Modelling the Affinity Maturation of B Lymphocytes in the Germinal Centre

By

ADAM THOMAS REYNOLDS

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To my Mum and Dad

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Abstract

This study involved the successful development of a computer model to simulate the affinity maturation of B lymphocytes in the germinal centre in 3-D because certain behavioural aspects cannot currently be examined experimentally. The software tool recreates the structure of the germinal centre that enables the B cells to move and interact with other cells inside the germinal centre. This allows the modelling of the affinity maturation of the B cells to produce high affinity antibodies.

The model was validated against the available published population dynamics data that shows population sizes on different days post-immunisation. The modelling results show a reasonable correspondence with published data. They also suggest that the high affinity plasma cells that are produced from the germinal centre reaction displace the antibodies on the immune complexes on the follicular dendritic cell. This feed-back drives the selection of high affinity centrocytes to be selected and become high affinity plasma blasts and memory B cells. This conclusion has been partially confirmed by recent experimental results (Toellner, unpublished).

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GLOSSARY

Antibody/ Immunoglobin (Ig) :	Any of a large number of proteins of high molecular weight that are produced normally by specialized B cells after stimulation by an antigen and act specifically against the antigen in an immune response, that are produced abnormally by some cancer cells, and that typically consist of four subunits including two heavy chains and two light chains.[1]
Antigen :	Any substance (as an immunogen or a hapten) foreign to the body that evokes an immune response either alone or after forming a complex with a larger molecule (as a protein) and that is capable of binding with a product (as an antibody or T cell) of the immune response.[1]
B lymphocyte / B cell :	Any of the lymphocytes that have antigen-binding antibody molecules on the surface, that comprise the antibody-secreting plasma cells when mature, and that in mammals differentiate in the bone marrow.[1]
Centroblast (CB) :	A lymphocyte with a large noncleaved nucleus.[2]
Centrocyte (CC) :	A lymphocyte with a cleaved nucleus.[2]
Dark zone (DZ) :	The region of the germinal centre that appears darker as there is a large concentration of dividing centroblasts present.[3]
Fc receptor :	Receptors present on a variety of cells for the Fc fragment of immunoglobulins. These receptors recognize immunoglobulins of the IgG and IgE class.[3]
Follicular dendritic cell (FDC) :	Cells found in germinal centres of the lymphoid follicles of the spleen and lymph nodes; contain Fc receptors able to trap antigen bound to antibodies.[3]
Germinal centre :	The region of tissue where B cells undergo affinity maturation to produce high affinity antibodies.[3]
Immune-complex (IC) :	Any of various molecular complexes formed in the blood by combination of an antigen and an antibody that tend to accumulate in bodily tissue and are associated with various pathological conditions (as glomerulonephritis, vasculitis, and systemic lupus erythematosus).[3]

[1] - Medline Plus (2009) Web resource, available at http://medlineplus.gov/
[2] - mediLexicon (2009) Web resource, available at *http://www.medilexicon.com/medicaldictionary.php*. *http://www.nlm.nih.gov/medlineplus/mplusdictionary.html*
[3]- Kindt, T., Goldsby, R., Osborne , J. and Kuby, J. (2007) Immunology Sixth Edition.
[4] - Kroese, F. G., Wubbena, A. S., Seijen, H. G. and Nieuwenhuis, P. (1987) Germinal centers develop oligoclonally, Eur J Immunol, 17, 1069-72.

Immunoglobulin gene / Ig gene :	A gene encoding one of the protein chains (light or heavy) of an immunoglobulin.[3]
Light zone (LZ) :	The region of the germinal centre where centrocytes try to bind with the antigen being presented by the follicular dendritic cell.[3]
Lymphatic system / lymphoid system / lymph system :	The part of the circulatory system that is concerned especially with scavenging fluids and proteins that have escaped from cells and tissues and returning them to the blood, with the phagocytic removal of cellular debris and foreign material, and with immune responses, that overlaps and parallels the system of blood vessels in function and shares some constituents with it, and that consists especially of the thymus, spleen, tonsils, lymph, lymph nodes, lymphatic vessels, lymphocytes, and bone marrow where stem cells differentiate into precursors of B cells and T cells.[3]
Mantle zone / follicular mantle MZ)	The outer ring of small lymphocytes surrounding a germinal centre, includes transient lymphocytes.[3]
Memory B cell / mature B cell :	B lymphocytes that mediate immunologic memory; allow for enhanced immunologic reaction when an immunologically competent organism is re-exposed to an antigen.[3]
Monoclonal development	A single precursor cell initiates the germinal centre.[4]
Naïve B cell :	A type of B cell which has not been exposed to an antigen.[3]
Oligoclonal development	A few precursor cells initiate the germinal centre[4]
Plasmablast / plasmacytoblast (PB) :	A precursor of a plasma cell.[3]
Plasmacyte / plasma cell :	A lymphocyte that is a mature antibody-secreting B cell. [3]
Polyclonal development	Many precursor cells initiates the germinal centre. [4]
Secondary follicles :	A structure composed of a mantle and germinal centre located within all parts of the lymphatic system. [3]
Somatic hypermutation (SHM) :	A high frequency of mutation that occurs in the gene segments encoding the variable regions of antibodies during the differentiation of B lymphocytes into antibody producing plasma cells. [3]

[1] - Medline Plus (2009) Web resource, available at http://medlineplus.gov/
[2] - mediLexicon (2009) Web resource, available at *http://www.medilexicon.com/medicaldictionary.php*. *http://www.nlm.nih.gov/medlineplus/mplusdictionary.html*
[3]- Kindt, T., Goldsby, R., Osborne , J. and Kuby, J. (2007) Immunology Sixth Edition.
[4] - Kroese, F. G., Wubbena, A. S., Seijen, H. G. and Nieuwenhuis, P. (1987) Germinal centers develop oligoclonally, Eur J Immunol, 17, 1069-72.

