, Markiel A, Ozier O, et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504.
14. Garg A, Di Cara A, Xenarios I, et al (2008) Synchronous versus asynchronous modeling of gene regulatory networks. Bioinformatics.
15. Kubo M, Motomura Y (2012) Transcriptional regulation of the anti-inflammatory cytokine IL-10 in acquired immune cells. Front Immunol.
16. Hayashi SI, Rakugi H, Morishita R (2020) Insight into the Role of Angiopoietins in Ageing-Associated Diseases. Cells
17. Franco RF, De Jonge E, Dekkers PEP, et al (2000) The in vivo kinetics of tissue factor messenger RNA expression during human endotoxemia: Relationship with activation of coagulation. Blood.
18. Opal SM, Girard TD, Ely EW (2005) The immunopathogenesis of sepsis in elderly patients. In: Clinical Infectious Diseases
19. Serna M, Giles JL, Morgan BP, Bubeck D (2016) Structural basis of complement membrane attack complex formation. Nat Commun.
20. Kumar A, Roberts D, Wood KE, et al (2006) Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med.

21. Kim I, Kim JH, Ryu YS, et al (2000) Tumor necrosis factor-α upregulates angiopoietin-2 in human umbilical vein endothelial cells. Biochem Biophys Res Commun 269:361–365.
22. Cambien B, Bergmeier W, Saffaripour S, et al (2003) Antithrombotic activity of TNF-α. J Clin Invest.
23. Pircher J, Merkle M, Wörnle M, et al (2012) Prothrombotic effects of tumor necrosis factor alpha in vivo are amplified by the absence of TNF-alpha receptor subtype 1 and require TNF-alpha receptor subtype 2. Arthritis Res Ther.
24. Nosaka M, Ishida Y, Kimura A, et al (2018) Contribution of the TNF-α (tumor necrosis factor-α)-TNFrp55 (tumor necrosis factor receptor p55) axis in the resolution of venous thrombus. Arterioscler Thromb Vasc Biol.
25. Diaz JA, A.T. O, D.D. MJ, et al (2012) Critical review of mouse models of venous thrombosis. Arterioscler Thromb Vasc Biol
26. Stokes KY, Granger DN (2012) Platelets: A critical link between inflammation and microvascular dysfunction. J. Physiol.
27. Friedman G, Jankowski S, Marchant A, et al (1997) Blood interleukin 10 levels parallel the severity of septic shock. J Crit Care.
28. Yanagawa Y, Iwabuchi K, Onoé K (2009) Co-operative action of interleukin-10 and interferon-γ to regulate dendritic cell functions. Immunology.
29. Sun H (2006) The interaction between pathogens and the host coagulation system. Physiology
30. Gupta N, Sahu A, Prabhakar A, et al (2017) Activation of NLRP3 inflammasome complex potentiates venous thrombosis in response to hypoxia. Proc Natl Acad Sci U S A.
31. Ridker PM, Everett BM, Thuren T, et al (2017) Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med.
32. Najem MY, Couturaud F, Lemarié CA (2020) Cytokine and chemokine regulation of venous thromboembolism. J. Thromb. Haemost.
33. Zhang Y, Zhang Z, Wei R, et al (2020) IL (interleukin)-6 contributes to deep vein thrombosis and is negatively regulated by miR-338-5p. Arterioscler Thromb Vasc Biol.
34. Downing LJ, Strieter RM, Kadell AM, et al (1998) IL-10 regulates thrombus-induced vein wall inflammation and thrombosis. J Immunol
35. Christiansen SC, Næss IA, Cannegieter SC, et al (2006) Inflammatory cytokines as risk factors for a first venous thrombosis: A prospective population-based study. PLoS Med.
36. Portielje JEA, Kruit WHJ, Eerenberg AJM, et al (2001) Interleukin 12 induces activation of fibrinolysis and coagulation in humans. Br J Haematol.
37. Eichenberger EM, Dagher M, Ruffin F, et al (2020) Complement levels in patients with bloodstream infection due to Staphylococcus aureus or Gram-negative bacteria. Eur J Clin Microbiol Infect Dis.
38. Flierl MA, Rittirsch D, Nadeau BA, et al (2008) Functions of the complement components C3 and C5 during sepsis. FASEB J.

