Modelling Two Possible Mechanisms for the Regulation of the Germinal Center Dynamics

Joana S. Moreira and Jose Faro

J Immunol 2006; 177:3705-3710; doi: 10.4049/jimmunol.177.6.3705
http://www.jimmunol.org/content/177/6/3705

References
This article cites 30 articles, 15 of which you can access for free at:
http://www.jimmunol.org/content/177/6/3705.full#ref-list-1

Why The JI? Submit online.
• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Fast Publication! 4 weeks from acceptance to publication

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Modelling Two Possible Mechanisms for the Regulation of the Germinal Center Dynamics

Joana S. Moreira and Jose Faro

Research on the germinal center has tried to unravel the mechanisms that control its dynamics. In this study we focus on the termination of the germinal center reaction, which is still an open problem. We propose two hypothetical biological mechanisms that may be responsible for the control of germinal center dynamics and analyze them through mathematical models. The first one is based on the differentiation of follicular dendritic cells and/or T cells. Interaction of these cells in the differentiated state with germinal center B cells would promote B cell differentiation into memory B cells and Ab-forming cells, ending the germinal center reaction. The second mechanism applies only to a scenario without recycling and consists of the decay of a hypothetical proliferation signal for centroblasts that limits the number of cell divisions. Each of the models makes predictions that can be experimentally tested. The Journal of Immunology, 2006, 177: 3705–3710.

Germinal centers (GCs) are transient structures that develop in B cell areas of secondary lymphoid organs. They constitute preferential sites for B cell interactions that are crucial for an efficient Ag-elicited humoral immune response. Observations concerning the physiology of the GC have accumulated in the last 50 years (1–4). A primary GC reaction starts within 3 to 4 days after Ag administration and lasts for ~3 wk. During this period of time, the GC presents a dynamical spatial structure (5). Indirect evidence suggests that GCs are seeded by a few B cells (6, 7). These B cells evolve through different phenotypes during the GC reaction. In the beginning, only large proliferating cells with decreased expression of surface Igs, called centroblasts, are observed. Somatic hypermutation in Ig V regions occurs during centroblast proliferation. Centroblasts differentiate later into smaller, nonproliferating cells called centrocytes that express surface Igs. Finally, centrocytes undergo a process of selection of high affinity cells that seems to involve interactions with follicular dendritic cells (FDCs) and helper T cells (8–10). This selection is accompanied by the differentiation of centrocytes into memory B cells and precursors of Ab forming cells (AFCs) (2). The affinity for Ag measured in serum Abs increases after the 14th day after immunization, which corresponds approximately to the peak in the number of cells in the GC (2, 11, 12).

It is known that Ag persists in the GC environment long after the reaction has ended (13). The amount of Ag presented on the membrane of FDCs measured 30 days after immunization may be reduced to half of the initial value (14). This finding indicates that the termination of the GC reaction is unlikely to be caused by lack of Ag. Moreover, in the last 5 years experimental reports have raised questions about the regulation of GC dynamics by Ag. Studies reveal that there is no significant difference in GC kinetics, affinity maturation, and serum Ab formation for a wide range of Ag deposition on the membrane of FDCs (15). Other experiments with mice that lack detectable immune complexes (ICs) on the surface of FDCs (16) indicate that the presence of ICs is not necessary for normal GC formation, kinetics, and termination. Furthermore, it has been shown that, once B and T cells are primed by Ag, the presence of ICs on the membrane of FDCs is not necessary for the GC reaction to proceed (17). Thus, although Ag priming of B and T cells is necessary to initiate the GC reaction, the above observations indicate that the presence of Ag in the follicles is not required for the maintenance or termination of GCs. Therefore, Ag dynamics does not seem to control the dynamics of the GC, and understanding the mechanism responsible for this regulation is an open problem.

However, affinity maturation and differentiation into plasma and memory B cells seem to require the interaction of Ag plus FDC with B cells in the GC (17, 18, 19). Furthermore, it has been reported that FDCs change their phenotype during the GC reaction (5) and that B cell interaction with both FDCs and T cells provides survival signals to B cells and may induce FDC and T cell differentiation. Although for GC T cells there is still not much information, it is known that T cells are essential for early processes in the GC, such as triggering the hypermutation on B cells, and for different later processes, such as the differentiation of B cells into memory and plasma cells; this suggests that GC T cells may also change their effector state (20, 21).