CHAPTER 1 -INTRODUCTION

1.1 Overview

The aim of the thesis is to model the affinity maturation of B lymphocytes in the germinal centre because certain aspects of the behaviour cannot currently be examined experimentally and would benefit from analysis using a software tool to guide future experimental direction.

Diversification is a key factor in the success of the adaptive immune system because it provides the white blood cells with a large repertoire of possible antibodies that can potentially bind with new antigens. New B cells develop in the bone marrow and undergo class switching and selection events in which the class switching alters the type of antibody that the B cells produce. Consequently, each B cell that passes selection will be characterised by the presence of a unique B cell receptor that consists of a membrane-bound immunoglobulin molecule. The B cell subsequently becomes activated when it interacts with an antigen exhibiting a matching binding affinity. This antigen will then be presented to the T helper 1 cell. On receipt of the activation signal from the T cell, the B cell will then differentiate to yield either a memory cell or an antibody-producing plasma cell. The plasma cells produce large quantities of antibodies that capture the free antigen and these are, in turn, captured on the follicular dendritic cells in the germinal centres. This process provides the centrocytes in the germinal centre with an opportunity to try and capture the antigen in its native form.

1.2 Structure of the Germinal Centre

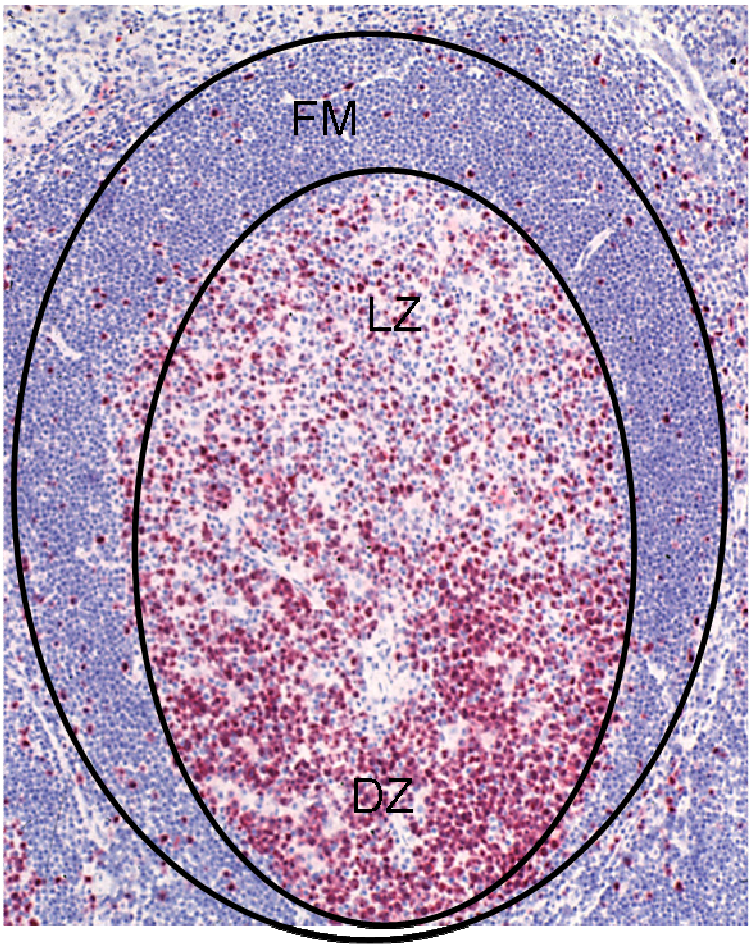

Figure 1: A photomicrograph illustrating the structure of a typical germinal centre consisting of an inner core of light (LZ) and dark (DZ) zones surrounded by a denser follicular mantle (FM). Image from (Toellner, 2006).

Germinal centres are located inside the secondary follicles in the spleen and in lymph nodes where they are surrounded by the rings of tightly-packed B cells that form the follicles (Kindt et al., 2007). The structure of a typical germinal centre is shown in Figure 1 and illustrates the relationships between the constituent two forms of B cells, T cells, and dendritic follicular cells. The population of dividing B cells is concentrated within the dark zone of the germinal

centre with the non-dividing B cells being located in the light zone. Within the germinal centre, B cells undergo affinity maturation in a process of division, mutation and selection. This process produces B cells that have a higher binding affinity to the antigen in comparison with the starting affinity of the antibodies produced by the starting population of activated B cells.

