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Unraveling Macrophage Metabolic Phenotypes in Cytokine Storms: A Systems Biology Perspective

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Unraveling Macrophage Metabolic Phenotypes in Cytokine Storms: A Systems Biology Perspective

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Abstract

Motivation:

The cytokine storm present in COVID-19 patients is one of the major concerns regarding COVID-19 illness. It has been linked to an increased risk of mortality and morbidity, with many patients requiring intensive care and mechanical ventilation. To better understand the mechanisms underlying this phenomenon, it is important to characterize macrophage responses to cytokine storms. This project aims to provide insight into macrophage response by investigating the effects of cytokine storms on non-activated macrophages. Here we want to provide a workflow capable of comparing metabolic differences between macrophages before and after the presence of a cytokine storm integrating transcriptomic and metabolic experimental data.

Results:

We found that after the cytokine storm, macrophages undergo a polarization towards a more glycolytic state, indicating a proinflammatory-like polarization. Additionally, we observed an intriguing enhancement in purine metabolism and one-carbon pool by folate, suggesting that these metabolic pathways could serve as potential targets for investigating the transition from a non-activated to a proinflammatory polarization.

Supplementary information:

Supplementary data are available at https://github.com/nuriaale/Macrophage-Metabolic-Phenotypes-in-Cytokine-Storms.git , Supplementary information materials https://docs.google.com/document/d/18gJ18QKW7HRKh-7vGVSwMh7uxqvXPyLJegrwbT4IRkk/edit?usp=sharing

1 Introduction

Introduction to Cytokine Storms in COVID-19 Patients

Cytokine storm syndrome (CSS) refers to an excessive and uncontrolled immune response, frequently self-perpetuating, leading to the overproduction of cytokines which can result in hyperinflammation and multiorgan failure[1,2]. Cytokine storms may occur in response to a variety of conditions, including infections (such as COVID-19), cancer, and genetic diseases[1]. Cell types of the innate immune system involved in cytokine storm include neutrophils, NK cells and macrophages[3].

Several studies have suggested a potential link between dysregulated host immune response, such as cytokine storm syndrome in lungs, and severe COVID-19 infection, such responses being distinguishable from conditions such as macrophage activation syndrome [1].
More specifically, some reports found evidence towards a link between increase in cytokines, in COVID-19 infected patients, to a progression to severe COVID-19 disease and proinflammatory cell death[1,4].

To provide an instance, studies found that genes enriched with inflammatory response and proinflammatory cytokine are upregulated in cell transcriptomics of patients with severe COVID-19[1,5,6].
Several attempts have been made to establish a criterion to predict risk for severe disease in COVID-19 patients. Among the variables proposed for obtaining the score were elevated ferritin concentration (hyperferritinemia) or hypercytokinemia observed in the patient's blood[1,7–11]. In all the studies, a high score on those criteria was associated with higher mortality risk and longer hospitalization risk. Therefore, it is assumed that the presence of inflammatory phenotype is related to worse outcomes in COVID-19 infection.

The Role of Macrophages in Cytokine Storms

The reason this cytokine storm may occur in COVID-19 patients is still not fully understood, making it a relevant topic of investigation. Since macrophages are one of the key immune cells that produce and release cytokines, dysregulations on macrophage activation state might contribute to cytokine storms [3]. Macrophages have the ability to change their phenotype, so their activation and function change in response to the environment they are in. This phenomenon is called polarization,[12] and is sustained by deep metabolic reprogramming. The most widespread categories used to describe macrophage states are non-activated (M0), pro-inflammatory state (M1) and an anti-inflammatory/pro-resolving state (M2).[12] Therefore, an excess of M1 macrophages can lead to Macrophage Activation Syndrome (MAS), ultimately ending up in a cytokine storm.[12]

The role of Macrophage in cytokines storm is a double-edged sword; On one hand, they are able to release cytokines, and on the other hand, they express a plethora of sensor molecules that detect changes occurring in the surrounding cells/tissue/extracellular milieu including changes in metabolites, antibodies and cytokines levels.[13,14] Among other factors, cytokines are able to trigger the state of macrophages into M1 state promoting chronic inflammatory state.[13] However, the macrophage's metabolic response to the cytokine storm remains unknown.

