Supplemental Material: A mathematical model of phagocytosis of *C. neoformans*

The major findings in this system that are consistently observed in experiments and reported in ([2, 13, 15]) are an increase in the phagocytic index with the increasing concentrations of free antibody and the *prozone-like* effect, which is a paradoxical decrease in the phagocytic index at higher antibody concentrations.

We assume that phagocytosis is a result of attachment of microbes to macrophages and subsequent ingestion and that both processes are facilitated by the antibody bound to the microbe capsule and, possibly, to Fc receptors on macrophages. Therefore, the processes involved in phagocytosis are *binding*, of free antibody to microbes, as well as binding of free antibody to Fc receptors on macrophages. *Attachment* is a process in which antibody-coated microbes attach to macrophages. *Ingestion* is a process in which microbes attached to macrophages are internalized. In general, we assume that microbes are attached to macrophages and subsequently internalized through mechanisms that involve binding of the antibody already bound to microbes to available Fc receptors on macrophages. This assumption is validated by the fact that in the absence of IgG1 opsonin, there is essentially zero attachment or ingestion. It is possible that the antibody bound to Fc receptors contributes to the attachment of microbes to macrophages through binding to available sites on the *C. neoformans* capsule. We expect that the contribution of the second type of the binding will

be very small due to the rigidity of the binding sites on the capsule as opposed to flexibility of the Fc receptors on the macrophage surface, but we did not rule out this possibility *a priori* since the experimental results indicated an increase in the phagocytic index at concentrations of free antibody high enough for most of Fc receptors to be occupied by antibody within minutes. Microbes can also attach to macrophages through complement receptors. In our phagocytosis system there is no phagocytosis mediated by complement component 3 (C3). However, antibody binding to the capsule is believed to produce a structural change that allows direct interactions of capsular polysaccharide with the C3 receptor [14]. Hence, attachment of antibody-opsonized cells to macrophages occurs through both Fc receptors interacting with microbe-bound IgG1, and CR3 receptors interacting with a polysaccharide structural motif. According to the published data, [1], it takes on the average 15 minutes for an attached microbe to be ingested. Here we investigate how the probability that a microbe will be attached to and eventually ingested by a macrophage depends on the amount of antibody present and/or bound to both macrophages and microbes.

We modeled the total amount of ingested microbes as a function of the antibody bound to the microbe capsule. The dependence of the rate of ingestion on the amount of antibody and percentage of attached microbes that are ingested have not been investigated to date and it is one of the possible future directions in which our model can be refined (see below).

We propose a mathematical description of phagocytosis based on differential equation *E-1* which describes the rate of change of the total number of ingested microbes P_I, $\frac{dP_I}{dt} = r_T P_F$.

We assume that the rate at which the microbes are ingested is proportional to the population of non-ingested (free) microbes P_F and introduce a function r_T, which is the relative $(\frac{1}{P_F}\frac{dP_I}{dt})$ rate of phagocytosis as a function of antibody bound to *C. neoformans* capsule and the number of receptors available. We call the function r_T the *efficacy of phagocytosis*.

Phagocytosis decreases the number of free microbes and the rate of change of the population of free microbes P_F is:

$$\frac{dP_F}{dt} = -\frac{dP_I}{dt} + \mu_P P_F \qquad (E\text{-}15)$$

The term $\mu_P P_F$ represents the growth rate of the population of unbound microbes. For the purpose of this study we neglected the growth of the population of microbes during the observed time of phagocytosis. We assumed that most microbes do not replicate while being phagocytized since the phagocytosis is much faster than the doubling time of *C. neoformans*. Furthermore, we assumed no intracellular growth since intracellular yeast replication occurs several hours after ingestion [17], while the experiments modeled here were limited to 2 h. Therefore we have that $P_F = P_0 - P_I$. The variables and parameters in our mathematical model are listed in Tables 1, 2 and 3.

Motivated by the experimental results that indicate that the efficacy of phagocytosis is a function of antibody bound to the *C. neoformans* capsule, we first studied binding of free antibody to the microbe capsule. Binding of free antibody to microbes and macrophages is

described by the following three differential equations

$$\frac{dA_P}{dt} = k_{FP}(L_P - A_P)c_F - D_{FP}A_P \qquad (E\text{-}16)$$

$$\frac{dA_M}{dt} = k_{FM}c_F X - D_{FM}A_M \qquad (E\text{-}17)$$

$$\frac{dc_F}{dt} = -\frac{1}{N_A V}(\frac{dA_P}{dt} + \frac{dA_M}{dt}) \qquad (E\text{-}18)$$

The variables and parameters that characterize the antibody binding processes we modeled are listed and described in Tables 2 and 3.