The red and blue dots in Figure 1 represent stained B cells with the germinal centre being defined by the inner ovoid zone. The dark and light zones within the germinal centre contain centroblasts, centrocytes, follicular dendritic cells and T cells that are involved in presentation, selection, and mutation. The antigen and immune complexes are very much smaller than the individual cells and are not resolved on the scale of Figure 1. The follicular mantle surrounding the germinal centre consists of naive B-cells, plasma blasts and T cells. Further differentiation of the centrocytes (non-dividing B cells) into memory and plasma blasts occurs along the boundary between the germinal centre and the follicular mantle.

1.3 Behaviour of the Germinal Centre

Our overall understanding the behaviour of the germinal centre has improved with time, particularly in recent years where the technique of two photon microscopy has provided detailed information on the length of time required for the centrocytes to interact with the antigen (Suzuki et al., 2009, Schwickert et al., 2007, Hauser et al., 2007). (Suzuki et al., 2009) concluded that this takes less than two minutes. In addition, (Allen et al., 2007a) concluded that the rate of centroblast division is 12 hours instead of the previously established 6-7 hours (Liu YJ et al., 1991).

No clear model has yet been developed that adequately accounts for the mechanism of B cell differentiation and the regulation of the dividing population. This is because of two main factors:

- It is not possible to view the system or a model of the system *in vitro* because of the very short life span of the centrocytes and,
- The resolution of population dynamics in which there are far more data available for the initial expansion phase relative to the sparseness of data after day seven.

1.4 Organisation of the Germinal Centre

The organisation of the germinal centre has been defined as a combination of two chemotactic signals inside the germinal centre. The dark zone (Figure 1) is regulated by the CXCL12 ligand that is predicted to be secreted by stromal cells in the dark zone and is highly localized in the base of the germinal centre (Allen et al., 2004, Tarlinton, 2008). The CXCR4 receptor for the CXCL12 ligand is secreted on the cell membranes of the centroblasts (Allen et al., 2004, Tarlinton, 2008). The light zone is regulated by CXCL13 ligand that is secreted by the follicular dendritic cells (Allen et al., 2004, Tarlinton, 2008). The CXCR5 receptor binds to the CXCL13 ligand (Allen et al., 2004, Tarlinton, 2008). Both the centroblasts and centrocytes secrete this receptor on their cell membranes. The CXCL12 ligand exerts a stronger effect on the centroblast with the result that they remain concentrated in the dark zone. The centrocytes are receptive to the CXCL13 ligand and will migrate towards the follicular dendritic cells that present the antigen.

The dark zone is populated by a group of dividing B cells. They are characterised by a large nucleus and size in comparison to the centrocytes. In addition, the centroblasts do not appear to express the B cell receptor (Allen et al., 2004, Tarlinton, 2008).

The light zone contains a population of non-dividing B cells (centrocytes) and hosts a network of follicular dendritic cells that hold the antigen in the immune complexes to the centrocytes. T helper cells are also present to perform the selection of the centrocytes and to provide the signals required to select the differentiation pathway of the B cell (Allen et al., 2007b, Hauser et al., 2007). The follicular mantle surrounding the germinal centre contains a large population of naïve inactivated B cells that are displaced by the expanding population of the germinal centre. Some evidence also exists to indicate that CXCL8/IL8 binds are secreted by germinal centre B cells to attract T cells to the germinal centre (Sims-Mourtada et al., 2003). Macrophages are also present and have the role of removing the apoptotic centrocytes that do not receive the survival signal.

1.5 Germinal Centre Timeline

Two population dynamics can be observed in the germinal centre reactions that result from primary and secondary responses to an antigen. Primary and secondary anti-hapten responses show a peak in cell population around days three and four in which the primary peak is 3 orders lower than the maximum peak in the secondary response (Liu YJ et al., 1991). The germinal centre is active for a period of 21 days after the host is exposed to an antigen (Ho et al., 1986, Hollowood K and J., 1991, Liu YJ et al., 1991). The germinal centres initially develop from a few active B blasts (typically 3-10 cells) (Kroese et al., 1987, Camacho et al., 1998) and are observed to be in contact with the follicular dendritic cell network (MacLennan,

1994). These blasts initially develop into centroblasts and begin to divide rapidly in the germinal centre activation (Hollowood K and J., 1991, Liu YJ et al., 1991).

The rapidly dividing centroblasts ultimately fill the secondary follicle thus displacing the naïve B cells that are present. In this initial period the light zone (Figure 1) is not present and the germinal centre will attain its maximum volume. This cell division rate was initially measured at 6-7 hours (Hollowood K and J., 1991, Liu YJ et al., 1991), but (Suzuki et al., 2009, Schwickert et al., 2007) subsequently measured this division rate at 12 hrs. This almost double increase in the division time appears to contradict the established behaviour of the germinal centre by indicating that a rapid increase in cell population occurs during the initial phase of the germinal centre reaction. For example, if it is assumed that cells divide at a rate of two divisions per day with a starting population of 10 cells, then by day 3 this will yield a population of only 640 cells. A total population of 40960 cells will therefore only be reached by day 6. This suggests that the rate of division may be controlled by an unknown factor because estimates of cell numbers range between $1x10^3$ – $10x10^4$ (Hollowood K and J., 1991, Liu YJ et al., 1991).