Using GSMM to Study Macrophage Responses to Cytokine Storms and Nitric Oxide Treatment

To simulate the behavior of an organism, cells or tissues, Genome-Scale Metabolic Models (GSMM) are developed as a mathematical

representation of a metabolic network that captures the stoichiometry and thermodynamics of each metabolic reaction in the network.[15]

These models can be solved using a variety of computational methods, including constraint-based modeling approaches such as flux balance analysis [15] which are described below in the methods section. The mentioned methods allow predicting the flow of metabolites through the network and the production rates of specific metabolites and/or biomass reaction. In this project, the biomass reaction serves as a way to ensure cell conservation since macrophages do not proliferate. Thus, linear programming is applied to solve for the optimal flux distribution that maximizes a particular objective function, taking into account the given set of reaction bounds. The objective function represents the primary metabolic goal of the cell or organism and provides a way to optimize the metabolic fluxes to achieve that goal. [15]
Typical objective functions used in cellular proliferation would be biomass production.
However, in our specific case, the biomass reaction is specific for macrophages. Since macrophages do not undergo proliferation like other cell types, the biomass reaction serves as a means to measure their overall metabolic activity and maintenance requirements. It includes the necessary components and reactions involved in sustaining the macrophage's essential cellular functions, such as energy production, protein synthesis, and membrane maintenance.

Therefore, in order to simulate metabolic reprogramming with the aim of treatment discovery to avoid the effects of the cytokine storms in macrophages we have developed a framework that applies several methods such as Flux Variability Analysis[16], Gene Inactivation Moderated by Metabolism, Metabolomics and Expression,[17] and Metabolic Transformation Algorithm[18].

2 Objectives of the Study

The main objectives of this project are:

- To develop a workflow for the study of macrophages metabolic flux reprogramming in response to an environmental challenge or drug intervention, based on COBRApy module integrating transcriptomic genomic-scale data, and available metabolic experimental measurements.

- To apply the developed workflow for the characterization of metabolic reprogramming in macrophages associated with SARS-CoV-2-triggered cytokine storm.

3 Methods

Genome-Scale Metabolic Models (GSMMs)

The process of creating a genome-scale metabolic model typically involves several steps. First, the genome of the organism is sequenced and annotated to identify genes, their functions, and their locations on the genome.[2] Gene expression data is key when building condition-specific GSMM, most times those are included considering the Gene Product Rule (GPR) of each reaction.[19] Then, the metabolic pathways present in the organism are reconstructed based on the known metabolic reactions and enzymes associated with each gene. Here, the GPR included in Recon3D[20] are the ones benign implemented.

The mathematical representation of the metabolic network as a GSMM consists of a stoichiometric matrix (*S*), a matrix of *n* rows and *m* columns where *n* is the number of metabolites in the network and *m* is the number of reactions and transport processes.[15] (F*ig. 1).*

The mathematical expression that GSMM represent would be[15]:

$$S \times v = 0$$

Being *S* the stoichiometric matrix and *v* the flux vector containing the reaction fluxes:

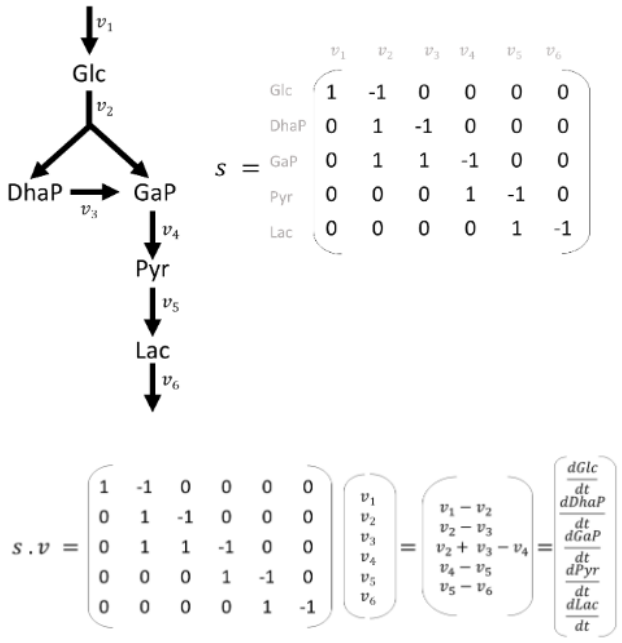

Figure 1. *Mathematical representation which includes the stoichiometric matrix referred as* ***S*** *and mass balance equations for a simplified metabolic network of glycolysis. Note that* ***v*** *is the flux vector containing the reaction fluxes whose length corresponds to the number of reactions.*

Experimental techniques before the workflow

The experimental data used in the present study to build up the human macrophages GSMM have been provided by the laboratories of Dr Lisardo Bosca (CSIC, Madrid) and Dra Marta Cascante (Barcelona University). The initial cell culture media metabolites content was used to constraint the maximal fluxes of each metabolite consumption. The experimental quantification of glucose consumed and lactate production in the cell culture experiments with or without cytokines treatment, has been performed by a method based in NADPH or NADH coupled reactions using a COBAS Mira Plus spectrophotometer (Horiba ABX).[21]