## Supplemental materials

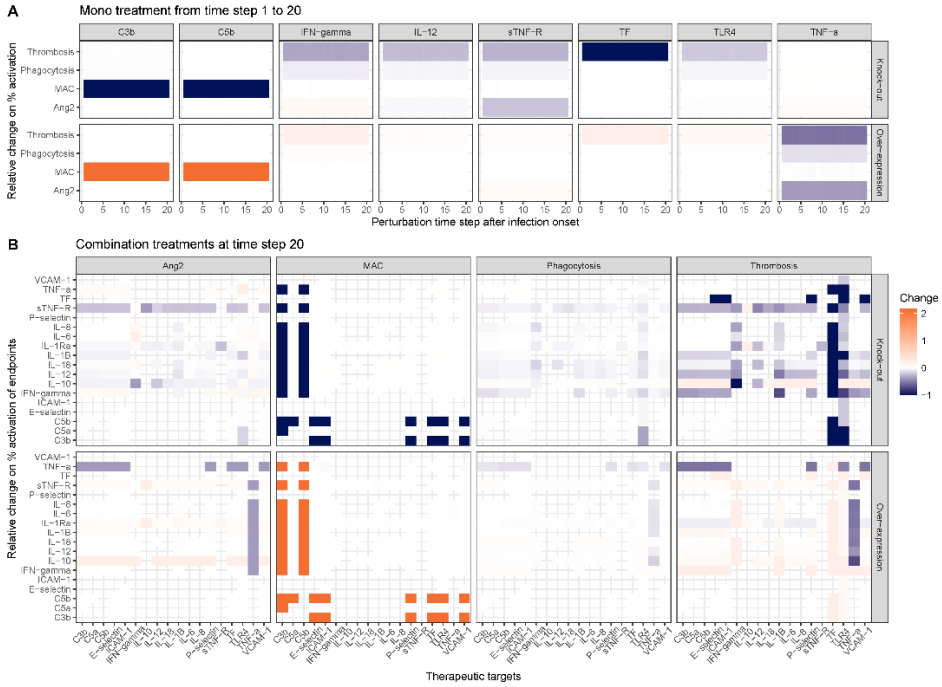



**Fig S4.1 Relative changes of four selected endpoint activation under mono and combined perturbations on mediatory molecules.** Upper heatmap (A) showed the effect of knock-out or over-express of identified mono therapeutic targets on four endpoints over different perturbation initiation time steps; below heatmaps (B) showed the an example of effect of knocking-out or over-expressing combined therapeutic targets on four endpoints at time step 20. Colors of the heatmap represented the negative, neutral and positive relative changes of endpoints activation with blue, white and orange, respectively.

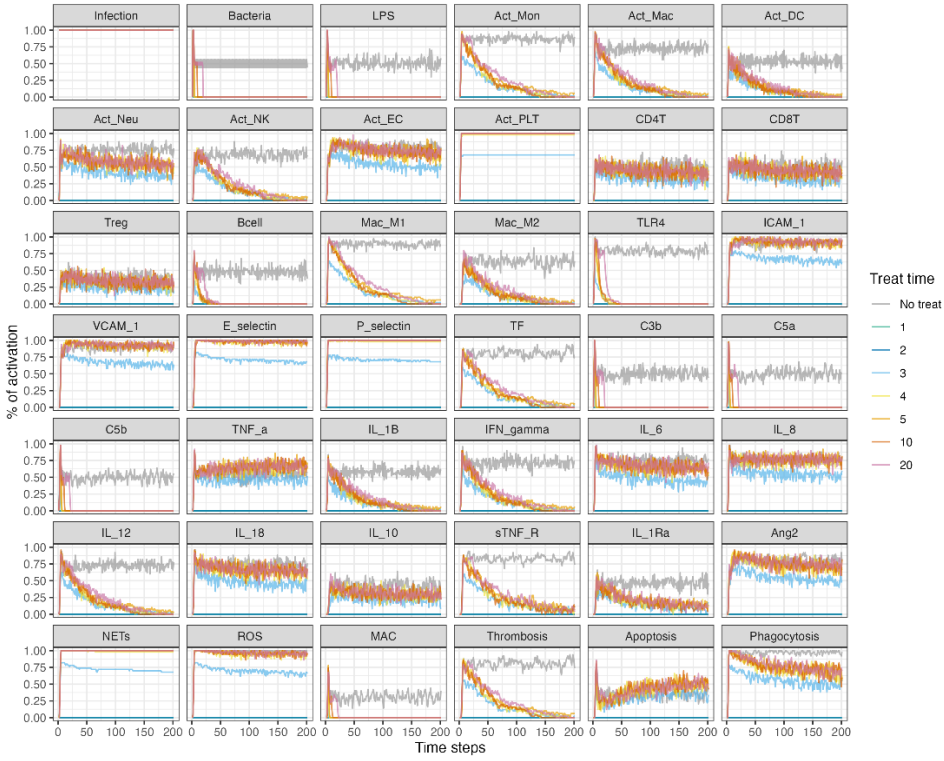



**Fig S4.2 Average activation profiles for each node under antibiotic treatment (i.e. knocking out node *Bacteria*) at different time step with 100 repetitions.** When removing bacteria at an early phase (before time step 4), most nodes were not activated or returned back to baseline immediately; when removing bacteria at a later phase, it showed varying decline patterns for different nodes. Colors of the lines represented different perturbation initiation time steps.

