Are T cells at the origin of B cell lymphomas?

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Abstract

Lymphoma pathogenesis is at least in some cases related to transformed B cells (BCs) arising from germinal centre reactions (GCRs). In this article possible deregulations of GCRs are investigated using in silico simulations. It is found that the final differentiation of BCs as regulated by helper T cells (TCs) is the best candidate mechanism for such a deregulation. This shifts the paradigm of BC lymphoma pathogenesis from BC transformations to an emphasized role of TC–BC interactions.

Keywords: Germinal centre; B-cell lymphoma; Pathogenesis; Mathematical model

1. Introduction

Lymphomas are rather diverse in appearance and in histoimmunological characterisation. Gene-translocation events are often associated with lymphomas and in this way oncogenes (like bcl-2) are activated. Also their morphology and their sites are diverse. A suitable way of classification of lymphomas is to use these characteristics as a basis. Two such classifications are widely used, the REAL and the WHO classification (Harris et al., 1994; Jaffe et al., 2001). Both are based on specific markers and specific morphologies and sites of malignancy without providing a causal relationship between different lymphoma subtypes.

One may ask whether all lymphoma types develop differently or if there exists a common starting point for some forms of lymphomas. The latter hypothesis is supported by the clinical observation that lymphomas of different histologic type frequently develop in the same anatomic site and in parts genetically depend on each other (Pileri et al., 2005).

A rather general source for lymphoma development is the germinal centre reaction (GCR) (Küppers, 2005). This is also supported by the evolutionary correlation of the appearance of Hodgkin lymphomas with germinal centres (GCs) (Seitz et al., 2003). Also the frequently observed translocation of bcl-6 in non-Hodgkin lymphomas leads to the overexpression of BCL-6 which plays an important role in GC development by regulating differentiation to plasma cells (Cattoretti et al., 1995; Staudt and Wilson, 2002). Presently the dominant view is that B cells (BCs) in GCRs are subject of various pathogenetic transformations that are at the origin of lymphoma development. Alternatively, one might state that the relevant question to lymphoma pathogenesis is not why do neoplastic gene-translocation happen, but why do they survive selection in the GCR?

In this alternative view neoplastic transformations of BCs in GCRs are considered to be normal, and these BCs are in general eliminated by follicular dendritic cell (FDC) and/or T cell (TC) regulation. TCs act with a given perfection and bear the risk of false selection of BCs. Lymphoma development could be seen as the result of such an accidental or induced error in BC selection. The risk to get false selection increases in deregulated GCRs.

In follicular lymphomas (FLs) 80% of the cases in human show the translocation t(14;18) in BCs (Seitz et al., 2003), which is related to the blocking of apoptosis by activation of the proto-oncogene bcl-2 (Jäger et al., 2000). Thus there is a common genetic basis for FLs that is not
found in other types of lymphomas. This seems to support these transformations to be at the origin of FL pathogenesis (the remaining 20% set a little question mark) and to exclude other lymphoma types not showing the same translocation to be derived from FLs. However, it is also possible that this translocation only leads to a specific appearance of lymphoma disease, namely FLs in this case, without being a necessary cause for lymphoma development, which might be found at a different level of GCRs.

In the case of Epstein–Barr virus (EBV) associated endemic Burkitt’s lymphomas a translocation of c-myc to the heavy chain gene is almost always found (Küppers, 2005). These translocations do not occur during Ig recombination in the bone marrow, but in the course of somatic hypermutations in GCRs (Goossens et al., 1998). Deletions and insertions are found with equal frequency in GC–BCs and in malignant Burkitt’s lymphoma BCs, and these transformations make up 6% of all mutations. This suggests that deletions and insertions are part of the normal somatic hypermutation machinery and might well include translocation (Dunn-Walters et al., 2001). The results of the simulations in the present paper support the view that these translocation happen with increased risk in the late course of specifically deregulated GCRs.

This article addresses the question what kind of deregulation in GCRs might be at the origin of lymphoma development. The implications of various deregulation are discussed and compared to pathological characteristics of lymphomas. We propose the final differentiation of BCs as regulated by helper TCs to be a major player in GC deregulation. This corresponds to a shift of paradigm from the humoral to a combined humoral-cellular immune defect that is realized in defective TC–BC interactions. A similar shift occurred in the context of hyper IgM syndrome (Lougaris et al., 2005). Here also the disorder was attributed to BC defects until the TC–BC interaction mediated by CD40L-CD40 was shown to play an essential role in the pathogenesis. Meanwhile different forms of hyper IgM can be attributed to be related to BC defects (CD40L-related) or TC defects (CD40L-related), respectively.

Hyper IgM2 is characterized by lymph node hyperplasia with giant GCs (Lougaris et al., 2005). These are the result of several BC mutations including CD40 and activation induced cytidine deaminase. Thus a deregulation of the interaction with TCs by changed properties of BCs can lead to uncontrolled growth of GC. It seems that giant GCs are not found in the case of deregulated CD40L in TCs (Hyper IgM1), such that CD40L-CD40 interaction is probably not the relevant mechanism in the pathogenesis of lymphomas. This interaction is more important for the class switch from IgM to IgG (Cerutti et al., 1998).

Different possible deregulations of GCRs are carefully discussed using in silico simulations based on four different models for healthy GCRs. The emerging follicular content is characterized and compared to properties as found in lymphoma diseases. It results that the deregulation of final differentiation signals as provided by TCs is the most realistic scenario for lymphoma initiation. Alternatives, like deregulation of centrocyte (CC) life time or of CC apoptosis, rely on rather specific assumptions on the selection mechanism acting in GCs. As this computer simulation cannot be considered as a proof for a specific GCR deregulation being at the origin of lymphoma development some quantitative predictions are proposed that allow different deregulations to be distinguished in experiments.

2. Methods

We introduce a set of models relying on previously introduced ordinary differential equation and agent-based models (Meyer-Hermann et al., 2001; Meyer-Hermann, 2002). All used cellular agents and parameters are listed in Table 1 and the general concept is explained in the following and in Eq. (2). The models are extended in this article according to the needs for lymphoma pathogenesis.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \phi )</td>
<td>Denotes the clones in the shape space</td>
<td>Index</td>
</tr>
<tr>
<td>( \phi^* )</td>
<td>Denotes the optimal clone (complimentary to the antigen)</td>
<td>Index</td>
</tr>
<tr>
<td>( B(\phi) )</td>
<td>Centroblasts (CBs)</td>
<td>Number</td>
</tr>
<tr>
<td>( C(\phi) )</td>
<td>Centrocytes (CCs) without contact to follicular dendritic cells (FDCs)</td>
<td>Number</td>
</tr>
<tr>
<td>( C_{\text{FDC}}(\phi) )</td>
<td>CCs in contact to FDCs</td>
<td>Number</td>
</tr>
<tr>
<td>( C^*(\phi) )</td>
<td>CCs rescued from apoptosis without contact to T cells (TCs)</td>
<td>Number</td>
</tr>
<tr>
<td>( C_{\text{TC}}(\phi) )</td>
<td>CCs in contact to TCs</td>
<td>Number</td>
</tr>
<tr>
<td>( O(\phi) )</td>
<td>Plasma and memory cells (output cells)</td>
<td>Number</td>
</tr>
<tr>
<td>( \zeta )</td>
<td>Apoptotic cells</td>
<td>Number</td>
</tr>
</tbody>
</table>

\( m \) | CB proliferation rate | 1/h |
\( m_p \) | Mutation probability per division | Probability |
\( \theta(\Omega) \) | CB to CC differentiation rate | 1/h |
\( \bar{\gamma}_{\text{FDC}} \) | Rate for CCs to find FDCs | 1/h |
\( \gamma^* \) | CCs apoptosis rate (inverse life time) | 1/h |
\( \rho_{\text{FDC}} \) | Ratio of FDC network density to the density in healthy GC | Ratio |
\( \theta^{\text{FDC}} \) | Inverse duration of CC–FDC interaction | 1/h |
\( \bar{\gamma}_{\text{TC}} \) | Rate for FDC-selected CCs to find TCs | 1/h |
\( \gamma^* \) | Apoptosis rate of FDC-selected CCs | 1/h |
\( \rho_{\text{TC}} \) | Ratio of TC density to the density in healthy GC | Ratio |
\( \theta_{\text{TC}} \) | Inverse duration of CC–TC interaction | 1/h |
\( q \) | Probability of final differentiation to output cells for CCs in stage \( C_{\text{TC}} \) | Probability |
\( a_0 \) | Probability of positive selection by FDCs for the optimal CC clone | Probability |
\( \rho(\phi) \) | Binding probability function for CCs of clone \( \phi \) (see Eq. (1)) | Probability |
\( \Gamma \) | Width of binding probability function | Factor |
The set of equations of each model as introduced in the Cells with larger distances have negligible affinity to the highest affinity clone with respect to the antigen. with a limited distance (assumed to be 13 mutations here) impact on the result). It is sufficient to only model clones analysed for their consequences (see also Fig. 1): selection to allow different deregulations of the GCR being represented in the model using a fourth order Runge–Kutta method with adaptive step size control.