The differential equation *E-16* describes the change in the total amount A_P of the antibody bound to microbes. The first term describes the binding of free antibody to microbes, and the second term antibody dissociation. c_F is the concentration of free antibody (in mol/L) and L_P is the total capacity of microbes for binding the antibody, that is the number of antibody binding sites on the *Cryptococcus neoformans* capsule.

We adopted a general convention that the *on* rate constants are denoted by the letter k with two subscripts, say k_{XY}, where the subscripts indicate the reaction in question. Similarly, *off* rate constants are denoted by the letter D with two subscripts, say D_{XY}, where

the subscripts again indicate the reaction in question. Therefore k_{FP} stands for the forward rate constant in the binding process of free antibody to microbes and k_{FM} for the forward rate constant constant in the binding process of free antibody to macrophages. D_{FP} and D_{FM} are the corresponding dissociation rate constants and $k_{FP} = K_P D_{FP}$, $k_{FM} = K_M D_{FM}$.

The differential equation *E-17* describes the binding of free antibody to macrophages; c_F is the concentration of free antibody and X is the number of available Fc receptors on macrophages. The first term on the right hand side is the rate of the forward reaction, while the second term is again a dissociation term.

We are aware that there are at least three types of Fc-gamma receptors that could interact with IgG1 [1]. At this time there is not sufficient data on their relative prevalence and binding affinities for murine IgG1 to model them individually. Hence, we use the term Fc receptor to denote all the receptor types that can interact with IgG1 through its constant region.

The differential equation *E-18* describes the decrease in the concentration of free antibody through binding of free antibody to microbes and to Fc receptors on macrophages. N_A is Avogadro's number and V is the volume of a well (0.2 ml).

The range of antibody concentrations used in the experiments modeled here is well above the binding capacity of both macrophages and microbes (see *Results*). Therefore, in our preliminary analysis of the binding dynamics and discussion of the experimental setup we assumed that the concentration of the free antibody was constant, substituting $c_F = c_0$

in the remaining equations of our model, where c_0 is the initial concentration of the free antibody. This assumption enabled us to solve the differential equation *E-16* analytically. We also neglected the possible reduction in available binding sites on *C. neoformans* due the attached macrophages. The solution is the exponential function given by equation *E-12*. Equation *E-11* was obtained in the similar way from equation *E-17*. In addition to considered A_F to be a constant, when deriving *E-11* we also assumed that the number of available Fc receptors X is $L_M - A_M$ (see discussion below).

The last two equations in our model describe the rate of change of Fc and complement receptors. Published results [7] imply that Fc receptors ingested during phagocytosis of live cells are degraded. The expression of the Fc receptors in such conditions is not restored to the level prior to phagocytosis for hours. Therefore we can consider that the Fc receptors initially present are effectively removed from the cell after phagocytosis and for the duration of our experiments. It is possible that some Fc receptors are replenished during the course of our experiments, since Fc receptor activation triggers synthesis of Fc receptors [12]. However, since the published data are contradictory on this point, we did not include synthesis of new Fc receptors in our model.

Given that one macrophage can ingest numerous yeast cells, the number of microbes used in our experiments is considerably smaller than the phagocytic capacity of the macrophages involved. Hence, for the purposes of this study we neglected the possible influence of the decrease in the number of available receptors, both Fc and complement, due to their removal

through phagocytosis on the efficacy of phagocytosis.

We also assumed that the population of macrophages is constant, neglecting the rather small growth rate: the doubling rate of normal J774 cells is approximately 12 hours [6].

Binding of free antibody reduces the number of available Fc receptors for binding of microbe-associated antibody. Differential equation *E-19* describes the decrease in the number of available Fc receptors X due to binding of free antibody

$$\frac{dX}{dt} = -k_{FM} X A_F + D_{FM} A_M. \qquad (E\text{-}19)$$

We assume that the number of complement receptors does not change significantly due to phagocytosis, therefore Z is constant in our model.