The purpose of this work is to help clarify which biological mechanisms may be responsible for the control of the GC dynamics. In the light of the above experimental evidence, we hypothesize that the signals produced by undifferentiated FDCs and T cells, in the beginning of the GC reaction differ from those produced later by the differentiated cells. Whereas the undifferentiated FDCs and T cells would provide survival signals to B cells, the differentiated cells would provide signals to induce B cell differentiation into memory B cells and AFCs. We identify this as a potential biological mechanism for terminating the GC reaction, and we analyze it by mathematical modeling. Alternatively, we propose and model another mechanism for the termination of the...
GC reaction. This mechanism is based on the assumption that B cells receive a proliferation signal that decays as cells divide. After this signal has reached a certain level, B cells stop proliferating, are selected, and differentiate into memory B cells or AFCs, terminating the reaction. Predictions of each model are presented, and experimental tests for validating the models are proposed.

Materials and Methods
The numerical computations were performed with the software Mathematica 4.2 (Wolfram Research). The models were tested against two data sets obtained from the literature (Table I; see Refs. 11 and 12).

Results
Two types of models have already been proposed for approaching the problem of the GC dynamics and termination. In the first type of model it is assumed that B cell survival depends on the presence of Ag and that Ag depletion by interaction with centrocytes is the mechanism of GC regulation (22, 23). However, the dependency of the GC termination on the Ag level is not compatible with experimental evidence, as explained above. The second type of model considers that the mechanisms of generation of AFCs and memory B cells are time dependent and, therefore, the number of output cells from a GC is also time dependent. The hypothesis states that, after a certain time, production of output cells becomes faster than centroblast proliferation, leading to GC decay. This hypothesis has been studied through mathematical models with recycling (24, 25). In both models, the time dependency of the output production is modeled through the definition of three different phases of the GC dynamics, which are introduced to reproduce the experimental data. The models reproduce GC termination independently of Ag level, although the biological mechanism that accounts for the three phases of the GC reaction is missing. In this work, we model two hypothetical biological mechanisms that may control the GC dynamics. The first one is based on the differentiation of FDCs and T cells. Interaction of these cells in the differentiated state with GC B cells would promote B cell differentiation into memory B cells and AFCs, ending the GC reaction. The second mechanism consists of the decay of a hypothetical proliferation signal for B cells, thus limiting the number of cell divisions in a proliferation cascade that ends with B cell differentiation into AFCs or memory B cells.

GC regulation by differentiation of FDCs and T cells
Interactions of centrocytes with FDCs and T cells provide survival signals to the centrocytes and possibly induce the differentiation to a distinct effector stage of FDCs (5) and T cells (20, 21). We propose that the interaction of centrocytes with differentiated cells promotes their differentiation into AFCs or memory B cells that leave the GC, ultimately terminating the reaction. We model this mechanism and analyze the parameter conditions required to reproduce the experimental data of GC kinetics in specific primary immune responses.

We consider a compartment of B cells, B, that can proliferate, die, and be selected into GC output (Fig. 1). It is not clear whether B cell proliferation depends on signals from undifferentiated FDCs and T cells or whether it depends on signals from both differentiated and undifferentiated types (8). We therefore define two submodels, A and B, accounting for both possibilities. In the first case (model A), the effective proliferation term (including proliferation and death) is proportional to the number of B cells interacting with undifferentiated FDCs and/or T cells, which is proportional to \( B(1 - F) \), where \( F \) is the fraction of differentiated cells present in the GC (Equation 1a); in the second case (model B), the effective proliferation term is proportional to B (Equation 1b). In both submodels, the proportionality constant is the maximum effective proliferation rate of B cells in a GC, denoted by \( p \). It includes the effects of cell proliferation and death and, therefore, should be smaller than the proliferation rate usually estimated in experimental reports. (The proliferation rate is usually obtained by assuming exponential growth of the B cell population during the first days of the GC reaction, when there is virtually no B cell death. The numbers of GC B cells experimentally determined for initial time points are fitted to an exponential function to determine \( p \).) Moreover, in both submodels B cell selection is proportional to the number of B cells interacting with differentiated FDC/T cells, which is proportional to \( BF \). The maximum rate of B cell selection is \( k \). When writing equations (1) as a function of the fraction of differentiated FDC/T cells instead of the number of differentiated cells, we are implicitly assuming that \( p \) and \( k \) depend on the number of FDC/T cells; therefore, the values of \( p \) and \( k \) may differ from GC to GC. Furthermore, in both submodel A and submodel B the differentiation of FDCs and/or T cells is promoted by interaction with B cells (Equation 2). After being selected into the rescued cells compartment, \( R \) (Equation 4), B cells differentiate into AFCs or memory B cells and leave the GC. Therefore, one can write the following.