In brief, at the start and at the end of the cellular incubation period, metabolites from the cell culture media were collected and the fluxes of glucose uptake or lactate production was calculated taking into account exponential cellular growth with the following formulas:

$$\mu = \frac{ln(N_f/N_o)}{\Delta t}$$

Where *N* is the number of living cells and Δt is the duration of cell incubation experiment. The consumption or production rate was then calculated using the following formula:

$$k_{PC_M} = \frac{\Delta M}{\Delta N} \cdot \mu$$

Where ΔM is the consumed or produced quantity of the metabolite *M*, and ΔN is the number of cells. Total cell RNA from both control and cytokine-exposed macrophages was isolated using the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. The isolated RNA samples were then sent to the transcriptomics unit at CSIC-UAM for RNA sequencing analysis (RNASeq). The raw data obtained was processed by the informatics unit of the ISCIII-Spanish Consortium of Biomedical research and Digestive Diseases (CIBEREHD) using the statistical DESeq2 package for R, which is based on the negative binomial distribution. This RNA-seq quantification software was used to summarize transcript expression levels for each gene as FPKM (fragments per kilobase of transcript per million reads mapped), which were then utilized for GSMMs reconstruction.

Flux Balance Analysis and Flux Variability Analysis

Flux Balance Analysis[15] (FBA) and Flux Variability Analysis[16] (FVA) are computational methods to predict and analyze the metabolic fluxes within a biological system. FBA[15] assumes that cellular metabolism is in a steady state, meaning that the rates of all metabolic reactions are balanced, and the concentrations of metabolites are constant over time. To represent this, FBA incorporates mass balance equation constraints, defining the network as a system of linear equations with upper and lower bounds for each reaction[15]. Using the FBA method, the distribution of fluxes in a metabolic network are computed under steady state conditions and taking into account these set of constraints, by optimization of a specific defined objective function[15]. To model cell metabolism, under different challenges, an artificial reaction that recapitulates the cell bioenergetic needs to survive (primary cells culture) or to proliferate (immortalized cells culture) is used as optimization function and is named “biomass reaction” and takes the role of the objective function. To simulate the metabolic, energetic, and reductive demands of macrophages, we implemented the macrophage biomass reaction described by Bordbar et al.[22] The mathematical expressions that define this method are[15]:

$$Maximize\ v_{obj}$$

$$S \times v = 0$$

$$LB_i \leq v_i \leq UB_i$$

For each reaction *i* being *LB* the lower bound constraint and *UB* the upper bound constraint of the reaction.

Another advantage of FBA is that it has the capability to integrate gene expression data into the metabolic network. However, one of the main drawbacks of FBA would be that most times, it does not provide a unique solution but rather an allowable solution space which tends to be quite big.

In contrast, Flux Variability Analysis[23] which is used in conjunction with FBA, evaluates the possible range of solution fluxes for each reaction by solving two optimization problems represented as[23]:

$$v_i^{max} = \max(v_i)$$

$$v_i^{min} = \min(v_i)$$

$$S \times v = 0\,;\ LB_i \leq v_i \leq UB_i\,;\ v_{obj} \geq f \cdot v_{obj}^{FBA}$$

Where *f* is the tolerance coefficient, which indicates the minimum percentage of the FBA optimized objective function value that needs to be achieved.

FVA provides flux boundaries values for each flux reaction and permit to estimate the FBA space of solutions that permit to achieve the desired FBA objective.[23] In addition, the resulting flux ranges provide a measure of the flexibility or robustness of the metabolic network, and it is used to identify metabolic pathways that are most sensitive to changes in environmental conditions or genetic perturbations.

Gene Inactivation Moderated by Metabolism, Metabolomics and Expression (GIM3E) algorithm

The Gene Inactivation Moderated by Metabolism, Metabolomics and Expression (GIM3E)[17] algorithm aims to integrate transcriptomics and metabolomics data to improve the accuracy of flux calculations in Genome-Scale Metabolic Models (GSMMs), resulting in more reliable solutions that align closely with experimentally validated data. Incorporating transcriptomics data into the

model introduces additional constraints, which reduces the solution space[17]. GIM3E applies gene expression data to assign weights to each reaction, setting the reaction's minimization rate for subsequent flux calculations. The lower the expression levels the higher the minimization weight, ensuring a reduced flux value for the less expressed reactions. The mathematical expressions are:

$$Minimize \sum_{i}^{N} w_i \cdot |v_i|$$

$$w_i = \max(ge_{th} - ge_i, 0)$$

Also subject to:

$$S \times v = 0;\ LB_i \leq v_i \leq UB_i;\ v_{obj} \geq f \cdot v_{obj}^{FBA}$$

Where ge_{th} is a threshold (100 FPKM in our workflow) that can be set by each user which sets the minimum gene expression for a reaction to be present in the model while ge_i is the gene expression of the *ith* reaction. These calculations are also subjected to the same constraints as described for FVA.

Metabolic Transformation Algorithm.