The basic model explicitly represents the CCs and their interactions with FDCs and TCs at different stages of selection to allow different deregulations of the GCR being analysed for their consequences (see also Fig. 1):

\[
\frac{dB(\phi)}{dt} = (p - 2pm - g(O))B(\phi) + \frac{pm}{4} \sum_{|\Delta\phi|=1} B(\phi + \Delta\phi) \]

\[
+ g_{TC}(1 - q)M_1 C_{TC}(\phi),
\]

\[
\frac{dC(\phi)}{dt} = g(O)B(\phi) - (\gamma_{FDC} \rho_{FDC} M_2 + \xi)C(\phi) \]

\[
+ M_3 g_{FDC}(1 - M_4)C_{FDC}(\phi),
\]

\[
\frac{dC_{FDC}(\phi)}{dt} = \gamma_{FDC} \rho_{FDC} M_2 C(\phi) - g_{FDC} C_{FDC}(\phi),
\]

\[
\frac{dC^*(\phi)}{dt} = g_{FDC} M_4 C_{FDC}(\phi) - (\gamma_{TC} \rho_{TC} + \xi^*)C^*(\phi),
\]

\[
\frac{dC_{TC}^*(\phi)}{dt} = \gamma_{TC} \rho_{TC} C^*(\phi) - g_{TC} C_{TC}^*(\phi),
\]

\[
\frac{dO}{dt} = \sum_{\phi} g_{TC} \cdot q \cdot M_1 C_{TC}(\phi),
\]

The width is determined to be \( \Gamma = 2.8 \) according to experimental data (Meyer-Hermann et al., 2001) (note that a different metric was used in this reference which has no impact on the result). It is sufficient to only model clones with a limited distance (assumed to be 13 mutations here) to the highest affinity clone with respect to the antigen. Cells with larger distances have negligible affinity to the antigen and thus negligible survival probabilities in GCs. The set of equations of each model as introduced in the following sections is solved for all 28561 clones \( \phi \) represented in the model using a fourth order Runge–Kutta method with adaptive step size control.

The GC model schematically: The GC models as described by Eq. (2) is illustrated schematically for a single clone. The differentiation states of the B cells are shown in the ellipses. The arrow denote a reaction and the corresponding rates are attributed to the arrows. Different selection mechanisms correspond to different values for the \( M_i \)'s. The connection to other clones is only given structurally.

\[
\frac{dC}{dt} = \sum_{\phi} \left[ \xi C(\phi) + (1 - M_3) g_{FDC}(1 - M_4) C_{FDC}(\phi) \right] \]

\[
+ \xi^* C^*(\phi) + g_{TC}(1 - M_3) C_{TC}^*(\phi),
\]

where the \( M_i \) (with \( i = 1, \ldots, 4 \)) distinguish the different selection mechanisms and are given in the subsequent sections. Please refer to Table 1 for the meaning of the symbols.

This model includes centroblast (CB) proliferation, mutation and differentiation to CCs. The CCs are going through a sequence of selection steps: First, CCs (C) have to get in contact to FDCs. The binding process may depend on the affinity of the BC receptor (BCR) to the antigen if \( M_2 \sim \rho(\phi) \) according to Eq. (1). CCs may die by apoptosis with rate \( \xi \) before they successfully bind. CCs bound to FDCs (\( C_{FDC} \)) get survival signals by the FDCs (which again depends on the affinity between BCR and antigen if \( M_4 \sim \rho(\phi) \)) and turn into the state \( C^* \). If \( M_3 = 1 \) CCs that did not succeed to get sufficient survival signals restart search for FDC binding sites. For \( M_3 = 0 \) they directly die by apoptosis. The positively selected CCs (\( C^* \)) search for TCs, bind to TCRs (\( C_{TC}^* \)), and get further differentiation signals from TCs which might depend on affinity if \( M_1 \sim \rho(\phi) \). Those CCs (\( C_{TC}^* \)) that survive all steps differentiate either to output cells (plasma or memory cells (O)) or recycle back into the state of proliferating and mutating CBs (B). In various steps CCs are lost by apoptosis or due to lack of sufficient survival signals. All these cells are collected in \( \xi \), where macrophage activity is ignored. Note that in every subsequently introduced model only a subset of these selection mechanisms is active.

Guided by agent-based model explicitly representing physical space (Meyer-Hermann, 2002; Meyer-Hermann and Maini, 2005) we assume a dynamic CB differentiation
rate \( g(O) \) which is regulated by the already produced amount of output cells. This implies that the CBs proliferate more (or differentiate slower) in the beginning of the reaction and mimics effects of space resolution in the cited agent-based model. The hypothesized feedback signal of produced output cells to the CB differentiation rate follows:

\[
g(O) = \frac{g_0}{1 - f g_0^\alpha + K_O}
\]

When sufficient output cells emerged from the GCR the differentiation rate is increased (effectively reducing the number of divisions per cell). \( g_0 \) is the initial differentiation rate (without output cells). The factor \( f_g \in [0, \ldots, 1] \) by default is set to 0.3 and determines the degree of increase of the differentiation rate for very large numbers of output cells. Note that only the total number of output cells enters the differentiation rate irrespective of their affinity to the antigen. Note also that the results do not depend on the specific choice of the function \( g(O) \) and that the same results are obtained for a constant differentiation rate. Eq. (3) mainly increases the robustness of healthy GCR against variations of other parameters. 

With \( K_O = 1000 \) output cells, the termination of the GCR becomes more robust while the intermediate part of the reaction is not affected. The feedback of output cells on the termination of the GCR might for example be mediated by a limited amount of recycling signals that can be provided by TCs to selected BCs or by antigen masking in the GC by plasma cell derived soluble antibodies. Both mechanisms, indeed, would directly depend on the amount of produced output cells.

The initial state of all simulations is defined by the optimal BC clone with respect to the antigen positioned in the centre of the shape space and 12288 BCs (three BCs proliferating for three days with cell cycle time of 6h) monoclonally derived from three different clones at a distance of five mutations from the clone that optimally binds the antigen. The GCR is assumed to last for 21 days. As we start the simulation after three days of monoclonal expansion the total simulation time is 18 days.

First the model types are introduced and then the parameters sets optimal for the description of healthy GCRs are identified and summarized in Table 2. These simulations will then serve as a basis for the analysis of GC deregulations. Note that most parameters were determined with the help of independent experiments or in previous modelling work (Meyer-Hermann et al., 2001; Meyer-Hermann, 2002; Meyer-Hermann and Maini, 2005) and are therefore not varied in the present simulations.

### 2.1. Model A: affinity-dependent selection by FDCs

It is assumed that selection of CCs relies on interaction of BCR with antigen held on FDCs. Both the binding process and the deliver of survival signals by FDCs (rescuing the CCs from apoptosis) depend on the BCR-antigen affinity. The interaction with TCs, in contrast, does not depend on affinity. This defines \emph{model A} and is realized using

\[
M_1 = 1, \quad M_2 = \rho(\phi), \quad M_3 = 0, \quad M_4 = a_0\rho(\phi).
\]

Thus in model A affinity maturation is enabled by two affinity-dependent selection steps both related to FDCs. These are investigated separately in models B and C.