\[
\frac{dB}{dt} = p(1 - F)B - kFB \quad (1a)
\]
\[
\frac{dB}{dt} = pB - kFB \quad (1b)
\]
\[
\frac{dF}{dt} = pB(1 - F) \quad (2)
\]
\[
\frac{dR}{dt} = kFB \quad (3)
\]
Notice that Equation 1a can be obtained from Equation 1b by replacing $k$ with $k + p$, which yields similar dynamics for B and F compartments in both submodels.

Equations 1 and 2 can only be solved numerically. Therefore, one must determine the range of parameters that includes biologically reasonable values. The maximum effective proliferation rate, $p$, has been estimated in several experimental reports (11, 12, 26, 27), and choosing $p \in [0.5d^{-1}, 2.5d^{-1}]$ (where $d$ stands for day) includes all reported values. The maximum rate of B cell selection, $k$, is not known; the only restriction to this parameter is $k > 0$ for model A and $k > p$ for model B to lead the GC reaction to an end. The parameter $a$ controls the speed of FDC/T cell differentiation. If $a = 0.01d^{-1}$, differentiation of FDCs/T cells is very fast and B cells have little time to proliferate, resulting in few B cells; if $a = 0.0001d^{-1}$, differentiation of FDCs/T cells is very slow and B cells proliferate during a long period, resulting in an excessive number of B cells. Therefore, choosing $a \in [0.0001d^{-1}, 0.01d^{-1}]$ should be appropriate. Combining all of the parameter values described above, it is easy to observe that $F(t)$ can be well approximated by a logistic function as shown in Equation 4,

$$f(t) = \frac{1}{1 + \exp\left(-\frac{t + \tau}{s}\right)} \quad (4)$$

where $\tau$ is the day by which half of the FDCs/T cells are differentiated. The parameter $s$ determines the steepness of the function $f(t)$ and, for the parameter range mentioned above, we have $s \in [0.4d, 1.1d]$ (for the examples below, we have chosen $s = 1d$).

Therefore, to simplify the problem, one can define new equations for the B cell compartment (Equation 5a for model A and Equation 5b for model B), replacing $F(t)$ by an explicit function of time, $f(t)$.

$$\frac{dB}{dt} = pb - (k + p)f(t)B \quad (5a)$$

$$\frac{dB}{dt} = pb - kf(t)B \quad (5b)$$

Equations 5 and 6 can be analytically solved in terms of $p$, $k$ and $\tau$. To determine these parameters, we used information from experimental reports that show the GC kinetics in primary immune responses to (4-hydroxy-3-nitrophenyl)acetyl (12) and sheep RBC (11) in mice. These data provide approximate values for the GC initiation and termination; the maximum number of GC B cells, as well as the day when that value is reached, can be approximately read from the original data (Table I). Furthermore, it has been estimated that the time of differentiation of a GC B cell into an APC or a memory B cell is ~2 days (17, 28). Therefore, one can assume that there is a delay of 2 days between the time when a cell enters the compartment of rescued cells and the time it actually exits the GC, which means that at time $t$ the number of rescued B cells still inside the GC is $R(t) - R(t-2d)$. This information is introduced in the model to calculate parameters $p$, $k$, and $\tau$ through the following conditions shown in Equations 7–9,

$$B'(t_p) + R'(t_p) - R'(t_p - 2) = 0 \quad (7)$$

$$B(t_p) + R(t_p) - R(t_p - 2) = C_p \quad (8)$$

$$B(t_j) + R(t_j) - R(t_j - 2) = C_f \quad (9)$$

where Equation 7 is the condition for having a maximum number of cells at time $t_p$, the GC peak; Equation 8 is the expression for the number of GC B cells at the peak, $C_p$ and Equation 9 is the condition for the end of the GC reaction, where $C_f$ is the final number of cells and $t_j$ is the corresponding time. For each of the data in Table I, we obtained the corresponding GC dynamics and estimated the parameters of models A and B. The results are shown in Figs. 2 and 3 and in Table II. The dynamics calculated by the FDC/T cell differentiation models fit the data of both experimental reports.
Proliferation cascade of centroblasts controls the GC dynamics