The Metabolic Transformation Algorithm MTA)[18] algorithm is used to mimic an aimed metabolic switch between two metabolic states by minimizing the difference between flux fold change (FC_r=FCK_r/FCT_r) and gene expression fold change (*FCg*) between the two conditions. This algorithm is based on the metabolic transformation algorithm (MTA).[18] To simulate the metabolic switch, the algorithm maps differentially expressed genes and then minimizes the differences in reaction fluxes. This mapping helps define a set of reactions whose fluxes should be increased and another set of reactions whose fluxes should be decreased to achieve the desired metabolic phenotype. Before running the qMTA it is necessary to compute the flux distribution for the reference condition which can be done with GIM3E or FVA.

The qMTA can be mathematically expressed as:

$$min \sum_{g \in DExp} \left(W_g \sum_{i \in R_g} \left(\frac{v_i^{ref} \cdot FC_g - v_i^{res,MTA}}{v_i^{ref}(FC_g - 1)} \right)^2 \right) + \sum_{j \in Rexp} \frac{\left(E_j - v_j^{res,MTA}\right)^2}{\sigma_j} + \sum_{i \in Ru} \frac{\left(v_i^{ref} - v_i^{res,MTA}\right)^2}{v_i^{ref}}$$

$$W_g = log_{10}\left(p_{th}/p_g\right)$$

$$S \times v^{res,MTA} = 0$$

$$LB < v^{res,MTA} < UB$$

Where:

$DExp$ is a set of differentially expressed genes between the conditions.

W_g is gene *g*'s the weight.

R_g is a set of reactions associated with gene *g*.

vi^{ref} is the reference flux value in the control condition for reaction *i.*

FC_g is the gene expression fold change relative for the g gene.

$vi^{res,\ MTA}$ is the flux value obtained by the qMTA for reaction i in the desired metabolic state.

$Rexp$ is a set of fluxes measured experimentally.

E_j is the mean flux for the experimental measure of reaction *j* in target condition.

σ_j is the standard deviation of the flux for the experimental measure of reaction *j* in target condition.

R_u is the set of reactions not associated with differentially expressed genes between the conditions.

p_{th} is the p-adjusted value threshold (previously set by the user) to consider whenever a gene is significantly expressed.

p_g is the p-adjusted values for the gene expression of gene g.

4 Results and Discussion

Computational workflow developed to generate macrophages GSMM before and after treatment with cytokines

A computational workflow was developed to generate GSMMs for macrophages under both control and cytokine-exposed conditions. The workflow integrates available transcriptomics (RNAseq for both conditions) and metabolic data (glucose consumption and lactate production of macrophages, cultured in standard conditions in presence/absence of cytokines, measured at different time points from 0 to 24 in standard cell culture conditions). Since the objective of the workflow is to characterize the metabolic differences between non-activated macrophages and macrophages after cytokine treatment, which is expected to induce a pro-inflammatory state (M1), it was necessary that the computational workflow was able to recreate the metabolic states as close as possible to the metabolic macrophage phenotypes. Taking this into account the GIM3E algorithm was chosen to be the main algorithm to determine the reaction fluxes due to its capability to integrate transcriptomic data and find more efficient solution fluxes for the reactions while maximizing the objective function.

The workflow involves the reconstruction of macrophage GSMMs and uses both experimental metabolomic and transcriptomic data as well as the composition of the commercial cell culture media (*Fig. 2).* The workflow consists of three computational blocks. In the first computational block, referred to as *model preparation block,* several inputs are used, including the Recon3D model, metabolite concentrations in the cell culture medium, production and consumption rates, and gene expression data. The primary objective of this block is to obtain condition-specific models. To achieve this objective, the model is optimized using the provided inputs. Flux Balance Analysis (FBA) is employed to calculate the optimal fluxes and exchanges within the metabolic network. This optimization process considers the medium concentrations and production and metabolite consumption rates, allowing the model to adapt and reflect the specific conditions under investigation.

In this step, a list of reactions is compiled for further gene knockout (KO) testing in the subsequent step of the workflow. The reactions included in this list fulfill two criteria: first, they are associated with genes that exhibit lower expression levels than a predefined threshold (FPKM below 1 in all conditions), and second, they have a flux value lower than a predefined threshold (in our study, $1 \cdot 10^{-8}$ pmol/(h·cell)). With the list of genes from the previous step, a consecutive gene knockout (KO) test is performed. Each gene in the list is individually knocked out from a test model, which is identical to the recon3D input model. The impact of each knockout on the biomass function is evaluated using FVA calculations. The results of FVA calculations are examined to ensure that the model can still produce at least

10% of the optimal biomass and maintain the measured rates of metabolite uptake and secretion even after the tested gene KO. The FVA calculations are also used to check that the tested gene KO is not affecting any of the previously defined essential reactions. The purpose of this evaluation is to verify that the removal of the specific genes does not significantly impair the model's ability to sustain essential metabolic functions. Finally, those genes which KO testing did not cause a biomass production to fall to 10% of the optimal biomass and did not affect the essential reactions are eliminated from the model. This process leads to the generation of condition-specific models. By deactivating reactions based on gene expression data, the resulting condition-specific model is more representative of the biological reality and provides insights into the metabolic behavior under the given conditions.