### 2.2. Model B: binding probability

In model A (Eq. (2) and (4)), a limited binding probability to FDC is a part of the CC selection process. Unbound CCs are subject to apoptosis and compete to bind antigen held on FDCs. If bound CCs fail to get sufficient survival signals (due to too low BCR affinity), they die by apoptosis, which corresponds to negative selection of CCs by FDCs. In model B, a GCR is proposed that solely relies on the binding process, thus ignoring affinity-dependence of survival signals delivered by FDCs. This emphasizes competition between life time and binding to antigen on FDCs. The parameters defining the selection

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Table 2

<table>
<thead>
<tr>
<th>Model</th>
<th>A</th>
<th>B</th>
<th>B'</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/(g_0) (h)</td>
<td>5.0</td>
<td>4.5</td>
<td>5.2</td>
<td>4.8</td>
<td>5.0</td>
</tr>
<tr>
<td>(f_g \in [0, \ldots, 1])</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>(K_O)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>1/(f_{BC}) (h)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>1/(f_{TC}) (h)</td>
<td>5.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>1/((V_{FDC}P_{FDC})) (h)</td>
<td>0.5</td>
<td>2.0</td>
<td>2.0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>1/((V_{TC}\rho_{BC})) (h)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>1/(\eta) (h)</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>1/(\zeta) (h)</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>(q \in [0, \ldots, 1])</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>(a_0 \leq 1)</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>(z^2)</td>
<td>0.20</td>
<td>0.24</td>
<td>0.15</td>
<td>0.27</td>
<td>0.32</td>
</tr>
<tr>
<td>(N_{BC})</td>
<td>755</td>
<td>317</td>
<td>673</td>
<td>568</td>
<td>424</td>
</tr>
<tr>
<td>(N_{BC})</td>
<td>3308</td>
<td>3410</td>
<td>2562</td>
<td>2877</td>
<td>1086</td>
</tr>
<tr>
<td>(p(BC))</td>
<td>0.57</td>
<td>0.45</td>
<td>0.30</td>
<td>0.60</td>
<td>0.58</td>
</tr>
<tr>
<td>(p(O))</td>
<td>0.67</td>
<td>0.44</td>
<td>0.60</td>
<td>0.63</td>
<td>0.76</td>
</tr>
<tr>
<td>(r_0 = N_{BC}/(N_{BC} + N_{BC}))</td>
<td>0.40</td>
<td>0.30</td>
<td>0.33</td>
<td>0.39</td>
<td>0.46</td>
</tr>
<tr>
<td>(r_1 = (N_{BC} + N_{BC})/N_{BC})</td>
<td>24.3</td>
<td>20.4</td>
<td>34.4</td>
<td>24.8</td>
<td>56.6</td>
</tr>
</tbody>
</table>

The best parameter sets (see Table 1 for the symbols) for healthy GCRs for different selection models A–D are shown in the upper part of the table. The lower part provides quantities generated by the simulations (thus these numbers are no parameters) that are considered to well characterize the GCR: \(\chi^2\) (see Eq. (9)) is a measure for the deviation of the GC kinetics from experimental data (Liu et al., 1991; Hollowood and Macarthy, 1992) (only simulations with \(\chi^2 < 0.5 \) have been included), \(N_{BC}\) and \(N_{BC}\) is the number of BCs at the end of the GCR and the accumulated number of output cells, respectively, \(\rho(BC)\) is the average affinity of all BCs (without output) at the end of the GCR, \(p(O)\) is the average affinity of all accumulated output cells, and \(N_{BC}/N_{BC}\) denotes the fraction of CBs within all BCs at the end of the reaction. \(r_0\) and \(r_1\) are measures of the density of output cells and apoptotic bodies, respectively.
mechanism in Eq. (2) read (model B):
\[ M_1 = 1, \quad M_2 = a_0 \rho(\phi), \quad M_3 = 0, \quad M_4 = 1. \] (5)

It is known that a first contact is established independently of affinity. However, the synapse-like structure is established only if a threshold-affinity is surpassed (Carrasco et al., 2004). This motivates the assumption made here. The competitive parameters are \( \gamma_{\text{FDC}} \) and \( \xi \). In order to get reliable affinity maturation \( 1/\gamma_{\text{FDC}} \) is chosen larger than in other models (see Table 2).

Alternatively model A can also be reduced to the CC–FDC binding process by keeping the CCs which fail to get the necessary affinity-dependent survival signals from FDCs. Instead of dying by apoptosis these cells repopulate the unbound CC pool. This explicitly describes multiple affinity-dependent attempts of CCs to bind antigen on FDCs. In this case
\[ M_1 = 1, \quad M_2 = \rho(\phi), \quad M_3 = 1, \quad M_4 = a_0 \rho(\phi). \] (6)
in Eq. (2) define model B'.

Both models Eqs. (5) and (6) are optimized for the same parameter set (with exception of the CB differentiation rate \( g_0 \) correcting for different population kinetics) and exhibit the same properties in deregulations of the GC. Note, however, that affinity maturation is less efficient in model B Eq. (5) while the degree of affinity maturation is comparable to other scenarios for Eq. (6) (see \( \bar{p}(O) \) in Table 2).

Thus, we concentrate on model B' Eq. (6) in the analysis of deregulations.

2.3. Model C: affinity-dependent FDC rescue signals

One may consider the CC–FDC binding probability to be sufficiently large, corresponding to the situation of abundant number of FDC sites loaded with antigen. Then the affinity-dependence of the binding process is less important because even the low affinity cells will get the chance to bind an antigen. We define model C by choosing
\[ M_1 = 1, \quad M_2 = 1, \quad M_3 = 0, \quad M_4 = a_0 \rho(\phi). \] (7)
in Eq. (2). Then the binding process of CCs to FDCs becomes independent of affinity while the deliver of rescue signals to already bound CCs remains affinity-dependent. Thus, the process of negative selection by FDCs is emphasized for affinity maturation while the CC life time \( 1/\xi \) is not an affinity driving parameter in this model.

2.4. Model D: affinity-dependent CC–TC interaction

Starting from model C one may add an hypothetical affinity-dependent selection of BCs by TCs, resulting in model D
\[ M_1 = \rho(\phi), \quad M_2 = 1, \quad M_5 = 0, \quad M_4 = a_0 \rho(\phi). \] (8)

As already found before in agent-based models (Meyer-Hermann et al., 2006) this implies a substantial improvement of affinity maturation as measured by the average affinity of output cells (see Table 2). Like in model C the CC life time \( 1/\xi \) is not an affinity-relevant parameter.

2.5. Healthy GCs

In the previous sections different models have been introduced and characterized for the embedded selection mechanism. The parameters are optimized for the population kinetics according to
\[ \chi^2 = \sum_{i=1}^{k} \frac{1}{k-1} \left( \frac{x_{\text{exp}}(t_i) - N_{\text{BC}}(t_i)/N_{\text{max}}}{x_{\text{exp}}(t_i)} \right)^2, \] (9)

where \( k \) experimental values \( x_{\text{exp}}(t_i) \) for the GC volume (Liu et al., 1991; Hollowood and Macartney, 1992) (given as the fraction of the maximum volume) at time \( t_i \) are used and compared to the volume of the simulated GC (given by the sum of all BCs \( N_{\text{BC}} \), excluding output cells, rescaled with the maximum volume in the simulation \( N_{\text{max}} \)). The data provided in Liu et al. (1991) are given as the ratio of the follicle centre volume to the total splenic volume after primary anti-hapten response in rats. In Hollowood and Macartney (1992) the absolute GC volume was measured after primary immunisation of mice. Both sequences of experimental values are normalized to the maximum GC volume to be compared with the simulation results.

The parameters are simultaneously optimized for the GC kinetics, the affinity level of output cells, while respecting that CCs dominate over CBs. Most parameters have been analysed previously and are strongly restricted in the range of possible values (see Meyer-Hermann et al., 2001; Meyer-Hermann and Maini, 2005 and references therein). These parameters are set by hand. Concerning the finding rates \( \gamma_{\text{FDC,TC}} \) no values were assumed in former agent-based models. However, these rates are a read out of the agent-based models, leading to values around 0.5 h. Longer values do not allow for the required domination of CCs over CBs (see also Section 2.6). We apply Powell’s algorithm (Brent, 1973) to find the best parameter set (minimising the deviation from the GC kinetics and maximising affinity maturation while respecting CC domination) for the undetermined subset of parameters, namely the differentiation rates \( g_0, g_{\text{TC}} \) and the finding rate \( \gamma_{\text{FDC}} \). The latter has to be larger in models B and B' to ensure affinity maturation. The best (rounded) parameter sets are collected in Table 2.