There are no inconsistencies with the observed behaviour showing the expanding cell population reaching a maximum after which it slowly declines (Hollowood K and J., 1991, Liu YJ et al., 1991). At some point around day 3, centroblasts start to differentiate into centrocytes and these will begin to migrate towards the follicular dendritic cells following the CXCL12 ligand because they have down-regulated the CXCR4 receptors (Suzuki et al., 2009). At this stage the centrocytes also start expressing their modified B cell receptor (mIg). The centrocytes need to receive a survival signal within 10 hours (Liu YJ et al., 1991)

otherwise they will become apoptotic and be removed by the macrophages in the germinal centre. Their task during this period is to bind their B cell receptor with the antigen in the immune complexes attached to the follicular dendritic cells (Suzuki et al., 2009). The current average contact time for this exchange is approximately two minutes (Suzuki et al., 2009).

If the centrocyte is able to bind with the antigen, the cell will internalise and process the antigen (MacLennan, 1994). If the centrocytes receive a survival signal from the follicular dendritic cell in the interaction, this will extend the 10 hour time limit (MacLennan, 1994). However, the surviving centrocytes need to interact with the T cells in the germinal centre to select the final differentiation process (Schwickert et al., 2007). The T cell selects whether the centrocyte will become either a plasma blast or a memory B cell (MacLennan, 1994). The plasma blast will leave the germinal centre and will further differentiate into a plasma cell. Currently there is some evidence that the antibodies that hold the antigen in the immune complexes on the follicular dendritic cell change during the course of the germinal centre reaction (Toellner, unpublished).

1.6 Somatic Hypermutation

Somatic hypermutation occurs when the B cell divides and a mutation occurs in the immunoglobulin gene. This mutation can replace, insert or delete a single base. This mutation rate is higher than other DNA sequence with a rate of $1x10^{-4}$ bp/division (Kindt et al., 2007). The effect of this mutation is observed in the higher affinity antibodies produced in the plasma and memory cells that are produced in the germinal centre. When sequence analysis is performed on the immunoglobulin locus of these cells and compared with the starting B cells,

the mutation is shown to be located in specific regions of the gene that are referred to as the complementarily determining regions (CDR) (Odegard and Schatz, 2006, Smith et al., 1997b). These hyper-variable regions correspond to the sequences that are in direct contact with the target antigen (Jacob et al., 1991). It is thought that the mutation is uniform across the Ig locus (Kindt et al., 2007, Smith et al., 1997b). However, because of selection, only successful mutations are observed in the output cells (Odegard and Schatz, 2006, Smith et al., 1997b). Unfortunately, because of their short life span it is not possible to record the centrocyte sequences to view the unsuccessful mutations (Liu YJ et al., 1991).

1.7 B Cell Stages Inside the Germinal Centre

B cells differentiate in a set sequence of events to become either plasma or memory cells (Schwickert et al., 2007). Inside the germinal centre, a microenvironment exists that controls the rapidly dividing centroblasts and mutant centrocytes to reduce the risk of creating harmful B cells. After B blast cells enter the secondary follicle they are observed to be in contact with the follicular dendritic cells (MacLennan, 1994). At some point these they differentiate into a population of dividing centroblasts, these B cells do not express any surface immunoglobulin and remain in the cell cycle (Suzuki et al., 2009). The centroblasts are attracted to the dark zone (in Figure 1) and there is no evidence that the cells apoptose (MacLennan et al., 2000). The centroblasts undergo somatic hypermutation as they divide which rapidly creates a population of B cells with slightly modified immunoglobulin genes (Odegard and Schatz, 2006). The centroblasts subsequently leave the cell cycle and differentiate into a centrocyte (Schwickert et al., 2007). The process that regulates this differentiation into a centrocyte is not

well understood and a number of different hypothesis have been investigated in computational models (Meyer-Hermann, 2007, Meyer-Hermann et al., 2006, Iber and Maini, 2002).

When the centroblast differentiates into a centrocyte the cell shrinks and expresses the B cell receptor that includes the mutated immunoglobulin molecule that is expressed on the membrane of the cell created from the somatic hypermutation of the immunoglobulin gene (Faili et al., 2002). The centrocyte is then attracted to the light zone (Figure 1) where it tries to receive the survival signal. however the centrocyte has a short life span of only 10 hrs (Liu et al., 1994, MacLennan et al., 1990) within which to achieve this. The centrocyte needs to bind the antigen in immune complexes being presented on the follicular dendritic cell (Suzuki et al., 2009).The centrocyte will receive a survival signal from the follicular dendritic cell if is able to displace the antigen from the immune complexes (Suzuki et al., 2009, Wu et al., 2008). This survival signal extends the survival time of the centrocyte by several hours beyond the 10-hour limit (Suzuki et al., 2009). The centrocyte will apoptose within the 10-hour limit if it is unsuccessful in capturing the antigen (Schwickert et al., 2007).