In the second block, named as the *sampling block,* GIM3E is used to weigh the minimization of fluxes based on gene expression as well as the production and consumption experimental rates, resulting in a more accurate reconstruction. Subsequently, flux variability analysis is used to identify the solution space within 99% of the GIM3E optimal solution. Once the solution space is identified, it needs to be sampled. For this purpose, in this block, the Artificial Centering Hit-and-Run (ACHR) algorithm[24] is used to sample the solution space of the model. This algorithm allows us to determine the minimum and maximum ranges of flux for each reaction, as well as the average value of flux.

In the third computational block, referred to as *comparison block*, the qMTA algorithm starts having as an input the mean flux values obtained after the sampling. This block is considered the most important one because its main objective is to characterize the metabolic differences between the control and cytokine-exposed conditions, that are represented by their respective model solutions. In order to facilitate the comparison of phenotypes, the reaction data is organized in well-established KEGG pathways (*Fig. 2*) to link model reactions and the pathways they are part of. Once the differences are determined, it would be possible to identify targets that are more likely to facilitate the transition towards the desired state.

It is worth noting that the model preparation block is very important because it reduces the computational time and costs of the next blocks by trimming the deactivated reactions without affecting significatively biomass. In addition, the first two computational blocks also provide information in other output files (in excel format) about how well the model is able to adapt the exchange reactions related to the experimental consumption rates to have the desired experimental flux. In cases where adaptation is not possible, it may indicate an incompatibility between the media constraints file, which set the upper and lower bounds for all the reactions, and the experimental consumption rates.

Study of the Macrophage metabolic flux changes in response to cytokines treatment

We applied the developed computational tool to study metabolic flux changes of macrophages in response to cytokines exposure. The experimental data used as inputs to run our workflow for GSMMs, have been provided by Dra. Cascante (University of Barcelona) and Dr. Bosca (CSIC, Madrid) laboratories.
This data consists of transcriptomic data of macrophages (non-treated and treated with cytokines) and glucose consumption and lactate production data from macrophages cell culture media from both experimental conditions (see methods section for details).

We conducted an analysis of flux distributions through the metabolic pathways defined by the KEGG database. In *Fig. 3,* which represents the top 10 pathways with higher flux. We can observe that the pathways with higher fluxes are glycolysis, purine metabolism, TCA cycle, oxidative phosphorylation, one carbon pool by folate, fatty acid metabolism, pyruvate metabolism and several nucleotides metabolism.

The pathway that has the highest flux is glycolysis, in both control and cytokines-treated macrophages, and a 2.5-fold increase of flux is observed in treated cells *vs* control cells. It has also been found that the purine pathway is considerably enhanced upon cytokines treatment compared to the pyrimidine pathway, which presents a slightly decreased flux. Furthermore, the pentose phosphate pathway presents almost no flux variation between the two conditions (see figur*e Fig. 4)* and it is not shown in figure *Fig. 3.* since it falls into the top 25 pathways with higher flux. Finally, we observed that the other pathways in the top-10 flux ranking are those related to fatty acids and amino acids metabolism.

To compare the metabolic differences between the two sets of macrophages, we are going to measure log2 fold change ($Log_2\ FC_i$) where FC_i is calculated with the expression:

$$FC_i = FCK_i \,/\, FCT_i$$

Where FCK is the determined reaction flux for the cytokine-exposed macrophages and FCT is the determined reaction flux for the non-activated macrophages.

Figures 4 and 5 show the obtained log2 fold change for the glycolysis, TCA cycle and Pentose Phosphate pathways.

Based on literature[12,25,26] we expect macrophages exposed to cytokines to exhibit M1 polarization, which tend to have a higher level of glycolytic and pentose phosphate metabolism. M1 macrophages also tend to synthesize more fatty acids and rely on specific signaling molecules such as succinate (to maintain a balance with akg), citrate (for transport to the cytosol), and itaconate.

