MAXIMUM LIKELIHOOD FACTOR ANALYSIS IN MALARIA CYTOKINES ANALYSIS AND MODELLING

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ABSTRACT

A maximum likelihood factor analysis (MLFA) of the dynamics of the human immune system in malaria, based on the observation of a few cytokine in plasma, suggests that an increased overproduction of cytokines: IL-12, IL-6, IFN-γ, IL-2, and IL-5, over the cytokines: TGF-β, TNF-α, IL-10 and IL-1β has protective effects against severe malaria and cerebral malaria.

According to a simplified model of the human adaptive immunity and the MLFA results, basal levels of cytokine IL-6 may play a key role in the favorable resolution of a malaria infection.

1. INTRODUCTION

In spite of the research efforts made in the fight against malaria, we are far from achieving an effective vaccine [1]. A possible explanation to this fact would be the partial knowledge of how natural malaria immunity is acquired in humans. The residents of regions where malaria is endemic, develop some form of natural partial immunity that maintains asymptomatic even if they are parasite carriers. The speed and intensity that the immunological system responds to malaria infection is determining for a favorable outcome of the infection [2].

A usual way of characterizing the dynamics of the immunological human system is based on the observation and analysis of certain variables, such as the cytokine concentration in plasma. Given that the number of cytokines to measure can be very large, which cytokines are relevant for correctly characterizing the dynamics of the immunological human system in malaria?

2. CYTOKINES ANALYSIS IN MALARIA

A usual way to answer this question in dynamic systems modeling is using the "trial and error" strategy. A model is proposed and tested if it fits enough to the reality. If the proposed model does not fit enough to the reality, the proposed model and their associated parameters are modified iteratively until we achieve a model that characterizes with sufficient fidelity the observations.

2.1. Maximum Likelihood Factor Analysis (MLFA)

One of the methods that provided a good degree of fidelity modeling the behavior of the cytokine levels in plasma, and the several degrees of severity of malaria, has been the maximum likelihood factor analysis (MLFA) [3]. The MLFA method, computes the maximum likelihood estimation (MLE) of the factor loadings matrix $\underline{\underline{\Lambda}}$ of the factor analysis model:

$$\underline{x} = \underline{\underline{\Lambda}}\,\underline{f} + \underline{\mu} + \underline{e}, \qquad (1)$$

where the vector $\underline{x}$ contains the values of the diverse cytokine observed values (d=10). $\underline{\underline{\Lambda}}$ is a constant matrix (d x m) of factor loadings (or mixing matrix) to be estimated. $\underline{f}$ is a vector (length m=number of factors) of independent and standardized common factors with distribution $N(0,\underline{\underline{I}})$. $\underline{\mu}$ is a constant vector of means, and $\underline{e}$ is the noise vector with distribution $N(0,\underline{\underline{\psi}})$. The iterative numerical algorithm to estimate $\underline{\underline{\Lambda}}$ and $\underline{\underline{\psi}}$ is described in [4, 5].

When the sample covariance matrix is of full rank, the ML solution always exists because the likelihood function is bounded (the covariance matrix is of full rank when the number of observations is greater than the number of variables).

2.2. MLFA in malaria cytokines analysis

The MLFA (with two factors) has been applied the to the analysis of the datum contained in one of the most large study undertaken to date [6]. In this study, several cytokines levels in plasma (IFN-γ, IL-2, IL-5, IL-6, IL-12, IL-4, TGF-β, TNF-α, IL-10, IL-1β) are related with several degrees of malaria severity: endemic control (EC), ex cerebral malaria (Ex-CM), mild malaria (MM), severe noncerebral malaria (SM) and cerebral malaria (CM). Due to that we want to focus our analysis only on the most serious malaria degrees (CM, SM and MM) we have grouped the individuals belonging to the classes EC and Ex-CM, in a new class called no-malaria (NM).

The results of MLFA, are shown graphically in Figure 1, where all the data has been transformed into the new 2D space (with the inverse matrix of the ML estimation of factor loadings matrix: $\underline{\underline{\Lambda}}^{-1}$) and the boundary between classes can be established with simple lines (Voronoi diagram). From this result we can observe that all the measured cytokines are sufficient, in the sense

that they are sufficient for clearly recognize the four classes of malaria severity that we are considering: NM, CM, SM and MM (there is no overlapping between classes in the new 2D transformed space).