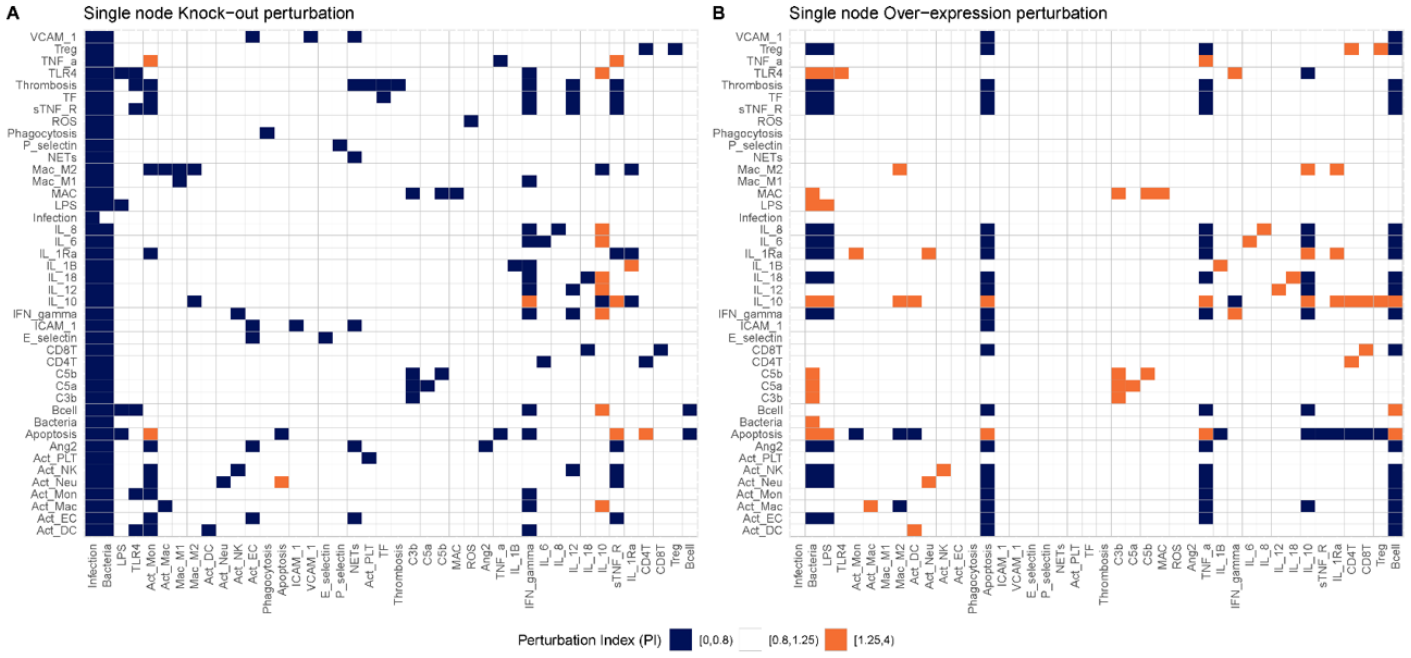



**Fig S4.3 Overview of single node perturbation analysis of the network.** The heatmaps indicated the effect of entire knock-out (A) or over-expression (B) of each node (columns) in every network node (rows). Colors of the heatmap represented the Perturbation Index (PI) with the negative (PI < 0.8), neutral (0.8 < PI < 1.25) and positive (PI > 1.25) changes being blue, white and orange, respectively.