Note that model A which includes two affinity checks as well as model B' which relies on limited CC–FDC binding probability with multiple possible searches for antigen on FDCs exhibit the most satisfying affinity maturation (\( \bar{p}(O) \)) and population kinetics (\( \chi^2 \)). The simulation based on model A will serve as a reference simulation for the investigation of possible deregulated mechanisms in lymphomas. Note also that the affinity of output cells (\( \bar{p}(O) \)) is substantially increased when affinity-dependent selection by TCs is assumed instead of another selection mechanism (model D). This is in agreement with previous
modelling work (Meyer-Hermann et al., 2006) However, the differences between the best fits are subtle as far as only healthy GCRs are considered.

2.6. Parameter analysis

In the following the specificities of the most important parameters and their impact on the healthy GC development are discussed. Most of the values are well established in previous agent-based models (see Meyer-Hermann and Maini, 2005 and references therein) and are either not subject to variation in the present simulations or have to respect a certain range of possible values.

**CB differentiation g**: This rate turns out to be rather small. Population kinetics are most sensitive to the differentiation rate. Fast differentiation is required to guarantee the end of the reaction. At the same time differentiation should take a minimum duration to allow CBs to sufficiently proliferate. Otherwise affinity maturation is not observed. The assumption of a dynamic differentiation rate in Eq. (3) allows to find a robust compromise between these tendencies. However, also a constant g can be tightly chosen to find satisfying results (data not shown).

The ratio of 1/g to the sum of all other reaction times approximately determines the ratio of CBs to CCs (sum in all substrates of CCs) in the late stage of the GCR.

**Duration of CCs to find and bind FDCs 1/τFDC**: This parameter in most models acts as retardation of the reaction only. Long durations keep cells in the stage of free CCs for a while. However, it also competes with the life time of CCs ξ, and the relevance for the GC depends on the question whether the binding process is assumed to depend on BCR affinity or not (thus whether the factor ρ(φ) comes with τFDC or not).

If the binding process is independent on BCR affinity (models C, D), cell death by apoptosis is a background effect. Binding sites on FDCs are abundant. This implies that the ratio of 1/τFDC to 1/ξ determines the proportion of CCs that will have the chance to be selected for high affinity in the next step (getting survival signals from FDCs). Thus a slow binding of CCs to FDCs leads to the elimination of several BCs from the system without impact on affinity. If binding of CCs to FDCs depends on BCR affinity (models A, B, B') then BCs have to be rescued within their life time, where the selection pressure on low affinity clones is increased.

Another effect of τFDC is that it is a main regulator of the total throughput of cells through the different substrates of the BCs. As the interaction times (after onset of binding) of CCs with FDCs or TCs are known from experiment it is the search- and binding-times 1/τFDC/TC that are the most important players. Fast binding times lead to high throughput which increases the number of recycled BCs but also reduces the CC to CB ratio in the GC.

**CC–FDC interaction time 1/τFDC**: The interaction time of CCs with FDCs has been estimated in experiment by measuring how fast the CCs get the survival signals from the FDCs. This time should be chosen between 1 and 2 h (Lindhout et al., 1995).

**Duration of CCs to find and bind TCs 1/τTC**: Here the same applies as for τFDC (see above). However, in all models it is assumed that the binding of CCs to TCs is independent of affinity and that the FDC selected CCs 1/ξ* are long-living cells. Thus, this parameter is mainly a retardation parameter with impact on the CC to CB ratio and the number of recycled BCs.

**CC–TC interaction time 1/τTC**: The CC–TC interaction is expected to be of the same order than the CC–FDC interaction. However, the model does not explicitly include the differentiation process initiated by this interaction (either to CBs or to output). Therefore, this time has to be interpreted as a combined duration of interaction with TCs and differentiation (after dissociation from TCs). This implies far longer times than 2 h. Indeed, in order to be in agreement with the observation of CCs to dominate the GC population in later stages of the reaction (MacLennan, 1994), longer CC–TC interaction times of the order of 5 h had to be chosen.

3. Results

The analysis of GC deregulations is guided by characteristic quantities and properties of lymphomas, in particular of FLs: Ongoing selection and mutation, amounts of apoptotic bodies, population kinetics, density of proliferating and non-proliferating cells, and density of output cells.

The fraction of CBs \( N_{CB}/N_{BC} \) in deregulated GCRs is compared to the value of 0.5 or smaller in healthy GCRs (MacLennan, 1994). The ratio \( r_0 = O/O + BC \) is taken as a measure for the density of output cells in the healthy or malignant follicles. Similarly, the ratio of accumulated apoptotic cells to the sum of accumulated output cells and BCs at the end of the simulation \( r_z = \xi/O + BC \) is used as a measure for the density of apoptotic bodies. Note that both quantities by no means are quantitative predictions for these densities. The first ratio \( r_0 \) ignores emigration of output cells out of the GC area. The second ratio \( r_z \) ignores the macrophage activity digesting apoptotic bodies. Therefore, all ratios will be interpreted in relation to typical values in healthy GCRs which are \( N_{CB}/N_{BC} = 0.4 \), \( r_0 = 0.81 \), and \( r_z = 24.3 \) in the case of model A (see Table 2).

Model A is used as a reference simulation for healthy GCs with the parameters as given in Table 2. For some deregulation mechanism it turns out that the assumed selection mechanism is relevant to the outcome such that different models will be compared to each other. The results are cross-checked for robustness against changes in the model assumptions. The most relevant results are summarized in Table 3, where also acronyms for the deregulation scenarios are introduced.
3.1. Deregulation of CC life time

The CCs are in a state of activated apoptosis after differentiation from CBs. If apoptosis is deregulated in CCs this might happen in different CC states. Here, the earliest deregulation is investigated: a prolonged CC life time of CCs not yet having encountered FDCs. Such prolonged CC life times do not prevent CCs from apoptosis in general. It is only the conditions of rescue that are altered. So this deregulation has to be distinguished from a general deregulation of CC apoptosis which makes the CCs independent of any further rescue signal provided by FDCs or TCs. The latter is investigated in Sections 3.2 and 3.3.

Prolonged CC life times are simulated by setting the CC life time to 1/\(z = 200\) h in model A (see Table 3 1A). The GC is characterized by far too many cells but with a constantly decreasing (thus not exploding) population. The quality of output cells is high (0.65) but the average BC quality in the GC is not substantially improving (0.19 average binding probability at day 18) but steadily improving (0.30 at day 28). Thus there is still some selection ongoing. More precisely the BC quality is improving less than in a healthy GCR because longer CC life times increase the total CC–FDC binding probability and keep low affinity CCs (0.02 in average) in the early CC state (C). However, in model A the FDC derived survival signals are also assumed to be affinity-dependent thus still driving some affinity maturation. Consequently, later stages of CC development acquire higher affinity: 0.54 in average binding probability at day 28; 0.35 in average binding probability at day 18. This shows that the deregulation of CC life time in GC has only an important effect when the CC–FDC binding process is critical within the CC compartment.

In model C rescue of CCs solely relies on affinity-dependent signals provided by FDCs, thus modelling negative selection of CCs by FDCs (Verbeke et al., 1999). We find an almost normal GCR (see Table 3 5C). The same is true in model D which relies on TC based affinity maturation (see Table 3 4D). This shows that the deregulation of CC life time in GC has only an important effect when the CC–FDC binding process is critical within CC life times.