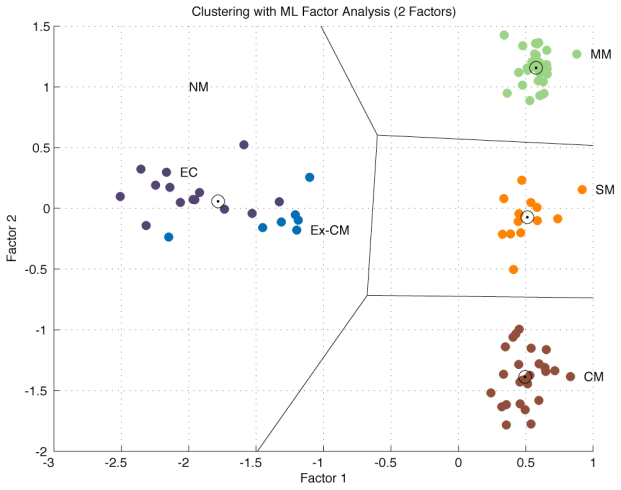

Figure 1. Results of MLFA, k-means clustering and the centroid and boundary for each class.

If we consider the absolute value of the vectorial contribution of each cytokine in the new transformed 2D space (see figure 2), we can observe that all measured cytokines are relevant (sorted by magnitude: IL-1β, IL-2, IL-12, IL-10, IFN-γ, TNF-α, TGF-β, IL-5, IL-6), with the exception of IL-4, which is quasi irrelevant compared with other contributions (very similar results are obtained without considering IL-4).

Now, if we observe the direction of the vectorial contribution of each cytokine individually (column vectors of $\underline{\underline{\Lambda}}^{-1}$) in the new transformed 2D space (figure 2), we can observe that there are two differentiated groups of cytokines. The group 1 (G1) formed by: IL-12, IL-6, IFN-γ, IL-2 and IL-5, and the group 2 (G2) formed by: TGF-β, TNF-α, IL-10 and IL-1β.

Roughly speaking, in terms of malaria severity degree, this means that:

- An increase of one or several cytokines levels included in the G1, and at the same time a similar increase of G2 cytokines, we are evolving from a no malaria state (NM) to the severe malaria state (SM).
- A predominance of the increase of one or several cytokines levels included in the G1 over G2 cytokines, we are evolving from a no malaria state (NM) to the mild malaria state (MM).
- A predominance of the increase of one or several cytokines levels included in the G2 over G1 cytokines, we are evolving from a no malaria state (NM) to the cerebral malaria state (CM).
- Within each cytokines group (G1 or G2) not all he cytokines have the same contribution in order to evolve to a determinate state. For example, in G1 group, the IL-12 contribution is greater than IL-2 or IFN-γ in order to reach the MM state. Analogously in G2 group, the contribution of TGF-β or TNF-α is greater than IL-1β in order to reach the CM state.

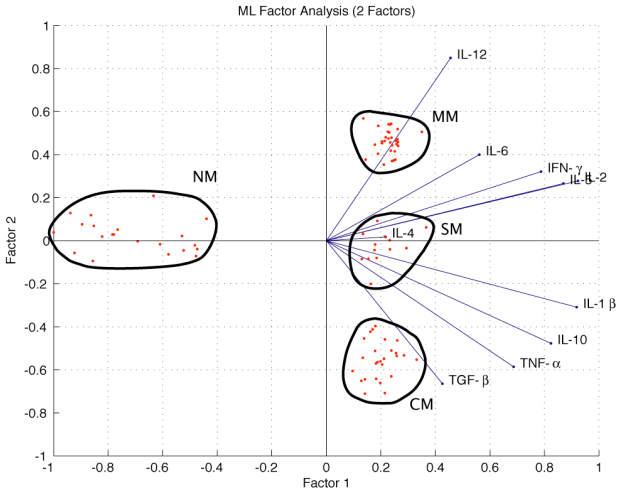

Figure 2. MLFA superposed to of the cytokines vectorial contribution in the new 2D space.