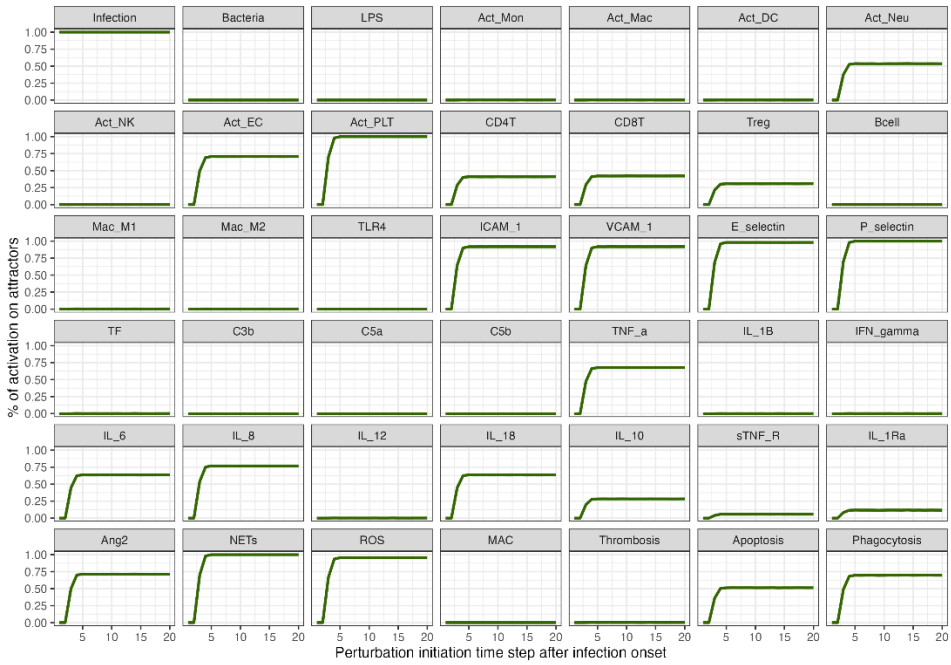



**Figure S4.4 Activation of each node on attractors under antibiotic treatment (i.e. knocking out node *Bacteria*) at different time steps.** The activations on attractors stayed unchanged when removing bacteria at a later phase (i.e. after time step 4).

**Table S4.1** Definitions of all nodes and related regulatory interactions in the final Boolean network

| Node | Explanation | Dependency | Interaction | Refs |
|---|---|---|---|---|
| **Infection** | Bacterial infection | Infection | Initial node, always ON after sepsis onset | |
| **Bacteria** | Gram-negative bacteria | Infection | Sepsis is mostly caused by Gram-negative bacteria infection. Infection ON means there are bacteria | |
| | | MAC | MAC can form cytotoxic pores on the surface of microbes and directly kill Gram-negative bacteria | [1] |
| | | Phagocytosis | Phagocytosis is a cellular process for ingesting and eliminating microorganisms, foreign substances, and apoptotic cells | [2] |
| | | ROS | The first discovered function of ROS in neutrophils was their microbicidal activity | [3] |
| | | NETs | NET killing can be as effective as phagocytosis | [4] |
| **LPS** | Lipopolysaccharides | Bacteria | The LPS is a central component of the outer membrane in Gram-negative bacteria and plays a key role in pathogenesis | [5] |
| **TLR4** | Toll-like receptor 4 | LPS | Several Pathogen-associated molecular patterns can stimulate TLR4 including LPS | [6] |
| | | IFN-gamma | In human monocytes and macrophages, IFN-γ augmented mRNA and surface expression of TLR4 | [7] |
| **Act-Mon** | Activated Monocyte | TLR4 | The number of monocytic TLR-4 expression were markedly increased in sepsis | [8] |

| | | | | |
|---|---|---|---|---|
| | | IL-1B | Primary blood monocytes are activated by mature IL-1β | [9] |
| | | IFN-gamma | IFNγ-primed monocytes showed increased responsiveness to LPS | [7] |
| | | Apoptosis | Apoptosis of stimulated monocytes is inhibited by IFN-γ | [10] |
| **Act-Mac** | Activated Macrophage | TLR4 | TLRs are highly expressed on macrophages | [11] |
| | | Act-Mon | During inflammation, circulating monocytes migrate into tissues, differentiate into macrophage | [12] |
| | | C5a | Complement anaphylatoxin C5a trigger macrophage activation | [13] |
| | | TNF-a | TNF-α amplifies inflammatory cascades by activating macrophages | [14] |
| | | IFN-gamma | IFN-γ promotes innate immune responses by activating macrophages | [15] |
| | | IL-1B | IL-1β induces the rapid differentiation of monocytes into CD209+ macrophages | [16] |
| | | IL-10 | IL-10 inhibits cytokine production by activated macrophages | [17] |
| | | Apoptosis | Apoptosis of stimulated macrophages is inhibited by IFN-γ | [10] |
| **Mac-M1** | M1-like Macrophage | Act-Mac | M1-like macrophage is a subset of macrophages | [18] |
| | | TNF-a | TNF-α promotes the activation and differentiation of macrophages | [19] |
| | | IL-1B | IL-1β promotes the differentiation of monocytes into M1-like macrophages | [16] |
| | | IFN-gamma | M1 macrophages are typically induced by Th1 cytokines, such as IFN-γ and TNF-α | [19] |