The successful, centrocyte will navigate to a T cell, present its B Cell receptor and undergo final differentiation (Schwickert et al., 2007). In this interaction, the T cell provides the survival CD40L ligand required for extending the B cell's life span (Suzuki et al., 2009). It is also believed that the T cell will select the centrocyte to become either a centroblast, plasma blast or memory cell (Suzuki et al., 2009). If the centrocyte is recycled into a centroblast, it will be able to undergo further generations of mutation and selection (Suzuki et al., 2009). This recycling mechanism that turns centrocytes back into centroblasts was predicted by Meyer-Hermann (Meyer-Hermann, 2002a) and partially proved by Schwickert, Lindquist

et al. (Schwickert et al., 2007) who show centrocytes migrating back into the dark zone (Figure 1) after interacting with T cells, the plasma blasts leave the germinal centre and develop into plasma cells that produce soluble antibodies in the surrounding tissue (MacLennan et al., 2000). The memory cells also leave the germinal centre and wait until it is again activated by the same antigen at a future date (MacLennan et al., 2000). The computational models often group the plasma blast and memory cells in the same output population. However, computational estimates of the number of output cells may not be valid because plasma cells can produce large numbers of antibodies from only a small number of cells in a short period. In addition, it is not currently possible to measure the total number of output cells over the duration of a germinal centre reaction.

1.8 Summary of Biological System

The germinal centre is an enclosed system in which the components are well defined making this an ideal system for modelling purposes. However, some unanswered questions remain that would benefit from investigation using another software model. A number of different computational approaches have been developed to reproduce the behaviour in the germinal centre. These consist either of a differential equation model focusing on reproducing the population response (Kesmir and De Boer, 1999, Meyer-Hermann et al., 2001, Iber and Maini, 2002, Meyer-Hermann, 2007) or a discrete system that focuses on individual cells to recreate the population dynamic (Meyer-Hermann and Beyer, 2002, Meyer-Hermann, 2002b, Kesmir and De Boer, 2003, Meyer-Hermann and Beyer, 2004, Meyer-Hermann and Maini, 2005, Meyer-Hermann et al., 2006, Meyer-Hermann et al., 2009, Beyer et al., 2002). In addition to these two types of model, research has been undertaken to try and accurately represent and

model the antibody affinity dynamics during the germinal centre reaction (Oprea et al., 2000, Perelson and Oster, 1979, Smith et al., 1997a, Kleinstein et al., 2003). The next section examines the key aspects of these models and concludes with a summarised list of published parameters based on either the results of the simulated models or biologically measured values.

1.9 Generic Germinal Centre Model

The germinal centre models can be summarised as illustrated in Figure 2. By examining the key parameters of the different stages of the germinal centre, it is possible to examine previous methods and compare the existing modelling approaches.

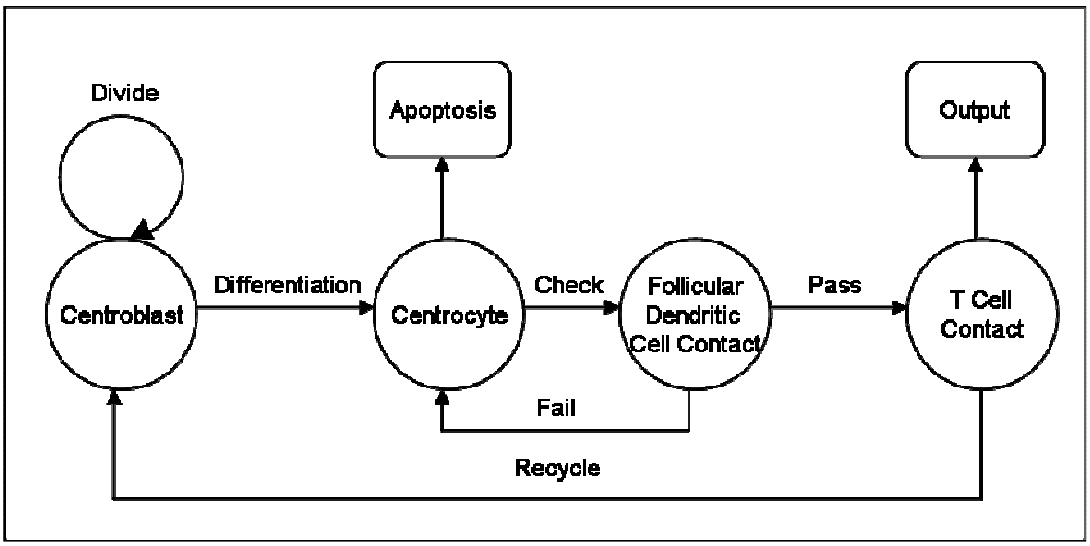

Figure 2 : A schematic diagram summarising the stages that the B cell undergoes in the germinal centre. The arrowed lines show the direction of the stage. The centroblast is the starting point for the B cell. The centroblast can then either divide or differentiate. If the centroblast divides, a new cell is usually created. If the centroblast differentiates, it will become a centrocyte. The check option performed is a selection metric that the centrocyte can either pass or fail. If the centrocyte fails, it is able to retry or undergo apoptosis and is removed from the simulation. If the centrocyte passes it will need to interact with a T cell to determine whether the centrocyte becomes an output cell or is recycled to become a centroblast again. The centroblast, centrocyte, recycled and output populations can be represented either by discrete cells or by differential equations. The selection metric that represents the centrocyte selection is usually a abstract representation of the antigen binding the antigen.