If centrocytes do not recycle back into centroblasts, the GC dynamics can be controlled by the dynamics of the centroblast compartment. The decay of cellular concentrations of molecules with successive cell divisions is a signaling mechanism. This mechanism could be used to control cell division itself if the signal at stake is a proliferation signal. Therefore, the decay of a hypothetical proliferation signal for centroblasts would limit the number of successive cell divisions is a signaling mechanism. This mechanism could be used to control cell division itself if the signal at stake is a proliferation signal. Therefore, the decay of a hypothetical proliferation signal for centroblasts would limit the number of centroblast divisions. B, centroblasts; C, centrocytes; R, selected centrocytes; M, output cells. The output compartment, M, comprises memory B cells and AFCs that have left the GC (Equation 15).

\[
\frac{dB_0}{dt} = -p_B B_0
\]  

(10)

\[
\frac{dB_i}{dt} = 2p_B B_{i-1} - p_B B_i, \text{ for } i = 1, 2, \ldots, n - 1
\]  

(11)

\[
\frac{dB_n}{dt} = 2p_B B_{n-1} - k_B B_n
\]  

(12)

\[
\frac{dC}{dt} = k_B B_n - (k_c + d_c)C
\]  

(13)

\[
\frac{dR}{dt} = k_c C - k_R R
\]  

(14)

\[
\frac{dM}{dt} = k_R R
\]  

(15)

Equations 10–15 can be analytically solved, although the complicated form of the solutions does not contribute to an intuitive understanding of the model dynamics. Therefore, we introduce parameter values taken from experimental reports and solve the system numerically. From experimental data, the rate of centrocyte apoptosis and the rate of differentiation into output cell can be fixed as \(d_c = 4d^{-1}\) (centrocyte half-life of 4 h) (29) and \(k_R = 0.35d^{-1}\) (equivalent to a time of differentiation into output cell of 48 h) (17). The rate of centroblast proliferation can be estimated by using data on the number of GC B cells from initial time points in the GC reaction where only centroblast proliferation occurs. For the calculation, we assumed exponential growth of the centroblast compartment and applied a linear regression to the natural logarithm of the number of proliferating centroblasts, \(\ln[B(t)] = \ln[B(t_0)] + p(t - t_0)\), with \(B(t_0) = 4\). The time point at which differentiation into centrocyte starts should be before the GC peak, when the initial B cell kinetics starts deviating from an exponential growth law, which can be roughly taken from the data. With this time point and the proliferation rate, one can estimate the total number of centroblast divisions, \(n\) (Table II). The rates of centroblast differentiation and centrocyte selection, \(k_B\) and \(k_c\), respectively, were chosen to obtain dynamics that fit the experimental data. Examples of GC dynamics obtained with this proliferation cascade model, using particular sets of parameters that fit the data, are shown in Figs. 5 and 6 in comparison with the respective experimental data.

Discussion

We propose two alternative hypotheses for the termination of the GC reaction and present two corresponding mathematical models for the GC. The models were applied to two sets of experimental data from different reports, namely primary immune responses against (4-hydroxy-3-nitrophenyl)acetyl (12) and against sheep RBCs (11) in mice.

The first hypothesis considers that the mechanism of GC termination is based on the differentiation of FDC/T cells driven by their interaction with centrocytes. This hypothesis is supported by experimental evidence that show a change in the FDC phenotype during a GC reaction (5) and by the fact that T cells play different roles in different phases of the GC (20, 21). Under this hypothesis, we propose two submodels corresponding to B cell proliferation

\[
B_0 \xrightarrow{p_B} B_1 \xrightarrow{p_B} \ldots \xrightarrow{p_B} B_n \xrightarrow{k_B} C \xrightarrow{d_c} \bullet
\]

and output cells.