We therefore expect to find a drastic effect if affinity maturation relies on the CC–FDC binding process alone, thus in model B or B'. We use a moderately increased life

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**Table 3**

Characteristics of deregulated GCRs

<table>
<thead>
<tr>
<th>Sim</th>
<th>Deregulation</th>
<th>(\chi^2)</th>
<th>(\theta(BC))</th>
<th>(N_{BC})</th>
<th>(\theta(BC))</th>
<th>(\theta(C_1))</th>
<th>(\theta(C_2))</th>
<th>(\theta(O))</th>
<th>(N_O)</th>
<th>(N_{CB}/N_{BC})</th>
<th>(r_O)</th>
<th>(r_z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0A</td>
<td>Healthy GCR</td>
<td>0.20</td>
<td>0.57</td>
<td>755</td>
<td>–</td>
<td>0.31</td>
<td>0.73</td>
<td>0.67</td>
<td>3308</td>
<td>0.40</td>
<td>0.81</td>
<td>24.3</td>
</tr>
<tr>
<td>1A</td>
<td>CC life time</td>
<td>9.6</td>
<td>0.19</td>
<td>7794</td>
<td>–</td>
<td>0.54</td>
<td>0.72</td>
<td>0.65</td>
<td>6768</td>
<td>0.12</td>
<td>0.46</td>
<td>7.4</td>
</tr>
<tr>
<td>2A</td>
<td>CC life time</td>
<td>54.7</td>
<td>0.34</td>
<td>61.515</td>
<td>–</td>
<td>0.38</td>
<td>0.22</td>
<td>0.45</td>
<td>59015</td>
<td>0.21</td>
<td>0.49</td>
<td>1.6</td>
</tr>
<tr>
<td>2B</td>
<td>CC life time</td>
<td>55.7</td>
<td>0.42</td>
<td>41.602+</td>
<td>–</td>
<td>0.48</td>
<td>0.29</td>
<td>0.62</td>
<td>26999</td>
<td>0.20</td>
<td>0.39</td>
<td>2.6</td>
</tr>
<tr>
<td>3A</td>
<td>CC life time</td>
<td>40.8</td>
<td>0.27</td>
<td>916.554+</td>
<td>–</td>
<td>0.43</td>
<td>0.17</td>
<td>0.54</td>
<td>191480</td>
<td>0.11</td>
<td>0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>3B</td>
<td>CC life time</td>
<td>0.30</td>
<td>0.64</td>
<td>752–</td>
<td>–</td>
<td>0.58</td>
<td>0.58</td>
<td>0.75</td>
<td>78</td>
<td>0.41</td>
<td>0.71</td>
<td>35.0</td>
</tr>
<tr>
<td>4A</td>
<td>CC life time</td>
<td>0.58</td>
<td>0.62</td>
<td>1507–</td>
<td>–</td>
<td>0.56</td>
<td>0.56</td>
<td>0.72</td>
<td>669</td>
<td>0.36</td>
<td>0.76</td>
<td>14.7</td>
</tr>
<tr>
<td>5A</td>
<td>CC life time</td>
<td>0.39</td>
<td>0.17</td>
<td>0.67</td>
<td>0.66</td>
<td>14 102</td>
<td>0.51</td>
<td>0.32</td>
<td>5.4</td>
<td>0.21</td>
<td>0.17</td>
<td>0.8</td>
</tr>
<tr>
<td>5B</td>
<td>CC life time</td>
<td>0.35</td>
<td>0.18</td>
<td>0.65</td>
<td>0.65</td>
<td>6812</td>
<td>0.41</td>
<td>0.76</td>
<td>12.8</td>
<td>0.36</td>
<td>0.48</td>
<td>1.6</td>
</tr>
<tr>
<td>6A</td>
<td>Specificity</td>
<td>56.4</td>
<td>0.38</td>
<td>64.102+</td>
<td>–</td>
<td>0.35</td>
<td>0.27</td>
<td>0.44</td>
<td>0.40</td>
<td>99988</td>
<td>0.39</td>
<td>61.0</td>
</tr>
<tr>
<td>7A</td>
<td>Negative selection</td>
<td>43.1</td>
<td>0.35</td>
<td>306.402+</td>
<td>–</td>
<td>0.35</td>
<td>0.18</td>
<td>0.43</td>
<td>0.41</td>
<td>280386</td>
<td>0.36</td>
<td>48.8</td>
</tr>
<tr>
<td>8A</td>
<td>Negative selection</td>
<td>32.3</td>
<td>0.03</td>
<td>3.7 \times 10^8+</td>
<td>–</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>1.7 \times 10^3</td>
<td>0.34</td>
<td>31.0</td>
</tr>
<tr>
<td>9A</td>
<td>Negative selection</td>
<td>0.23</td>
<td>0.58</td>
<td>801–</td>
<td>–</td>
<td>0.53</td>
<td>0.53</td>
<td>0.71</td>
<td>0.63</td>
<td>4027</td>
<td>0.42</td>
<td>83.3</td>
</tr>
<tr>
<td>10A</td>
<td>Proliferation</td>
<td>35.7</td>
<td>0.37</td>
<td>5.2 \times 10^6+</td>
<td>–</td>
<td>0.35</td>
<td>0.15</td>
<td>0.65</td>
<td>0.65</td>
<td>1.6 \times 10^6</td>
<td>0.43</td>
<td>23.5</td>
</tr>
<tr>
<td>11A</td>
<td>Feedback</td>
<td>43.8</td>
<td>0.41</td>
<td>30.231+</td>
<td>–</td>
<td>0.39</td>
<td>0.17</td>
<td>0.67</td>
<td>0.66</td>
<td>14102</td>
<td>0.51</td>
<td>32.2</td>
</tr>
<tr>
<td>12A</td>
<td>FDC density</td>
<td>0.33</td>
<td>0.59</td>
<td>1282–</td>
<td>–</td>
<td>0.55</td>
<td>0.28</td>
<td>0.72</td>
<td>0.67</td>
<td>4749</td>
<td>0.40</td>
<td>79.7</td>
</tr>
<tr>
<td>13A</td>
<td>FDC density</td>
<td>1.1</td>
<td>0.60</td>
<td>2194–</td>
<td>–</td>
<td>0.55</td>
<td>0.19</td>
<td>0.72</td>
<td>0.67</td>
<td>6812</td>
<td>0.41</td>
<td>76.1</td>
</tr>
<tr>
<td>14A</td>
<td>TC density</td>
<td>0.20</td>
<td>0.57</td>
<td>813–</td>
<td>–</td>
<td>0.55</td>
<td>0.32</td>
<td>0.73</td>
<td>0.67</td>
<td>3584</td>
<td>0.41</td>
<td>82.2</td>
</tr>
<tr>
<td>15A</td>
<td>TC density</td>
<td>0.32</td>
<td>0.58</td>
<td>432–</td>
<td>–</td>
<td>0.53</td>
<td>0.53</td>
<td>0.73</td>
<td>0.76</td>
<td>1177</td>
<td>0.46</td>
<td>73.5</td>
</tr>
<tr>
<td>16A</td>
<td>Differentiation</td>
<td>10.2</td>
<td>0.60</td>
<td>6690+</td>
<td>–</td>
<td>0.57</td>
<td>0.35</td>
<td>0.74</td>
<td>0.71</td>
<td>5541</td>
<td>0.40</td>
<td>45.4</td>
</tr>
<tr>
<td>17A</td>
<td>Differentiation</td>
<td>64.4</td>
<td>0.60</td>
<td>23.584+</td>
<td>–</td>
<td>0.57</td>
<td>0.36</td>
<td>0.74</td>
<td>0.72</td>
<td>6531</td>
<td>0.40</td>
<td>22.8</td>
</tr>
<tr>
<td>18A</td>
<td>Differentiation</td>
<td>33.1</td>
<td>0.46</td>
<td>6.8 \times 10^5+</td>
<td>–</td>
<td>0.44</td>
<td>0.22</td>
<td>0.69</td>
<td>–</td>
<td>0</td>
<td>0.52</td>
<td>0.26</td>
</tr>
</tbody>
</table>
time of 24 h (see Table 3 2B and 2B). Indeed, in simulation 2B the BC population is going through a minimum (see Fig. 2) and then grows without limits. There is still moderate affinity maturation found (average of all BCs is 0.42, see also Fig. 3). A characteristic property of this malignant GCR deregulation is the comparably small number of CBs which account for 20% of the BC population (without output cells). The density of apoptotic bodies as measured by \( r_z = 2.6 \) adapts a rather small value compared to 24.3 in a healthy GCR. Despite the absolute number of output cells to be substantially increased, the output density is about half of the density expected in healthy GCRs.

If the CC life time is deregulated more drastically (to 20000 h) the general tendency remains the same but the characteristic properties adapt even more extreme values (see Table 3 3B). In particular affinity maturation is further weakened and the density of apoptotic bodies is very small. The output density does not fall below \( r_O = 0.17 \).