| | | | | |
|---|---|---|---|---|
| | | Mac-M2 | "Re-polarization" of differentiated macrophages includes M2 macrophages to M1 signals, or vice versa | [19] |
| **Mac-M2** | M2-like Macrophage | Act-Mac | M2-like macrophage is a subset of macrophages | [18] |
| | | IL-10 | IL-10 promotes M2 polarization | [20] |
| | | IL-1Ra | IL1Ra control the polarization of macrophages toward a M2 phenotype | [21] |
| | | Mac-M1 | "Re-polarization" of differentiated macrophages includes M2 macrophages to M1 signals, or vice versa | [19] |
| **Act-DC** | Activated Dendritic Cell | TLR4 | TLR4 is essential for dendritic cell activation | [22] |
| | | Act-Mon | Monocytes can further differentiate into a range of tissue dendritic cells | [12] |
| | | Treg | Regulatory T-cells mediate their suppressive action by acting on dendritic cells | [23] |
| | | IL-10 | IL-10 is a critical cytokine that blocks the maturation of dendritic cells (DCs) | [24] |
| | | Apoptosis | Apoptotic cell death plays an important role in inflammatory processes | [10] |
| **Act-Neu** | Activated Neutrophil | TLR4 | Activation of TLR4 caused changes in adhesion molecule expression on neutrophils | [25] |
| | | C5a | C5a generated during systemic inflammatory processes activates neutrophils | [26] |

| | | | | |
|---|---|---|---|---|
| | | IL-8 | IL-8 was originally discovered as chemokines activating neutrophil granulocytes | [27] |
| | | Apoptosis | Apoptotic cell death plays an important role in inflammatory processes | [10] |
| **Act-NK** | Activated Natural Killer Cell | IL-12 | IL-12 directly generated high lymphokine-activated killer cell activity in CD56+ NK cells | [28] |
| | | IL-18 | The combination of IL-18 and IL-12 induced extremely high amounts of IFN-γprotein secreted by NK cells | [29] |
| | | Act-DC | Both immature and mature DCs activate resting human natural killer cells | [30] |
| | | Apoptosis | Apoptotic cell death plays an important role in inflammatory processes | [10] |
| **Act-EC** | Activated Endothelial Cell | NETs | NETs promote the activation of Endothelial Cells | [31] |
| | | TNF-a | Type II activation of endothelial cells mediated by pro-inflammatory cytokines such as tumor-necrosis factor | [32] |
| | | Apoptosis | Apoptotic cell death plays an important role in inflammatory processes | [10] |
| **Phagocytosis** | Phagocytosis | Act-Mon | Professional phagocytes accomplish phagocytosis with high efficiency, including Macrophages, neutrophils, monocytes, and dendritic cells | [2] |
| | | Act-Mac | Professional phagocytes accomplish phagocytosis with high efficiency, including Macrophages, neutrophils, monocytes, and dendritic cells | [2] |

| | | | | |
|---|---|---|---|---|
| | | Act-Neu | Neutrophils are typically the first non-resident immune cells that arrive at a site of inflammation and its phagocytosis is an early event | [33] |
| | | Act-DC | Professional phagocytes accomplish phagocytosis with high efficiency, including Macrophages, neutrophils, monocytes, and dendritic cells | [2] |
| | | Bacteria | Complement labels bacteria with C3-derived products (C3b and C3bi) stimulate engulfment of bacteria by phagocytes. | [34] |
| | | C3b | Complement labels bacteria with C3-derived products (C3b and C3bi) stimulate engulfment of bacteria by phagocytes. | [34] |
| | | IFN-gamma | Complement receptors of mononuclear phagocytes are up-regulated by IFN-γ to promote receptor-mediated phagocytosis of opsonized extracellular pathogens. | [35] |
| | | IL-18 | IL-18 restored the burn-related decrease in activity of neutrophils and enhanced phagocytosis | [36] |
| **Apoptosis** | Apoptosis | TNF-a | TNF-induced apoptosis primarily through the activation of type I receptors | [37] |
| | | Bcell | Mechanisms of BCR-mediated apoptosis have been widely studied in B cells | [38] |
| | | LPS | LPS induces cell death as measured by caspase-3 activation and DNA fragmentation | [39] |

| | | | | |
|---|---|---|---|---|
| **ICAM-1** | Intercellular Adhesion Molecule 1 | Act-EC | Cytokine-activated venular endothelial cells increase their expression of ICAM1 | [32] |
| | | TNF-a | TNF-α enhances the expression of adhesion molecules | [14] |
| **VCAM-1** | Vascular cell adhesion molecule 1 | Act-EC | Cytokine-activated venular endothelial cells increase their expression of VCAM1 | [32] |
| | | TNF-a | TNF-α enhances the expression of adhesion molecules | [14] |
| **E-selectin** | E-selectin | Act-EC | E-selectin expressed on activated endothelial cells | [40] |
| | | ROS | Generation of ROS in endothelial cells is a critical signal mediating E-selectin expression. | [41] |
| **P-selectin** | P-selectin | Act-EC | P-selectin expressed on activated endothelial cells | [40] |
| | | Act-PLT | P-selectin expressed on activated platelets | [40] |
| **NETs** | Neutrophil extracellular traps | ROS | ROS can activate granular proteases and induce the formation of NETs | [42] |
| | | Act-Neu | Activated neutrophils release nuclear DNA and form a network structure NETs | [31] |
| | | C5a | C5a pre-stimulated neutrophils enhanced their ability to generate NETs | [31] |
| | | Act-PLT | The ability of platelets to promote NET formation was also observed in human cells | [43] |
| **Act-PLT** | Activated platelet | LPS | LPS can initiate the non-classical activation of platelets | [31] |
| | | TLR4 | TLR4 was found to activate platelet through different pathways | [44] |