As a result of MLFA, in the 2D new space, the factor-1 axis (abscises) measures the no-malaria/malaria degree (from negative for no malaria, to positive values for malaria), while the vertical axis measures the malaria severity degree: CM, SM and MM. (range values for factor-2 axis: (-∞, -0.7), [-0.7, 0.6], and (0.6, ∞) corresponds to CM, SM and MM respectively).

The main difference between our results, obtained with MLFA and the results obtained with LDA in [6], is the estimation of the contribution of each cytokine to the different degrees of malaria severity (for example, while in [6] show that TGF-β does not contribute to any malaria state: SM, MM, CM, we observe that TGF-β is the most contributive cytokine to CM state).

If we conclude that the measured cytokines (with the exception of IL-4) are sufficient to clearly discriminate between: NM, CM, SM and MM, which is the mechanism that makes that a malaria infected inhabitant of endemic malaria zones, evolve to a SM or a CM in spite of to MM?

3. ADAPTIVE IMMUNITY MODEL

The response of the human immune system to the presence of different pathogens is mediated by the T helper cells (Th cells or naive CD4 + cells) [7].

The selective activation of different subsets of Th cells (Th1, Th2, Treg, Th17) is mainly controlled by the activation of the Th cells receptors, the cytokines and other co-stimulatory molecules. The cells of the different cellular lines: Th1, Th2, Th17 and Treg, are specialized in the production of some cytokines depending on the stimuli.

3.1. A qualitative simplified model

The details of this regulation system in humans is partially known and based in mice models [8]. The model introduced in this section has the particularity to consider

the cytokines as state variables and consider four cellular lines: Th1, Th2, Treg and Th17. Considering the cytokines as the central point of the system, as state variables (fully observable), clarifies the role (stimuli or inhibitor) of each cytokine over the different cellular lines. Considering four cellular lines (rather than the classical Th1/Th2 used until now in malaria), we introduce two new freedom degrees, in order to interpret the results of previous section in terms of Th1/Th2/Treg/Th17 cellular activity (would be impossible to explain the results obtained in section 2 only in terms of Th1/Th2 activity).

The figure 3, shows the qualitative relations (presently known in humans) between cytokines, acting as stimuli or as inhibitor (through the corresponding transcription factor), and each cellular line. Note that IL-12 (canonical) is only supplied externally. While, in the regulatory system of the cellular lines, Th1, Th2 and Treg, the degree of similarity between humans and mice is very high, this is not the case of the cellular line Th17, where substantial differences have been observed [9-12]. In Th17 cellular line the stimulatory loop: IL-1β, IL-6, IL-23 (TGF-β, TNF-α) means that IL-1β is the necessary cytokine for Th17 cells production stimulation, IL-6 and IL-23 have co-stimulatory effects, but the maximum is obtained with a weighted combination of five cytokines: IL-1β, IL-6, IL-23, TGF-β and TNF-α (see [12]). RORC is the human ortholog of RORγt, the putative murine Th17 master regulatory transcription factor.

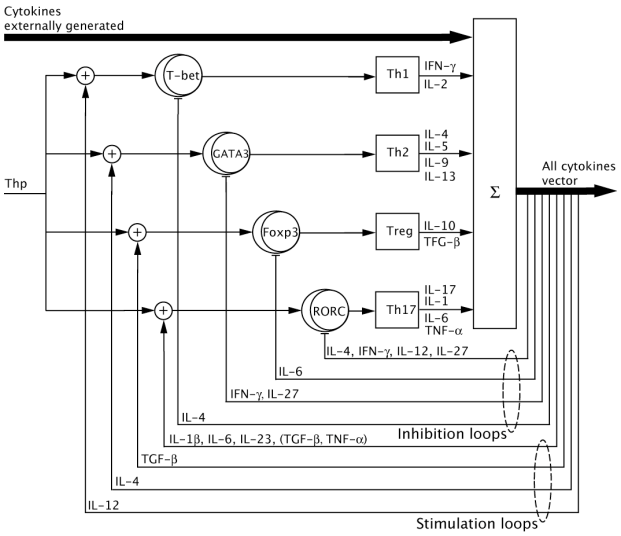

Figure 3. Schematic representation of qualitative relations between cytokines (stimulation, inhibition loops and externally supplied), and transcription factors, for each cellular line (Th1, Th2, Treg and Th17) in humans.