\[
R \xrightarrow{k_R} M
\]
either dependent exclusively on undifferentiated FDC/T cells or independent of the FDC/T cell effector stage. Using experimental information on the GC B cell kinetics, we calculate the model parameters that provide a good fit between the theoretical curves and the data points. The parameters are the maximum effective proliferation rate of B cells, \( p \), the maximum rate of B cell selection, \( k_c \), and the day when half of the FDCs/T cells are differentiated (Table II). These parameters are biologically meaningful and can be compared with experimental measurements. The results of the model are slightly different for each of the experimental data sets. A possible reason for this is that both rates of B cell proliferation and selection may depend on the following: 1) the number of FDC/T cells in a GC; 2) the type of Ag; and 3) other experimental conditions. Bearing this in mind, measuring the B cell effective proliferation rate and evaluating the number of differentiated vs undifferentiated FDC/T cells for several time points during the GC reaction would constitute an experimental test to this hypothesis.

The second hypothesis for the termination of the GC reaction considers that the GC dynamics is controlled through the dynamics of the centroblast compartment by allowing centroblasts to proliferate only a given number of times. This could be the result of a simple biological mechanism. For example, when B cells are stimulated to become GC B cells, they receive a proliferation signal; as the cells divide this signal is not renewed and therefore decays until a point is reached when the cells stop proliferating. Some parameters of the model are provided in the literature, whereas others have been estimated to obtain a good fit of the theoretical curves to experimental data. We underline the point that if one considers the possibility of centrocytes differentiating back into centroblasts, the proliferative compartment is always being renovated and GC termination cannot be explained by this hypothesis.

The models reveal key differences between the proposed mechanisms that could be experimentally tested. One of the main differences is the number of AFCs and memory B cells produced. In the model of FDC/T cell differentiation with B cell proliferation independent of the FDC/T cell differentiation state (model B), the number of output cells produced per GC is much higher than the maximum number of B cells in the GC. In the model of FDC/T cell differentiation with proliferation signals exclusively from undifferentiated cells (model A), the number of output cells is lower than in model B and is similar to the maximum number of B cells in the GC. In the model of B cell proliferation cascade, the number of output cells is even lower and is much lower than the maximum number of GC B cells. This difference arises from the behavior of the B cell proliferation rate in each model. In model B, the proliferation rate is constant during the whole reaction. In model A, the proliferation of B cells decreases depending on the fraction of undifferentiated FDC/T cells, eventually reaching zero. In the B cell proliferation cascade model, proliferation is limited to the time corresponding to a fixed number of cell cycles. Therefore, the total number of cells produced by a GC expected from model B is much larger than those from the other two models. This comparative result could be used to experimentally test which of these hypotheses for the GC termination is more likely. This task could be accomplished, for instance, by measuring in an immune response the ratio of the total number of output GC B cells (memory plus plasma cells) to the total number of GC B cells at the peak of the GC reaction. Because memory B cells and a large fraction of GC-derived plasma cells are long lived, the total number of output GC B cells could be estimated from the pools of memory B cells (in the spleen) and plasma cells (in the bone marrow) one or a few weeks after the ending of the GC reaction. Furthermore, models A and B can be distinguished by the value of the maximum rate of B cell rescue. If B cell proliferation does not depend exclusively on signals from undifferentiated FDC/T cells, the condition \( k > p \) must be verified, whereas if B cell proliferation depends on those signals, this condition is not necessary. Once again, experimental measurements could contribute to deciding between the two submodels.

In summary, we present two alternative hypotheses for the termination of the GC reaction along with the corresponding mathematical models. A set of parameters and an estimate of the number of AFCs and memory B cells produced per GC are obtained with each of the models. Comparison of these results with experimental measurements such as those mentioned above could contribute to clarify which of the hypotheses is closer to the biological mechanisms responsible for the termination of the GC reaction.

Acknowledgments

We are grateful to Jorge Carneiro and the Theoretical Immunology group (Instituto Gulbenkian de Ciência, Oeiras, Portugal) for valuable discussions and to Rui Gardner (Instituto Gulbenkian de Ciência, Oeiras, Portugal) for critically reading this manuscript. We also thank Prof. Ducla-Soares (Instituto de Biofísica e Engenharia Biomédica, Faculty of Sciences, Lisbon University, Lisbon, Portugal) for all the support.

Disclosures

The authors have no financial conflict of interest.

References