1.10 Modelling the Centroblast Population

The centroblast population is controlled by the division rate of the centroblast cells, the differentiation rate of centroblasts to centrocytes and the recycling rate of selected centrocytes back into centroblasts. Only these three parameters are required to control the number of centroblasts that are present in the germinal centre reaction. However, the overall germinal centre population will decline if too many cells differentiate into centrocytes, an inadequate number of centroblasts divide or an insufficient number of centrocytes are recycled back into centroblasts (Meyer-Hermann, 2002b, Meyer-Hermann et al., 2006, Iber and Maini, 2002). Centroblast division is limited to 8 divisions (Tangye and Hodgkin, 2004).

1.11 Modelling the Centrocyte Population

The centrocyte population is dependent on the number of centroblasts that differentiate into centrocytes. The centrocyte is removed from the simulation when it reaches the end of its finite lifetime. However, if the centrocyte has not reached the end of its lifetime, it has an opportunity to undergo a selection test that reproduces the antigen binding that occurs in the interaction with the follicular dendritic cell. If the centrocyte passes this test, it can then interact with a T cell that selected whether it can be recycled or become an output cell. If the centrocyte fails this selection test, it is able to retry again. In the different models, a parameter exists that adjusts the contact time with the centrocyte and the follicular dendritic cell. This regulates the number of times a centrocyte can undergo the test before the centrocyte becomes apoptotic (Meyer-Hermann, 2002b, Meyer-Hermann et al., 2006)

1.12 Modelling the Output Population

The output population is the total number centrocytes that successfully pass the selection metric and are selected to become output cells. This population will only appear after centrocytes appear in the germinal centre simulation and successfully pass the metric (Iber and Maini, 2002, Meyer-Hermann et al., 2006, Meyer-Hermann, 2002b).

1.13 Modelling the Overall Population Inside the Germinal Centre

The overall population within the germinal centre is the sum of centroblast and centrocyte cells less the number of cells apoptosing. The computational model should generate plots similar to the primary immune response shown by Hollowood K and J. (1991) and Liu YJ, Zhang J et al. (1991). These consist of log-normal plots of the number of cells inside the germinal centre against time and show an initial rapidly increasing population that reaches a maximum before decreasing.

1.14 Modelling the B Cells Antibody Affinity and Representing Somatic Hypermutation

The centroblast, centrocyte and output cell affinity is calculated from Equation 1 which was derived by Meyer-Hermann (2002b). Each B cell has a antibody that is represented by a 4-digit code in which each digit, varies between 1 and 10. For example, an antibody representation might have the form of (3,4,5,4). Somatic hypermutation is modelled by increasing or decreasing one of the four digits by a value of one. This attempts to reproduce the behaviour where a positive mutation results in a higher binding affinity of the resultant

antibody. Conversely a negative mutation results in a lower binding affinity of the resultant antibody.

$$a(\phi, \phi^*) = \exp\left(- \frac{\left\| \phi - \phi^* \right\|^n}{\Gamma^n} \right)$$

Equation 1: Euclidean distance affinity model (Meyer-Hermann, 2002b). φ, is the current antibody (For example it could have a value of (5,4,1,2)), φ^*, is the perfect antibody and has the value (5,5,5,5). $\|$ applying the Euclidean distance (Han and Kamber, 2001) using the two antibodies, n, is set to 2. Γ, is a constant with the value of 2.8. exp, is the exponential function.

Equation 1 reproduces the positive and negative mutation by having a perfect antibody that represents the highest possible binding affinity that the B cell can attain. The antibody of the B cell then mutates and Equation 1 compares the distance between this antibody and the perfect antibody using the Euclidian metric (Han and Kamber, 2001). This distance is then scaled to create an antibody affinity value [$a(\varphi,\varphi^*)$] between 0 and 1 where 1 is the highest possible binding affinity and 0 is the lowest. This initial starting position of the antibody of the B cell in the simulation is set to a distance of 5 units from the perfect antibody.

1.15 Modelling Chemotaxis inside the Germinal Centre

The chemotactic signal that appears in the germinal centre can be modelled using the diffusion equation that implements the finite difference method of Press et al., (1992), as shown in Equation 2. This method stores the concentrations in a lattice and calculates the new concentrations for the next incremental time step. The output for a series of time steps is shown in Figure 3. This approach has been previously used in modelling cell movement (Jabbarzadeh and Abrams, 2007).

$$u_{j,1}^{n+1} = u_{j,l}^{n} + \frac{1}{2}\alpha\left(\delta_x^2 u_{j,l}^{n+1} + \delta_x^2 u_{j,l}^{n} + \delta_y^2 u_{j,l}^{n+1} + \delta_y^2 u_{j,l}^{n}\right)$$