We conclude that if the selection mechanism of physiological GCRs relies on the CC–FDC binding process (models B and B'), CC life time prolongation leads to deregulated GCRs which are comparable to early stages of

Fig. 2. GC kinetics: The GC population kinetics as given by the number of CB and CC is shown during the GCR and compared to experimental values (Liu et al., 1991; Hollowood and Macartney, 1992) rescaled to the maximum GC volume (factor 1 for Liu et al., 1991 and 0.5 for Hollowood and Macartney, 1992). The black full line represents a typical healthy GCR (simulation 0A in Table 3) while all (coloured) dashed and dotted lines show the kinetics in several deregulated GCR (see the simulations marked bold in Table 3). Note that all deregulated GCR pass through a minimum between day 5 and 8 before uncontrolled population growth is entered.

Fig. 3. Time course of affinity maturation: The time course of affinity maturation is shown for GC–BC (excluding output cells) and for accumulated output cells. The quality of the output cells is almost unchanged with respect to healthy GCR (black full line and simulation 0A in Table 3) in the case of deregulation of proliferation (long dashed, magenta), feedback (dashed dotted, orange) or differentiation (wide dashed dotted, red), while all deregulation associated with CC life time (narrow dotted, blue), specificity (short dashed, green), or negative selection (wide dotted, cyan) exhibit impaired affinity of output. In the case of GC–BC only the deregulation of final differentiation (simulation 17A in Table 3) keeps normal affinity maturation (compare wide dashed dotted red and full black lines), which is strongly affected in all other deregulations.
lymphoma development in terms of population kinetics and ongoing BCR-dependent selection.

3.2. Loss of specificity of cell interactions

The process of CC rescue might be deregulated to become less affinity-dependent. To simulate this situation, the affinity function is calculated with a larger width using $\Gamma = 5.6$. The normal width of 2.8 is still used for evaluation of the cell quality. Only the CC–FDC binding and the rescue process are assumed to be less antigen-specific. Note that the specificity is reduced in all cell interactions such that this deregulation corresponds, for example, to the emergence of BCRs with higher cross-reactivity.

If we further increase the width of the affinity function in the simulations approach the result for deregulation of negative selection (see Table 3 8C) which is discussed in the next section. Here affinity maturation is not observed any more. In particular, affinity maturation is lacking when infinite width is used with reduced amplitude ($\rho(\phi) = 0.5$ for all clones), even though the kinetics of the GCR and the density apoptotic bodies become quite normal (data not shown). In terms of population kinetics flat affinity functions are turned from declining to exploding populations above $\rho(\phi) = 0.7$.

In summary a moderate deregulation of specificity of the rescue process leads to transformed GCs with weakened but ongoing selection. For rescue of CCs independent of the BCR affinity to the antigen, the affinity of the BCs is even declining.

3.3. Deregulation of negative selection

Deregulation of induction of apoptosis in CCs can act on different intracellular pathways. Deregulation of apoptosis in unbound CCs was discussed in Section 3.1. Now deregulation of apoptosis of CCs bound to FDCs via their interaction is discussed. In the models relying on affinity-dependent bound CC–FDC interaction (in models A, C, D)—to be distinguished from an affinity-dependent binding process—this interaction can be interpreted to represent negative selection by FDCs for example via Fas-FasL pathway (Verbeke et al., 1999). In order to deregulate negative selection by FDCs the rescue probability is set to $\rho(\phi) = 1$ for all clones $\phi$. Note that this does not affect affinity-dependence of the CC–FDC binding process (if included in the model). Using model A (see Table 3 7A) a result very similar to the result 2B and 6A (see Sections 3.1 and 3.2, and Fig. 3) is found, which are considered as deregulation relevant for lymphoma development.

It is interesting to compare result 7A and 2B in more detail: In 2B a single affinity-dependent selection step (binding process) is deregulated by prolonged cell life times. In 7A there are two affinity-dependent selection steps and only one of these is deregulated by inhibition of negative selection by FDCs. As affinity maturation is even more affected in 7A, we can conclude that in model A interaction of bound CC with FDC induce a stronger selection pressure than the CC–FDC binding process does.

If we now deregulate negative selection by FDCs in model C where this is the only affinity-dependent process we get a result characterized by strongly reduced density of apoptotic bodies ($r_c = 0.31$) and by lack of affinity maturation and lack of ongoing selection (see Table 3 8C). This is not in agreement with the observed ongoing selection in several lymphomas. Based on random mutations there is larger probability to find clones of less than of higher affinity. Therefore, even a continuously decreasing average affinity is found (0.03 at day 18 compared to 0.04 of the seeder cells). More generally, affinity maturation is lacking in all scenarios where the deregulation affects all affinity-dependent selection steps.

In contrast to deregulation of negative selection by FDCs, negative selection by TCs turns out to have minor effects on the GCR even when a part of affinity maturation relies on interaction with TCs (see Table 3 9D). The major difference is that FDCs are confronted with CCs of moderate or low affinity, while the CCs that reach selection by TCs are already of relatively high affinity. Thus only a minor part of CCs in contact to TCs is deleted by negative TC selection, implying this interaction to have only minor effect. In order to increase the impact of TC deregulation one would have to invent an affinity maturation process that relies solely on TCs (Meyer-Hermann et al., 2006) (i.e. FDCs would have no or only a minor effect on affinity maturation), which is generally considered to be unlikely.

We conclude that deregulation of CC apoptosis via impaired negative selection by FDCs is a GC deregulation relevant to lymphomas provided that at least two affinity-dependent selection steps exist. Deregulation of negative selection by TCs has no major effect on GCR unless the role of TCs in affinity maturation is strongly emphasized.

3.4. Deregulation of proliferation and feedback

Proliferation may be deregulated with an increased proliferation rate, which is unlikely as the cell cycle time of 6 h can hardly be surpassed. If the cell cycle time is reduced to 4 h in model A we get a transformed GC that, in principle, has to be considered as a possible scenario (see Table 3 10A, and Fig. 3): There is ongoing selection, a high fraction of CBs, relatively low density of output cells and of apoptotic bodies. Only affinity maturation of GC–BC is reduced.

The differentiation rate of CBs is assumed to by dynamic and to increase during the GCR (see Eq. (3)). This corresponds to a reduced proliferation rate during healthy
GCRs (Iber and Maini, 2002). One may think of a deregulation of the dynamically increased differentiation rate in later stages of the reaction. In model A this corresponds to a cut of the feedback from the produced output cells to the differentiation rate, thus to $f_2 = 0$. This deregulation exhibits similar characteristics as a direct deregulation of the cell cycle time (see Table 3 11A, and Figs. 3 and 2).

3.5. Alterations in the FDC density

The density of the FDC network determines the number of antigen presenting sites. The site number might be deregulated. If the density is decreased the rescue of CCs is less frequent, such that the GCR is terminated faster than normal. We test an increased FDC network density by setting $\rho_{FDC} = 2$ which corresponds to a doubled probability for a CC to find an FDC. The effect on the GCR turns out to be minor (see Table 3 12A). The effect is only slightly increased with a 10-fold FDC density ($\rho_{FDC} = 10$, see Table 3 13A), and finally saturates for a 50-fold FDC density ($\rho_{FDC} = 50$). Thus the GCR converges to a new dynamical state for abundant FDCs which shows no signs of malignant transformations.

This, however, is different if selection depends on the CC–FDC binding process only. Using $\rho_{FDC} = 5$ in model B or B’ we get a clear deregulation of GCRs very much similar to the results 2B and 2B’, respectively, in Table 3 (data not shown). If GC–BC selection relies on the CC–FDC binding process, deregulation by increased CC life time or by increased FDC network density turns out to be equivalent.

3.6. Changes in the specific or unspecific TC density

If CC rescue depends only on specific TCs then the number of unspecific TCs can be ignored in GCs. We deregulate the rescue process of CCs by TCs by increasing the specific TC density 10-fold ($\rho_{TC} = 10$). Note that an increased specific TC density may be a result of additional specific TC immigration or by unspecific TCs in the GC that acquire the capacity of specific TCs. There is no effect on the GCR that provides a hint to a malignant transformation (see Table 3 14A).