As stated, Glycolysis plays a crucial role in ATP production in the cytokine exposed macrophages. This phenomenon, known as the Warburg effect, is observed in M1-polarized macrophages redirecting pyruvate to lactic fermentation. Moreover, this increase in glycolysis can result in the production of more intermediates, which are crucial for the metabolic adaptation of the cell, that are essential for synthesizing nucleotides, amino acids, and fatty acids.
Therefore, the switch towards a more glycolytic state when the macrophages are exposed to cytokines is consistent with M1 polarization *(Figs. 3 and 4)*. Furthermore, in *Fig. 5* we can observe the Warburg effect by having a similar increase in glycolysis and lactate formation. The increase in glycolysis also explains the detected enhancement of nucleotides, amino acids and fatty acids pathways, as seen previously (*Fig. 3.)*

Another pathway that has a high flux and variation between conditions is the one carbon pool by folate which is reported by (Chen S et al., 2020)[27] to be involved in the production of IL-1β in proinflammatory macrophages. The study showed that serine, which is synthesized through the one carbon folate pathway, is a key nutrient that supports the production of IL-1β in macrophages by activating the mTOR signaling pathway. Inhibition of the one carbon folate pathway reduces the production of IL-1β in proinflammatory macrophages.

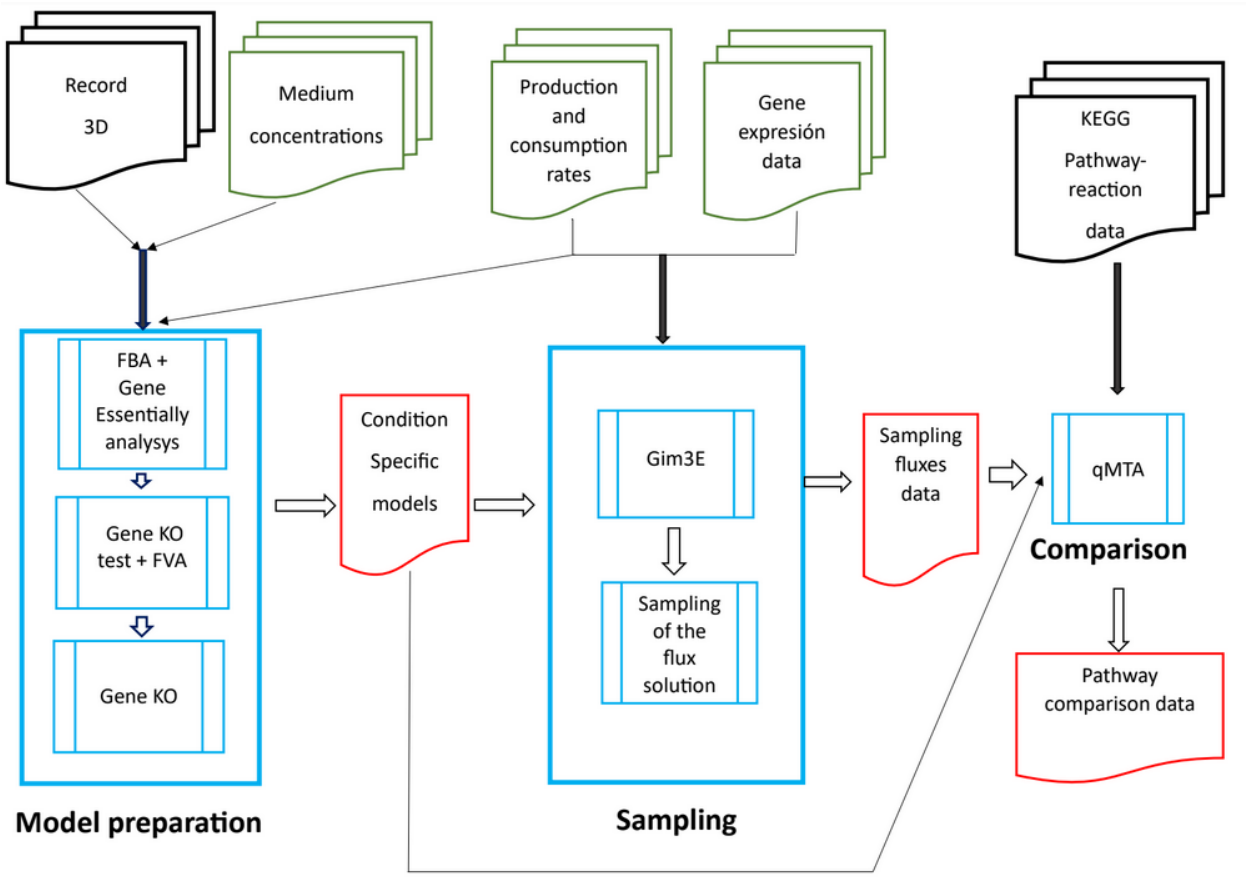

Figure 2. *Workflow developed to create the GSMMs for the study of cytokines treatment on macrophages metabolism. The Blue squares represent the computational blocks and tasks, and the red elements symbolize the most important outputs. The green and black elements represent the inputs used for the analysis, experimental and external data respectively.*