The cytokines, are not only produced by Th1, Th2, Th17 or Treg cells, they are also produced for other cells type such as macrophages or dendritic cells. This model also includes a possible contribution of any cytokine externally produced. In malaria, it has been observed in vitro that, in response to the Plasmodium, dendritic cells synthesize IL-12 and IL-6 and the macrophages secrete TNF-α IL-1, IL-6 and IL-8 [13, 14].

3.2. Relation between malaria cytokine analysis and the simplified model

Based on the data analysis of section 2 and its correspondence with the simplified model of the last section, we could say that:

- A high level of IL-6 may play a decisive role in a favorable resolution of malaria because they minimize the production/activation of anti-inflammatory cytokine TGF-β and IL-10 acting on the transcription factor Foxp3 (Forkhead Transcription Factor Winger Box3) [7, 15].
- With a low levels of TGF-β and IL-10 and given its negative cross-correlation with IL-12, a higher level of IL-12 would be expected, and as consequence a higher levels of IFN-γ and IL-2 (Th1).
- If the levels of IL-12 and IFN-γ are high, it means that a lower activity of the Th17 cellular line (IFN-γ and IL-12 inhibits Th17 activity acting on RORC transcription factor) and lower levels of IL-1β, TNF-α would be expected.

In conclusion, it means that the initial level of IL-6 can be determinant, in the role of steer the response of immunitary system towards a major G1 cytokines (IL-12, IL-6, IFN-γ, IL-2 and IL-5) production, in detriment of the G2 cytokine (TGF-β, TNF-α, IL-10 and IL-1β) production (and viceversa).

4. DISCUSSION

4.1. The hypothesis of the key role of the initial levels of IL-6 in malaria

Therefore in malaria, the IL-6 can be the "key cytokine" capable to bias the immunological system response towards a predominance of Th1 and Th2 cellular lines, over Treg and Th17. In the case of the malaria, the problem is that, the levels of IL-6 should be high before the parasites abandon the liver in order to inhibit the characteristic TGF-β activation and upregulation of Foxp3. It has been observed in humans, that the levels of the IFN-γ, IL-12, IL-10, TNF-α and IL-4 in plasma increase 4 or 5 days after the TGF-β burst [16].

It seems that an initial high level of IL-6 (and probably extended in time), should be a necessary initial condition to meet previously the parasite abandons the liver. Similarly we might think that people with symptoms of chronic inflammation, that clearly implies a high level of IL-6, could be examples of this bias in their immune system response. The sickle cell disease (SCD) and G6PD deficiency are examples of chronic inflammation and the patients with this disease can double the levels of IL-1 and IL-6 in respect control persons [17, 18]. Otherwise, the sickle cell disease and the G6PD deficiency, have been diseases traditionally associated to some degree of protection against severe malaria [19].

4.2. Signs of evidence of our hypotheses

Testing the hypothesized key role of the initial high levels of IL-6 in malaria infection is not a simple task, at

least "in vivo" and in humans. But, we believe that there are some research works that could help make our hypothesis more likely.

Interethnic studies made in Mali and Burkina Faso on the susceptibility to malaria in areas where it is hyperendemic, shows that the Fulani are less susceptible than their own neighbors of other ethnic groups. In a recent work [20] it was found that their immunity can be conferred by their genetic profile where the Fulani express higher amounts of RNA of some genes related to the Th1 and Th2, and a lower expression of the gene FOXP3 and CTLA4 compared with its neighbors, the Mossi ethnic group. Both FOXP3 as CTLA4 are directly involved in the activity regulation of Treg cell line and suggests that a functional deficit of Treg cells could be related to the lower susceptibility to malaria in this ethnic group.

5. CONCLUSION

To confirm the hypothesis presented here, on the role of an initial high level of IL-6, in the favorable outcome in a malaria infection, we would contributed to clarify some of the mechanisms by which the Plasmodium interacts with the human immune system.

Otherwise, all this work is based on an extended study of cytokines in malaria, and need to be properly weighted, because the samples were obtained in a very specific geographical area of India, and some of our results, observations and conclusions may not be valid, reproducible or extrapolated to other parts of the planet.

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