One might expect a larger impact of an increased TC density in a model with affinity-dependent selection by TCs (model D) combined with an affinity-dependent rescue of CCs by FDCs. A 10-fold TC density has only minor effects on the GCR (see Table 3 15D), which cannot be considered as a malignant transformation.

We conclude that in order to get a malignant transformation of the GC based on deregulated TC density or deregulated rescue probability by TCs, one would have to assume rather specific affinity-based TC-dependent selection mechanisms (Meyer-Hermann et al., 2006) and would also have to substantially weaken the role of FDCs in affinity maturation.

3.7. Deregulation of final differentiation

The final differentiation of rescued CCs is believed to depend on TC help. TCs provide signals for differentiation to memory BCs or plasmablasts. In the same way they may induce recycling to re-proliferating CBs (we do not consider recycling of CCs before TC encounter). Lymphomas may be induced by a deregulation of this process such that TCs favour to induce differentiation to CBs rather than to output cells. The probability for TCs to induce final differentiation in selected CCs is decreased to $q = 0.1$ (see Table 3 16A). We find ongoing affinity maturation, a steadily increasing population, normal fraction of CBs, slightly reduced densities of output cells ($r_O = 0.45$) and apoptotic bodies ($r_z = 12.5$). Using a stronger deregulation (with $q = 0.05$, see Table 3 17A) these two factors are further reduced ($r_O = 0.22$ and $r_z = 8.5$) and the population increases more drastically, while all parameters representative of affinity maturation remain unaltered (see Table 3 17A and Fig. 3).

In a more extreme scenario the final differentiation may not be deregulated but completely suppressed (i.e. $q = 0$, see Table 3 18A). Then affinity maturation is still present but in a weaker sense, the fraction of CBs is increased, the density of apoptotic bodies is strongly suppressed ($r_z = 2.6$), and there are no output cells at all.

A subtle deregulation of final differentiation signalling turns out to be a realistic candidate for a malignant transformation of GCRs towards lymphomas. The effect of this deregulation is widely independent of the assumed selection mechanism. This deregulation is the only we found that leaves affinity maturation unaffected.

3.8. Adhesion and migration

The migration of CBs and CCs is important for the selection process. Adhesion of BCs to other cells has an impact on BC motility. If CCs increase adhesion (and thereby loose their migration potential) the positive selection by FDCs is strongly suppressed and the GC dies out early. However, if the motility is increased by reducing BC adhesion this also increases the chance of positive selection, in particular when the CC–FDC binding process is relevant for selection. However, this corresponds to the scenario of increased FDC network density (see Section 3.5), which is not distinguishable from increased CC motility within the present models.

4. Discussion

4.1. Analysis of GC deregulation mechanisms

Assuming transformed BCs to frequently appear in healthy GCR we have asked for reasons that allow them to survive selection in GCs, which might be interpreted to be at the origin of lymphoma disease. This has been investigated on the basis of in silico simulations starting
from healthy GCR. We have found six realistic scenarios of deregulation of the GCR:

**CC Life time:** Increased CC life time (or denser FDC networks) in models based on limited CC–FDC binding probability (see 2B in Table 3).

**Specificity:** Partial loss of affinity-dependence of cell interactions (increased BCR cross-reactivity) (see 6A in Table 3).

**Negative selection:** Deregulation of negative selection by induction of apoptosis in one but not in all affinity-dependent selection steps (see 7A in Table 3), which restricts this scenario to GC with at least two affinity-dependent cell interactions.

**Proliferation:** Shortened CB cell cycle time in any model (see 10A in Table 3).

**Feedback:** Deregulation of an hypothetical feedback from output to CB differentiation (see 11A in Table 3).

**Differentiation:** Deregulation of induction of final differentiation by TCs in any model (see 17A in Table 3).

The bold acronyms are used in the following to identify the different deregulations. Note that some of the scenarios depend on specific assumptions about the selection mechanisms in GCR.

All scenarios are characterized by total GC-population kinetics that pass a minimum around day 8–11 after GC onset (day 5–8 in Fig. 2) and then strongly grow beyond the limits of normal GCs. Somatic hypermutation is kept in all scenarios, which is in agreement with data from diffuse large BC lymphomas (DLBCLs) (Lossos et al., 2000) and FLs (Bahler and Levy, 1992) and in many other types of lymphomas. This clinical observation strengthens the connection of lymphoma pathogenesis to the control of GCRs. In view of the observation of ongoing selection based on BCR expression in various types of lymphomas (Küppers, 2005), the scenarios chosen here all exhibit at least a minimum degree of ongoing selection mirrored in affinity maturation. In the following we discuss differences between the scenarios.

Concerning the cellular content of deregulated GCs we find normal fractions of CBs around 35–40% in most scenarios (with respect to simulated healthy GCRs, see also MacLennan, 1994). However, in the CC life time scenario the fraction of CBs in substantially smaller (20%) while it is increased to 51% in the feedback scenario and normal (around 40%) in all other scenarios. This difference can be considered as a quantitative prediction which allows the distinction between these pathogenesis scenarios.

Let us compare the relative amount of output cells which is strongly suppressed compared to healthy GCRs in all scenarios (see Fig. 4). If we take the ratio $r(O)$ (defined in Table 3) as a (relative) measure for the density of output cells we find 0.81 in a healthy GCR. Low output densities around 0.3 are found in the proliferation and feedback scenario. Higher output densities are found in the specificity scenario (0.61), in the differentiation scenario (0.45 or less), and in the CC life time scenario (0.39 or less). The density decreases with increasing deregulation in the latter three scenarios. However, the reduced density saturates at 0.3 in the specificity scenario, at 0.17 in the CC life time scenario and goes down to 0 in the differentiation scenario. This in silico result corresponds well to the fact that deregulated negative selection, CC life time or specificity do not affect differentiation signals as provided by TCs to selected CCs. Thus, the output cell density is even more reduced in the differentiation scenario. Indeed, a lack of plasma cells has been observed in FLs (Lampert et al., 2005; Dogan et al., 1998). CD138+ plasma cells are also rarely found in DLBCLs (Pileri et al., 2002, Table 3). This might be interpreted to support the scenario of a deregulation of final differentiation of BCs by TCs and the idea of lymphoma initiation by up regulation of recycling.

The statements that apoptosis is deregulated in GC during lymphoma pathogenesis and that clonal selection is ongoing (Küppers, 2005) in lymphomas turn out to be contradicting to some extend. BCR expression is observed in many lymphoma types (Gunven et al., 1980), there is ongoing V-region mutation during tumour clone expansion (Lossos et al., 2000), and the mutations are not random suggesting an ongoing selection process (Cleary et al., 1986) at least during onset of lymphomas. We find in silico that for the negative selection (provided there are two affinity-dependent selection steps) or the CC life time

![Fig. 4. Density of output cells: The relative density of output cells in the follicular area is calculated at 18 days after monoclonal expansion. Healthy GCR (full black line) exhibit highest output densities whereas the lowest output densities are found for deregulation of differentiation (wide dashed dotted red line and simulation 17A in Table 3). The ratio of output densities in deregulated to healthy GCR is predicted by the present simulations and might be subject of corresponding experiments.](image-url)
(provided there is limited binding probability only) deregulations have considerable impact on affinity maturation (see Section 3.3, simulation 7A, Section 3.1, simulation 2B, and Fig. 3). In the related specificity scenario (see Section 3.2, simulation 6A) affinity maturation is affected and can even be dropped to zero depending on the strength of the deregulation. In the negative selection scenario (provided the only affinity-dependent selection step is deregulated) (see Section 3.3, simulation 8C), there is no clonal selection found at all. Thus assuming ongoing affinity maturation to be a marker for ongoing selection, it seems unlikely that deregulation of CC life time, negative selection or specificity are major mechanisms driving lymphoma pathogenesis. The observed ongoing clonal selection suggests that the FDC–BC interaction remains intact but that it is the TC–BC interaction that turns deregulated. Indeed, the differentiation scenario is the only deregulation which exhibits normal affinity maturation (see Fig. 3, simulation 17A in Table 3), strong BCR-dependent selection independently of the degree of deregulation (see Table 3, 16A–18A), and a take over of specific clones.