| | | | | |
|---|---|---|---|---|
| | | TF | Platelet activation is stimulated by local prothrombotic factors such as tissue factor | [45] |
| | | Thrombosis | Thrombin activates platelets through protease-activated receptors | [45] |
| | | NETs | NETs further promote platelet activation | [31] |
| | | IFN-gamma | IFN-γ act on platelets to promote the degranulation of dense granules | [46] |
| **TF** | Tissue factor | Act-Mon | monocytes expressed TF mRNA in human model of endotoxemia | [47] |
| | | Act-EC | In ECs TNF-α and LPS are shown to up-regulate TF expression in vitro | [48] |
| | | LPS | LPS and TNF-a can also up-regulate TF expression from monocyte | [48] |
| | | TNF-a | LPS and TNF-a can also up-regulate TF expression from monocyte | [48] |
| **Thrombosis** | Thrombosis | NETs | The interaction of NETs with platelets mediates the formation of immune thrombosis | [31] |
| | | Act-PLT | The interaction of NETs with platelets mediates the formation of immune thrombosis | [31] |
| | | TF | In pathological conditions, elevated levels of tissue factor and deposition of platelets may conspire to trigger formation of a thrombotic clot | [49] |
| **C3b** | Component 3b | Bacteria | Bacteria have evolved several modulators of initial steps in complement activation to generate C3 convertases, the enzymes that cleave C3 to C3b | [50] |
| **C5a** | Component 5a | C3b | C3b binds to the C3 convertase, forming the C5 convertase enzyme cleaving C5 into C5a and C5b | [51] |

| | | | | |
|---|---|---|---|---|
| **C5b** | Component 5b | C3b | C3b binds to the C3 convertase, forming the C5 convertase enzyme cleaving C5 into C5a and C5b | [51] |
| **MAC** | Membrane attack complex | C5b | Stepwise MAC assembly pathway from soluble complement factors requires the large fragment C5b | [1] |
| **ROS** | Reactive oxygen species | Act-Neu | Neutrophils will release large amounts of ROS at the site of infection following the activation of surface receptors | [42] |
| | | Act-Mac | ROS formation in macrophages from Cytosolic or Mitochondrial sources | [52] |
| | | Mac-M1 | M1 macrophages participate in subsequent generation of ROS | [19] |
| | | Act-EC | Increased generation of ROS are among the major molecular changes associated with endothelial dysfunction | [53] |
| | | TNF-a | Mitochondrial ROS production increased after TNF-α stimulation | [54] |
| | | IL-18 | IL-18 strongly enhances the induction of ROS in phagocytes. | [36] |
| **Ang2** | Angiopoietin-2 | Act-EC | Expression profiling studies have identified endothelial cells as the primary source of Ang-2 and a dramatic transcriptional regulation of Ang-2 production on endothelial cell activation | [55] |
| **TNF-a** | Tumor necrosis factor alpha | Act-Mon | TNF-α produced predominantly by activated monocytes/macrophages | [56] |
| | | Mac-M1 | These M1 type macrophages produce and secrete higher levels of TNF-α | [19] |

| | | | | |
|---|---|---|---|---|
| | | Act-DC | DC maturation result in secretion of the inflammatory cytokines TNF-α. | [57] |
| | | Act-NK | NK cells also secrete several cytokines such as IFN-γ and TNF-α | [58] |
| | | CD4T | TNF is a product of effector CD4 and CD8+ T cells or innate cells | [59] |
| | | CD8T | TNF is a product of effector CD4 and CD8+ T cells or innate cells | [59] |
| | | IFN-gamma | The combination of IFN-γ/GM-CSF was consistently capable of inducing substantial TNF-α mRNA transcript levels and protein secretion | [60] |
| | | TNF-a | Autocrine action by TNFα promotes additional TNFα production by macrophages | [61] |
| | | ROS | ROS can stimulate the production of TNF-α | [62] |
| | | IL-1B | IL-1β were effective when given as a single stimulus for the production of TNF | [63] |
| | | IL-10 | IL-10 is a major anti-inflammatory cytokine and functions to inhibit production of TLR-induced proinflammatory mediators, such as TNF | [17] |
| | | sTNF-R | Soluble TNF receptors neutralize TNF-α induced cytotoxicity and immunoreactivity in vitro | [64] |
| **IL-1B** | Interleukin 1 beta | Act-Mon | IL-1β secretion in monocytes is central to the initiation of immune response | [65] |
| | | Mac-M1 | These M1 type macrophages produce and secrete higher levels of pro-inflammatory cytokines including IL-1β. | [19] |