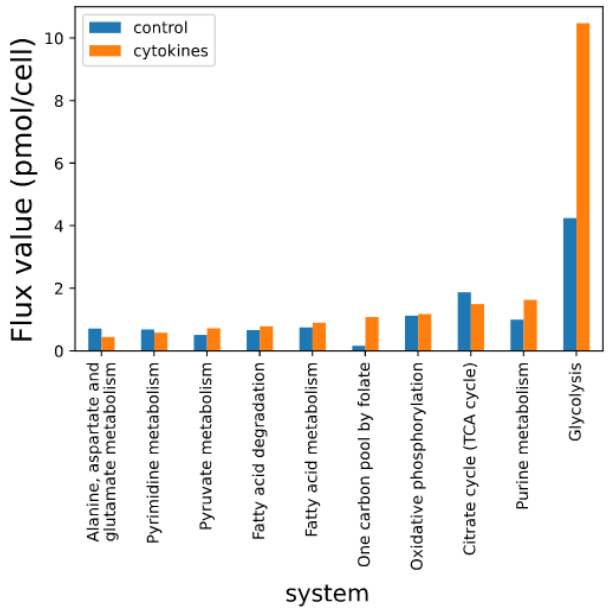

Figure 3. *Metabolic flux values predicted by GSMMs for control and cytokines treated macrophages*

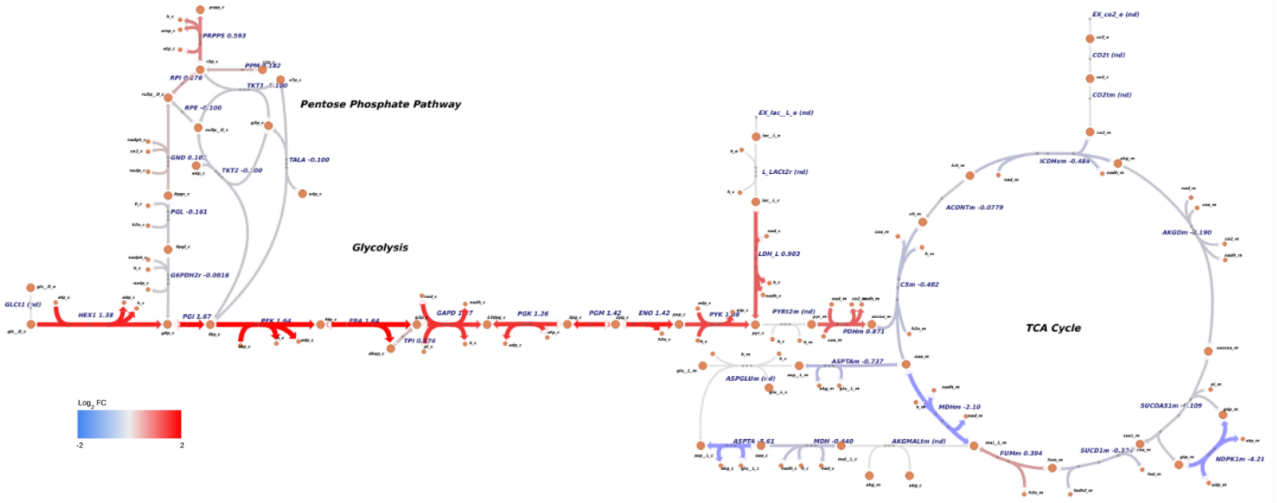

Figure 4. *Overview of the metabolic flux map differences of glycolysis, pentose phosphate pathways and TCA cycle between the conditions of control and cytokines-treated macrophages. Flux upregulation and downregulation are shown in red and blue, respectively. Each reaction is presented as the enzyme/transporter ID, the width of the lines is associated with log2FC value of the flux.*