In this context it might be interesting that in vitro FDCs inhibits Fas-induced apoptosis in malignant BC lines (Schwarz et al., 1999). The mechanism for that is unknown. However, this does not point to any essential role of FDCs in the pathogenesis of FLs: (1) FDCs inhibit apoptosis in normal GC–BCs as in malignant BCs. The result only shows that this mechanism is not lost in malignant BC–FDC interactions. (2) Bel-2 expression in malignant BCs was not altered in dependence on interaction with FDCs, such that the characteristic marker for FLs is not influenced by this process of inhibition of apoptosis.

In our simulations deregulation of final differentiation leads to far more relative apoptotic bodies than all other deregulations (compare $r^<_C$ for simulations 0A, 2B, 6A, 7A, 10A, 11A, and 17A in Table 3). If, indeed, the differentiation scenario is relevant to the lymphoma pathogenesis, we predict a factor 3 lower density compared to the healthy situation which can be clearly distinguished from the negative selection—or CC life time—scenario for which we predict a lower density of apoptotic bodies by a factor between 11 and 16 (with respect to the healthy GCR). Note that the exact factors assume macrophages to have constant activity. In reality macrophage activity might depend on the amount of apoptotic bodies. Due to lack of more precise data we have to make this rough estimate. Qualitatively DLBCLs show always numerous mitotic figures and display a huge amount of apoptotic bodies and great phagocytosis by macrophages (Pileri et al., 2002). This again favours a deregulation of final differentiation rather than negative selection or CC life time.

Thus, according to the model, deregulation of final differentiation induced by TCs is relevant to lymphoma pathogenesis. This result has not been presupposed at any stage of the model assumptions. The importance of final differentiation is a model prediction based on emerging properties of the uncontrolled follicles. This prediction is robust because it is independent of the assumed selection mechanism. This prediction may be wrong if the investigated observables (density of apoptotic bodies, density of plasma cells, diversity of BC clones) turn out not to be characteristic for at least as subset of lymphomas.

We consider a shorter CB cell cycle time to be unrealistic, for the cell cycle time of CBs is already the shortest known. A deregulation of an hypothetical feedback by which the CB differentiation time is regulated by the produced GC output, relies on this specific assumption. An experimental hint towards such a feedback mechanism is not known. For the moment it is only supported by the observation that the stability of healthy GCRs is increased when such a mechanism is included in the theoretical model. However, in these two scenarios affinity maturation is still observed at least for CCs in the late stage of development and for output cells, but substantially weakened for CBs and CCs in early stage (see Fig. 3 GC–BC quality). So they have still to be considered and are characterized by intermediate (compared to other scenarios) densities of apoptotic bodies, and by low densities of output cells in the same range than the differentiation scenario. The feedback scenario exhibits an abnormal increased fraction of CBs.

Which cells in the GC are putative lymphoma generating cells? If gene translocation is sufficient for lymphoma onset both, CBs and CCs might be at the origin of lymphoma development. However, if selection of transformed BCs by FDCs and TCs is a necessary requirement for lymphoma onset, pathogenesis is most likely related to CCs. As the BCR expressing CCs and not the BCR down regulated CBs are subject to interaction with TCs, only the recycled CCs may further develop into lymphomas. The latter is supported by a dominance of lymphoma subtypes that are characterized by cells that still carry the BCRs (Küppers, 2005), including FLs and DLBCLs.

One may support the idea of lymphomas not to be derived from CCs if one finds an experiment in TC deficient mice in which lymphomas develops. As known from TC-independent GCRs, CCs do not survive in these mice, so that the whole GC population is dominantly populated by proliferating CBs. An emerging lymphoma would therefore be most likely derived from CBs, or from a CBs differentiating to CCs which survives without further selection steps, and not from CCs.

Note that the CCs as lymphoma generating cell type in GCs does not imply that CBs will not be found in lymphomas. On the contrary: (1) The deregulation by TCs is supposed to be such that recycling to CBs is favoured. Therefore CBs would contribute a relevant part of the lymphoma cells. But the fatal interaction remains the one between the transformed CCs and the TCs. (2) The expression of BCRs as a marker for CCs is typically down regulated in later stages of differentiation in neoplastic cells (Küppers, 2005), such that this marker shall not necessarily be expected in lymphomas even when their origin lies in CCs.
4.2. Speculation on a common precursor in lymphoma pathogenesis

The present analysis relies on the assumption that translocations are not sufficient for the onset of lymphoma disease and that an early step in lymphoma pathogenesis is a deregulation of GCs. Let us now assume this hypothesis to be true. Then the control of the GC–BC population is lost which implies a considerably increased risk of malignant transformations in BCs. The translocation as observed in FLs, for example, could even happen after this deregulation. The GC deregulation acquires the status of a common origin of lymphomas. The development of lymphomas may then be interpreted as a race between a false selected malignant cell clone which expands and the ongoing selection which tries to delete the daughter cells. The diversity of lymphomas observed in later stages is a result of the exact type of transformation in the deregulated environment.

With respect to the general view that the BC transformation would be the initial step in lymphoma pathogenesis the deregulation of GCs would have to be considered as a secondary effect of the BC transformation. This would imply that GCR deregulations are initiated by a variety of different malignant transformations. According to the present in silico simulations, the deregulation of GCs is a subtle process which cannot be initiated by an arbitrary variety of different malignant transformations. However, the present simulations are not meant to rule out the BC transformation to happen first. Here, we aimed at pointing to the possibility, that, according to present knowledge, the malignant BC transformation might not be sufficient for lymphoma onset, which has to be supported if not preceded by a GC deregulation.

If GC deregulation is a necessary event, a natural classification of lymphomas would then be based on the phenotypical distance of the lymphoma type to normal GC. The dominating types of lymphomas are FLs and DLBCLs. These two share at the same time the most properties with GC. In particular FLs share with GC the microenvironment, the shape, the need for signals from FDCs and helper TCs to sustain BC proliferation (Umetsu et al., 1990; Dogan et al., 1998), and ongoing clonal selection. Large GCs showing the morphology (not necessarily the genetic characterisation) of FLs might be considered as a precursor stage towards other lymphoma subtypes.

Is there any marker for the primacy of FLs? PAG (Csk-binding protein) is found to up regulate Csk-kinases which, in turn, negatively regulate the activity of Src-kinases. These should be active in proliferating lymphocytes. Thus PAG-expression has a negative effect on the proliferative activity of lymphocytes. It was found that PAG is expressed on GC–BCs, on BCs in FLs, in parts on BCs in DLBCLs, but not expressed in any other kind of lymphomas (Svec et al., 2005). This suggests PAG being a marker for a successive BC differentiation into neoplastic cells. In this view, FLs would appear as a first stage of transformation, followed by DLBCLs which might be considered to recollect different lymphoma types that are effectively distinct.

CD10+/bcl6+ DLBCLs are thought to be derived either directly from GC or from preexisting FLs (Pileri et al., 2002). FLs and lymphocyte predominant Hodgkin’s lymphomas (Pileri et al., 2002) are also CD10+/bcl6+. Bcl-6 is specifically expressed on GC–BCs and also on GC–TCs. Thus the expression of bcl-6 might be considered as a possible marker for the distance of the lymphoma type to the GCR. This is further supported by the observation that bcl-6 negative lymphoma subtypes are rather diverse in other phenotypic properties (Pileri et al., 2002).

4.3. Conclusion

The present in silico simulations of GC deregulation mechanisms show that deregulation of CC life time or negative selection is a possible mechanism under specific assumptions about the GC selection mechanism. However, other deregulations scenarios like proliferation or feedback are more realistic. In summary, the results favour deregulation of TC induced final differentiation of positively selected CCs. Thus in early stages of lymphomas a deregulation of cytokines like II-10 which control final differentiation of GC–BCs is predicted by this result. The discussed scenarios can be distinguished in several morphological properties like the fractions of CBs, the density of plasma cells, and the density of apoptotic bodies compared to healthy GCRs.

Such deregulations of GCRs can be interpreted as an early step towards lymphoma pathogenesis. In this paradigm, malignant BC transformations are considered normal and only if supported by a deregulated GC environment would lead to lymphoma onset. The BC transformation is more relevant to the type of lymphoma which develops not to the onset as such. This suggests a novel classification of lymphoma disease according to their distance to the GC phenotype. Markers for this distance have been proposed.

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