| | | | | |
|---|---|---|---|---|
| | | TNF-a | Autocrine action by TNFα promotes additional production and release of IL-1 | [61] |
| | | Act-PLT | Platelet TLR4 induces enhanced splicing and translation of IL-1β | [44] |
| | | IL-10 | IL-10 inhibit production of TLR-induced proinflammatory mediators | [17] |
| | | IL-1Ra | IL-1Ra is specific for preventing the activity of IL-1α and IL-1β | [9] |
| **IFN-gamma** | Interferon gamma | Act-NK | Natural killer cells secrete cytokines such as IFN-γ | [66] |
| | | Mac-M1 | Human macrophages in vitro through stimulation with IL-12 and IL-18 were able to produce IFN-γ | [67] |
| | | CD4T | Naive CD4+ T cells can develop into cells that produced IFN-γ | [68] |
| | | CD8T | The ability of CD8+ T cells to produce IFNγ enhanced their ability to migrate to the site of antigen-presenting skin cells. | [69] |
| | | Act-DC | Human DCs were most consistently found to stimulate cytotoxicity and IFN-γ secretion of NK cells through IL-12 | [70] |
| | | IL-12 | IL-12 is a proinflammatory cytokine that facilitates IFN-γ production by Th1 cells particularly in conjunction with IL-18 | [36] |
| | | IL-18 | IL-18 is a proinflammatory cytokine that facilitates IFN-γ production by Th1 cells particularly in conjunction with IL-12 | [36] |
| | | IL-6 | Without a positive feedback loop through endogenous IFNγ, CD4+ T cells stimulated in the presence of IL-6 produce less IFNγ | [71] |
| | | IL-10 | This endogenous IL-10 reduces TNF, IFNγ, and MIP-2 levels | [72] |

| | | | | |
|---|---|---|---|---|
| **IL-6** | Interleukin 6 | Mac-M1 | These type 1 macrophages produce and secrete higher levels of IL-6 | [19] |
| | | Act-Mon | Activated human peripheral blood monocytes produce IL-6 | [73] |
| | | IL-1B | IL-1 induces the local production of Interleukin-6 | [74] |
| | | Act-EC | IL-6 is produced also by endothelial cells | [75] |
| | | Bcell | B lymphocytes can produce IL-6 | [76] |
| | | Act-DC | DC maturation result in secretion of the inflammatory cytokines IL-6 | [57] |
| | | IL-10 | IL-10 limits the production of proinflammatory cytokines and chemokines | [72] |
| **IL-8** | Interleukin 8 | Act-Mon | Monocytes/macrophages can produce IL-8. | [77] |
| | | Mac-M1 | Monocytes/macrophages can produce IL-8. | [77] |
| | | Act-EC | Endothelial cells are main producers of Interleukin 8 | [78] |
| | | TNF-a | TNFα induced IL-8 expression in human micro vessel endothelial cell line | [79] |
| | | IL-10 | IL-10 have a down-regulatory effect on IL-8 secretion | [80] |
| **IL-12** | Interleukin 12 | Act-Mon | LPS ablate the IL-12 productive capacity of primary human monocytes | [81] |
| | | Act-DC | DC should constitute a critical source of IL-12 | [82] |
| | | Mac-M1 | These M1 type macrophages produce and secrete higher levels of IL-12 | [19] |
| | | Act-NK | The activated NK cells then prime DC to produce IL-12 | [83] |
| | | IFN-gamma | IFN-γ provides a powerful stimulation signal for activated macrophages with a much enhanced potential to produce IL-12 | [84] |

| | | | | |
|---|---|---|---|---|
| | | IL-1B | IL-1β is identified as a new IL-12-inducing agent on human monocyte-derived DCs | [85] |
| | | IL-10 | IL-10 can inhibit IL-12 production in an autocrine manner. | [84] |
| **IL-18** | Interleukin 18 | Mac-M1 | Macrophages are the primary sources for the release of active IL-18. | [86] |
| | | Act-DC | Dendritic cells are the primary sources for the release of active IL-18. | [86] |
| | | Act-EC | Many investigators reported IL-18 production in endothelial cells | [36] |
| | | IL-10 | IL-10 limits the production of proinflammatory cytokines and chemokines | [72] |
| **IL-10** | Interleukin 10 | Mac-M2 | M2 macrophages have an anti-inflammatory cytokine profile, which characterized by high production of both IL-10 and TGF-β. | [19] |
| | | CD4T | IL-10 is expressed by many cells of the adaptive immune system, including TH1, TH2 and TH17 cell subsets, TReg cells, CD8+ T cells and B cells | [87] |
| | | CD8T | IL-10 is expressed by many cells of the adaptive immune system, including TH1, TH2 and TH17 cell subsets, TReg cells, CD8+ T cells and B cells | [87] |
| | | Treg | IL-10 is expressed by many cells of the adaptive immune system, including TH1, TH2 and TH17 cell subsets, TReg cells, CD8+ T cells and B cells | [87] |