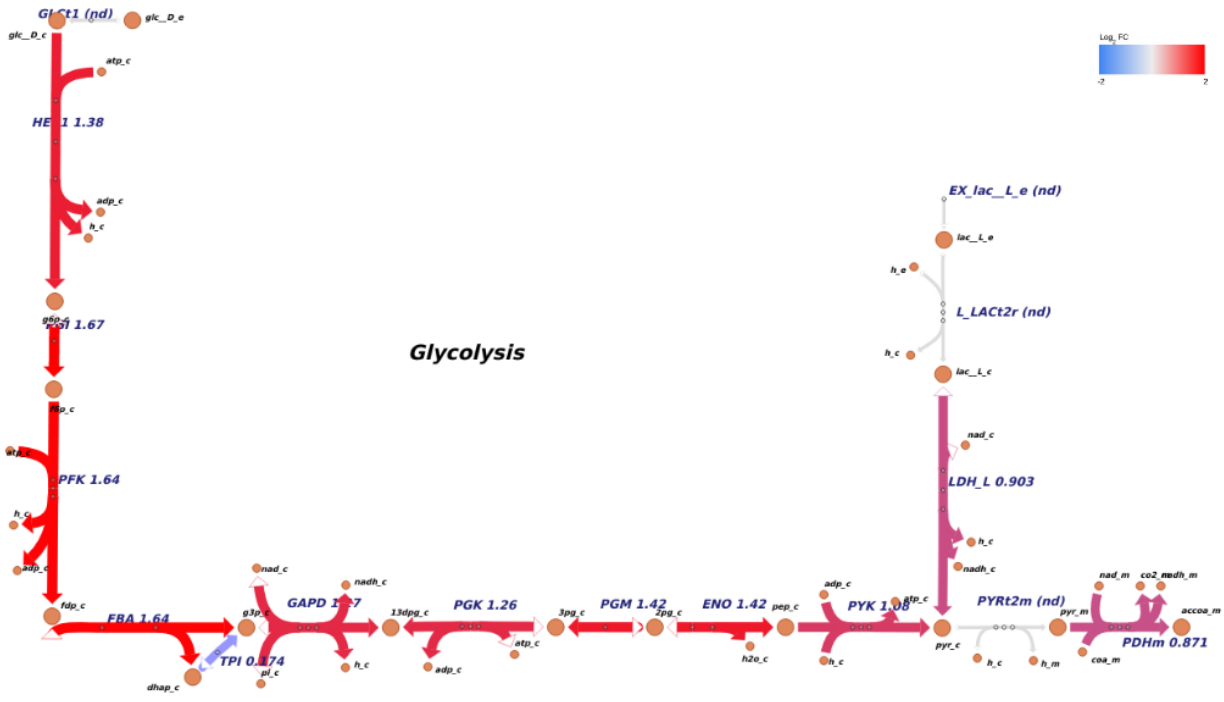

Figure 5. *Detailed metabolic flux map differences of glycolysis between control and cytokines-treated macrophages. Flux upregulation and downregulation are shown in red and blue, respectively. Each reaction is presented as the enzyme/transporter ID, the width of the lines is associated with log2FC value of the flux.*

Regarding flux variation between purine and pyrimidine metabolism *Fig. 3,* it has been reported that purine metabolism is enhanced in M1-like macrophages during M. tuberculosis infection[28].

Considering these evidences, we hypothesize that purine metabolism migth play an important role in the metabolic response of macrophages to cytokines treatment and that may contribute to the disparity between purine and pyrimidine fluxes, as the latter does not excert a significant effect on macrophages metabolism. However, further research and experimentation is needed to unravel the exact role of purine metabolism in macrophage metabolic reprogramming in response to cytokines storm.

A decrease in TCA cycle activity has been reported associated to the transition from M0 to M1-Macrophages, concomitant to the dramatic increase in glycolysis. This decrease has been related to downregulation of isocitrate dehydrogenase that results in accumulation of citrate and increased synthesis of itaconate, a metabolite that inhibits the conversion of succinate into fumarate leading to succinate accumulation. Succinate accumulation has been reported to stabilize (HIF)-1α which results in an increased dependence of glycolysis.[26] It has also been reported that the increased glycolysis in M1 macrophages results in a decrease of mitochondrial oxidative phosphorylation (OXPHOS) in the M1 pro-inflammatory phase and that OXPHOS decrease in the resolution of the inflammation state (M2). Our results are in accordance with these observations reported in the literature in the sense that we see a slight decrease in TCA cycle and 2.5-fold increase in glycolysis under cytokines treatment *Fig. 3.* Regarding OXPHOS the GSMMs predict a slight increase of OXPHOS upon cytokines treatment (see *Fig. 3*) which will be indicative of a failure in our model predictions or to the fact that macrophages start to transit from M1 to M2 state after the acute pro-inflammatory phase (M1 state).

Recently Dr. Boscà laboratory (CSIC, Madrid) performed such experiments and obtained a slight increase in OXPHOS after the treatment of macrophages with cytokines (results not shown) thus confirming our model prediction-.

5 Conclusions

We developed a workflow to study metabolic reprogramming in cells in response to an infection or drug intervention able to successfully predict the metabolic adaptations of macrophages in response to cytokines treatment. This workflow integrates transcriptomic genomic-scale data, and available metabolic experimental measurements.

We applied a developed workflow for the characterization of metabolic reprogramming in macrophages exposed to cytokine storm and we identified glycolysis, purine metabolism and one-carbon metabolism as the pathways with higher increase on flux upon cytokines treatment. The analysis of the GSMMs-predicted metabolic differences is in accordance with the metabolic characteristics of M1 macrophages reported in the literature, suggesting that exposure to cytokines storm, associated to Sars-Cv2, can dramatically increase the pro-inflammatory state of macrophages contributing to the exacerbated inflammatory response observed in acute Covid infection.

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