Where, $\alpha \equiv \frac{D\Delta t}{\Delta^2}$, $\Delta \equiv \Delta x = \Delta y$, $\delta_x^2 u_{j,l}^{n} = u_{j+1,l}^{n} - 2u_{j,l}^{n} + u_{j-1,l}^{n}$

and $\delta_y^2 u_{j,l}^{n} = u_{j+1,l}^{n} - 2u_{j,l}^{n} + u_{j-1,l}^{n}$

Equation 2: Diffusion equation in two-dimensions using the finite difference method (Press et al., 1992). $u_{j,1}^{n+1}$ is the new concentration at this lattice point. $u_{j,l}^{n}$ is the previous concentration at this lattice point. D is the diffusion constant, Δt, is the fixed time increment. Δx and Δy are the fixed lattice dimensions. $u_{j+1,l}^{n}$, $u_{j,l}^{n}$ and $u_{j-1,l}^{n}$ are the adjacent concentration values in the lattice for the respective x and y coordinates. Where j is set to the size of the lattice (0->N) where N is the length of the lattice.

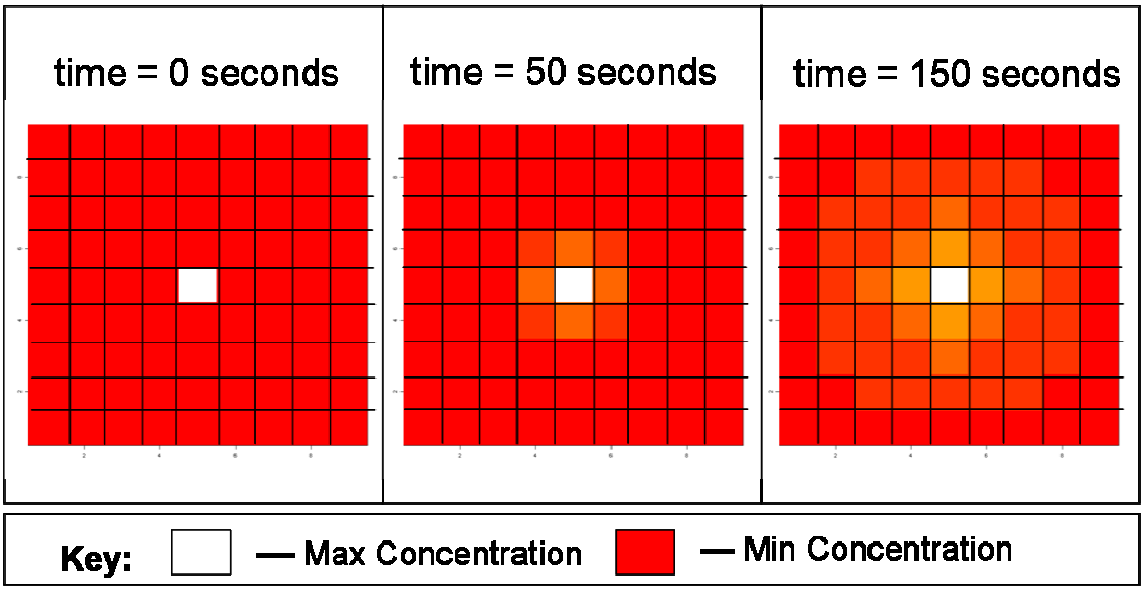

Figure 3: A heat map showing concentrations across the two-dimensional lattice at three time points: t=0s,t=50s and t=150s. The high concentration is shown in white and the low concentrations in red. The lattice dimensions are (0 →9). The initial concentrations in the lattice are zero except the central lattice point is equal to 10. The central lattice point has a fixed value of 10 to create a steady state concentration gradient that can then be used by the B cells movement algorithm to model the directed cell movement.

1.16 Conclusions

The germinal centre can be modelled using a lattice-based approach as previously used by Meyer-Hermann et al. (2006). As part of the current project, this approach was further developed by programming in C++ and using a three-dimensional lattice to better represent the structure of the germinal centre. Meyer-Hermann and Beyer (2002) did not conclusively prove that the simulation results from their two-dimensional model are comparable with the three-dimensional results of his model. It is therefore necessary to create a model in three-dimensions because the germinal centre is not a simple, two-dimensional slice. The additional

programming was undertaken using standard object-oriented design techniques (Holzner, 2001) and a full copy of the final code is supplied on the attached DVD.

Meyer-Hermann, Maini et al. (2006) summarised the list of key parameters that have been used in modelling germinal centres. This list of key parameters is summarised in Table 1, but has been up-dated to include more recently published data.