| | | | | |
|---|---|---|---|---|
| | | Bcell | Autocrine IL-10 promotes human B-cell differentiation and B-cell derived IL-10 | [88] |
| | | Act-DC | Il-10 is also expressed by cells of the innate immune system, including DCs | [87] |
| | | IL-10 | Autocrine IL-10 increases IL-10 production by DCs | [24] |
| | | IL-12 | IL-12 is capable of potently inducing its own inhibitor IL-10 | [89] |
| | | Apoptosis | Some apoptotic cells themselves are a potential source of IL-10 | [90] |
| | | IFN-gamma | IFN-γ suppresses IL-10 production by increasing the activity of glycogen synthase kinase 3β | [15] |
| **sTNF-R** | Soluble TNF receptor | Act-Mon | Both the p75 and p55 TNF receptors were measured for blood monocytes incubated in vitro | [91] |
| | | CD4T | Soluble TNF receptor production by activated T lymphocytes; p75 sTNF-R production upon stimulation is a feature to all subsets of T cells | [92] |
| | | CD8T | p75 sTNF-R production upon stimulation is a feature to all subsets of T cells | [92] |
| | | IL-10 | IL-10 increased surface and soluble p75 TNF receptor levels on monocytes | [91] |
| **IL-1Ra** | Interleukin 1 receptor antagonist | Act-Neu | Human neutrophils produce high levels of the interleukin-1 receptor antagonist | [93] |
| | | Act-Mon | Human monocytes secrete an interleukin-1 receptor antagonist | [94] |

| | | | | |
|---|---|---|---|---|
| | | IFN-gamma | IFN-γ also suppresses the expression of IFN-β- and IL-4-induced IL-1Ra | [95] |
| **CD4T** | CD4+ T cells | Act-DC | Naïve CD4+ T cells are activated by dendritic cell-derived exosomes | [96] |
| | | IL-6 | IL-6 was described as a costimulatory molecule for T cell activation | [97] |
| | | IFN-gamma | IFN-γ also upregulates cell surface MHC class II on APCs, thus promoting peptide-specific activation of CD4+ T cells | [98] |
| | | IL-12 | IL-12 substantially enhanced the ability of naive CD4+ T cells to develop into cells that produced IFN-γ upon re-stimulation | [68] |
| | | Treg | Regulatory T cells control immune activation by acting directly on conventional CD4+ and CD8+ T cells | [23] |
| | | IL-10 | IL-10 inhibits the ability of DCs and macrophages to stimulate antigen-specific CD4+ T cells | [99] |
| | | Apoptosis | Extensive lymphocyte apoptosis is seen in sepsis | [100] |
| **CD8T** | CD8+ T cells | IL-18 | IL-18 directly activates CD8+ T cells | [36] |
| | | Act-DC | DCs have a strong ability to activate CD8+ cytotoxic T lymphocytes | [22] |
| | | Act-NK | The activated NK cells prime DC to induce highly protective CD8+ T cell memory responses | [83] |
| | | Treg | Regulatory T-cells control immune activation by acting directly on conventional CD4+ and CD8+ T cells | [23] |
| | | IL-10 | IL-10 induces alloantigen-specific unresponsiveness in human CD8+ T cells | [101] |

| | | | | |
|---|---|---|---|---|
| | | Apoptosis | Extensive lymphocyte apoptosis is seen in sepsis | [100] |
| **Treg** | Regulatory T cells | CD4T | CD4+ T cells are commonly divided into regulatory T (Treg) cells | [102] |
| | | IL-10 | The presence of IL-10 leads to increased expansion of Foxp3+ Tregs. | [103] |
| | | IL-6 | IL-6 inhibits TGF-β-induced Treg differentiation | [104] |
| | | Apoptosis | Extensive lymphocyte apoptosis is seen in a sepsis | [100] |
| **Bcell** | B cells | TLR4 | The activation of B cells by microorganisms takes place also through the activation of TLRs | [105] |
| | | Act-DC | Antigen presentation by DCs lead to B cell activation | [106] |
| | | IL-6 | IL-6 has been shown to induce the final maturation of B cells | [107] |
| | | Treg | Tregs can directly suppress B cell response | [108] |
| | | IL-10 | IL-10 contribute to the differentiation of IL-10-secreting B cells into IgM- and IgG-secreting plasma blasts | [88] |
| | | Apoptosis | Extensive lymphocyte apoptosis is seen in sepsis | [100] |

## References

Supplemental references not included in this thesis can be found on the publication website:

https://link.springer.com/article/10.1007/s10928-022-09828-6