**What can we learn on germinal centre reactions from in silico experiments?**

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**Summary:**
We discuss initiation, morphogenesis, and mechanisms that terminates germinal centre reactions in the framework of in silico experiments.

**Introduction**

The germinal centre (GC) (MacLennan 1994) is an important part of the adaptive immune response. Here antibodies are optimised with respect to specific antigens presented by follicular dendritic cells (FDC): GC reactions (GCR) are initiated by activation of B cells. These cells (centroblasts) enter a phase of intense proliferation in the environment of FDC networks within follicles. With onset of somatic hypermutation in centroblasts and differentiation to antibody presenting centrocytes a selection process is started: The centrocytes are in a stage of activated apoptosis. They are rescued by successful interaction with antigen held on FDC and T cells and further differentiate to plasma and memory cells (Kosco-Vilbois 2003). GCR in that way give rise to memory and plasma cells with optimised affinity of the encoded antibodies to the antigen.

Even though the molecular mechanisms and cell interactions involved in this affinity maturation process have been widely investigated, concurring views of how the germinal centre works still hold. These concern the initiation of the GCR: Implications of the seeder cell quality for the GC development are to be clarified. The mechanisms leading to affinity maturation are still controversial. And even the reason for the end of GCR reaction is a matter of discussion. In the present work some of these open questions are discussed in the framework of in silico experiments.

**Methods**

We describe cells as individuals moving and interacting on a lattice. The model includes cell growth, migration, proliferation, differentiation, apoptosis, as well as molecules including differentiation signals, chemokines, and rescue signals. Signals are described by a reaction-diffusion-equation which is solved numerically on the lattice. The model type is stochastic and can be denoted as a generalized version of an hybrid

We start from an FDC network and three activated B cells. Then the in silico experiment evolves according to the assumed dynamics: The centroblasts differentiate into centrocytes, these die by apoptosis if not rescued by successful interaction with FDC and T cells. Positively selected centrocytes either re-proliferate or further differentiate to plasma and memory cells. All parameters are formulated in terms of rates which have physiological meanings. The assumed values are estimated on the basis of experimental data. Note, that the results are widely independent of the lattice dimension being two or three (Meyer-Hermann & Beyer 2003).

**Results**

All our in silico experiments reproduce the widely accepted properties and characteristics of GCR. Beside others this concerns the GC population kinetics, affinity maturation, the number of accumulated mutations, the dynamics of plasma and memory cell production, and also morphological properties as zoning dynamics (Meyer-Hermann 2002).

We find that a hypothetical FDC-derived differentiation signal for centroblasts is a sufficient condition for the morphogenesis of dark and light zones. Chemokines turn out to be less suitable because the shape of chemokine induced dark zones are ring shaped, wrapping around the light zone. FDC-contact- or self-induced differentiation of centroblasts do not induce dark zones at all or lead to wrong population dynamics, such that both mechanisms can be ruled out to be responsible for zoning.

In accordance with previous theoretical investigations (e.g. Kepler & Perelson 1993) affinity maturation occurs only if recycling of selected B cells is assumed (Meyer-Hermann 2002a). We quantitatively predict the probability of positively selected centrocytes to re-proliferate to be 0.8 (Meyer-Hermann et al. 2001). This result is based on experiments that considered the development of already established GC after application of soluble antigens with different affinity properties (Han et al. 1995). Note, that in contrast to the common picture, we rarely see recycled cells returning to the dark zone. Most of them re-proliferate in the light zone.

The relevance of seeder cell quality for the fate of GCR is analysed on the basis of 169 in silico experiments. The seeder cell quality is varied by assuming different numbers of phenotypically relevant mutations that are to be performed in order to find the optimal clone. We find that GCR develop very differently depending on the seeder cell quality. In fact the
resulting GCR can be divided into GCR that poorly develop and full intensity GCR. Intermediate GCR are rarely found such that GCR show an all-or-none distribution induced by seeder cell diversity.

GCR ends without further assumptions of limiting processes when the balance of proliferation rate and centroblast differentiation is chosen appropriately. The ratio of both rates has to be chosen in such a way that after the take over of good cells (8-10 days post immunization) cell birth is slower than death (including 0.8 probability for recycling). The inferred rates are in agreement with physiological constraints.

Conclusions
We have analysed several open questions concerning GCR with the help of in silico experiments. The reliability of the results is underlined by the in silico experiments being in agreement with a large amount of observations in real experiments.

We have analysed the importance of the initial seeder cell affinity of the antibodies to the antigen for a successful initiation of GCR and find an all-or-none behaviour of GC. It is worth investigating on a statistical basis whether this prediction is found in reality or not. In fact, such a result has already been suggested (Lentz & Manser 2001).

The morphogenesis of dark zones is most likely related to an FDC derived signal molecule that initiates centroblast differentiation to centrocytes. However, chemokines can not be ruled out: Ring shaped dark zones as found by chemotactic activity of centrocytes are found in chicken but not in mice where in general the dark zone is convex. One may suspect that the mechanisms for zoning are different in mice and chicken. More generally, species and even organ specific differences should be considered in great detail. For example dark zones are more frequently found in tonsils compared to lymph nodes (Steiniger et al. 2000).

There exist several hypotheses why GCR typically end after 21 days. Often, end of GCR is associated with competition for antigen as a limiting process that inhibits the rescue of centrocytes from apoptosis. This may be true in later stages of the reaction, in particular because of masking of antigen by newly produced antibodies within the GC. But this hypothesis is very unlikely to explain the very early break-down of the total population as at that time sufficient antigen is available. Our in silico experiments can not rule out other hypotheses as varying proliferation or recycling rates (Iber & Maini 2002). But it is worth mentioning that there are no additional assumptions necessary to explain the end of GCR. Our in
silico experiments claim that GCR will end without any active mechanism but simply according to a balance of parameter values that are adopted at the very beginning of the reaction.

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References