Table 1 – Key Parameters in Germinal Centre Models

Parameter	Function	Value	Reference
Lattice constant	Define the space the cells will occupy in the simulation.	10 μm	(Meyer-Hermann et al., 2006)
Lattice Dimension	The dimensions of the modelled space used in the model.	2	(Meyer-Hermann et al., 2006)
GC radius	The radius of the germinal centre	220 μm	(Breitfeld et al., 2000)
Number of seeder clones	Initial number of B blasts that will differentiate into centroblasts.	3	(Hermans et al., 1992, Kroese et al., 1987)
CB velocity	The speed the centroblasts move	2.0 μm/min	(Bajenoff et al., 2007, Wei et al., 2003, Suzuki et al., 2009, Schwickert et al., 2007)
Cell cycle time CB	Time taken for the centroblast to divide	6-12hrs	(Liu YJ et al., 1991, Allen et al., 2007a)
Mutation probability of CB	Probability daughter cell has mutated Ig gene	0.5	(Berek and Milstein, 1987, Nossal, 1992)
CC velocity	Velocity of the centrocytes	4.5- 6.0 μm/min	(Suzuki et al., 2009)
FDC –CC contact time	The time the centrocyte is in contact with the follicular dendritic cell	2-3 min	(Suzuki et al., 2009)
Recycling probability CC -> CB	The probability selected centrocytes will become centroblasts	0.8	(Meyer-Hermann, 2002b)
CC life time	Period of time the centrocytes are observed before apoptosing	10 hrs	(Liu et al., 1994)

1.17 Thesis Questions

The aim of the thesis is to model the affinity maturation of B lymphocytes in the germinal centre because certain aspects of the behaviour cannot currently be examined experimentally and would benefit from analysis using a software tool to guide future experimental direction. In order to achieve the required model the following approach is necessary:

- Create a software model that can accurately model the germinal centre.
    - The software model will have to employ a lattice-based approach to model the germinal centre in three-dimensions
    - The model needs to create a movement algorithm that represents the current understanding of how B cells navigate in the germinal centre.
- Once the software model has been developed, it will have to be tested and evaluated to determine whether it accurately represents the biological system. This will require the following:
    - Examination the overall population inside the germinal centre during the germinal centre reaction.
    - Examination of the total number of output cells produced by the germinal centre during the germinal centre reaction.
    - Comparison of the number of B cells in the dark and light zone regions during the germinal centre reaction.
    - Comparison of the population of centroblasts and centrocytes during the germinal centre reaction.
    - Evaluation of the antibody affinity dynamics of the centroblasts in the germinal centre.
    - Evaluation of the antibody affinity dynamics of the output cells.

- Does the simulated centroblasts and centrocytes move at the speed recorded in the germinal centre?
- Examination of the number of times the centroblasts divide.
- Examination of the number of times the centrocytes recycle.
- Evaluate whether the affinity maturation in the germinal centre can be driven using the resultant antibodies produced during the germinal centre reaction.

CHAPTER 2 -EXTRACTING GERMINAL CENTRE POPULATION DYNAMICS

2.1 Introduction

When creating any software model there is a requirement to validate the output of the model by comparing it with experimental data. These data can come from different sources and, in the case software simulations of the germinal centre, they are based predominantly on the population dynamics published by Hollowood K and J. (1991) and Liu YJ, Zhang J et al. (1991) who consider the behaviour of the germinal centre under different conditions. There is therefore an acute need for accurate population dynamics data for use in comparisons with simulation results.

2.2 Existing germinal centre datasets

A software simulation of the germinal centre reaction will generate data that record the total number of B cells produced in the germinal centre during the simulation. The resultant simulation data are not directly comparable with the published experimental results because of differences in the scale of the units used by different authors. For example, Hollowood K and J. (1991) express the germinal centre population in terms of units of total germinal centre splenic volume in mm^3. In contrast, Liu YJ, Zhang J et al. (1991) express the follicle centre volume in terms of percent total splenic volume. In both cases, the published units reflect the total of all the germinal centres in the spleen whereas the simulation results apply only to a single germinal centre.

(Meyer-Hermann et al., 2006) used a formula (Equation 3) that allowed comparisons to be made between their simulation results and the total germinal centre splenic volume. However, it is also necessary to include the two additional data sets from (Toellner et al., 1998, Toellner et al., 1996) in which the population is expressed as a measurement of a single germinal centre rather than in terms of total germinal centre splenic volume. The results of the experiments of Toellner, Luther et al. (1998) can, however, be used to accurately measure the radius of the germinal centre and estimate a lattice size for representing the germinal centre.

$$\eta = \sum_{i=1}^{N} \frac{1}{N-1} \frac{\left(x_{\exp}(t_i) - n(t_i)/n_{\max}\right)^2}{x_{\exp}(t_i)^2}$$

Equation 3: Germinal centre fitness equation source: (Meyer-Hermann et al., 2006), Where N is the total number of experimental points, $n(t_i)$ is the % volume of the simulated average germinal centre population at the time point i;. $n_{\max}$ is the average maximum % volume of the simulated germinal centre and $x_{exp}(t_i)$ is the measured % volume of the GC at time point i and η is the fitness score.

In the case of a perfect match between the simulated and experimental data, the deviation match would be equal to zero. However, (Meyer-Hermann et al., 2006) suggest that $\eta < 0.3$ is an acceptable fit.

The use of these different methods of presenting their results by the four research groups is summarised in Figure 4 from which the difficulties in making direct comparisons with the simulation data becomes apparent. Similarly, it is difficult to compare the results of the individual research groups. However, the additional data sets from from (Toellner et al., 1998, Toellner et al., 1996) can also be processed using Equation 3. The data set from Liu YJ, Zhang J et al. (1991) was derived from a study of rats and its application to mice is

uncertain. These data were therefore omitted from the combined data set that was used in the current study.

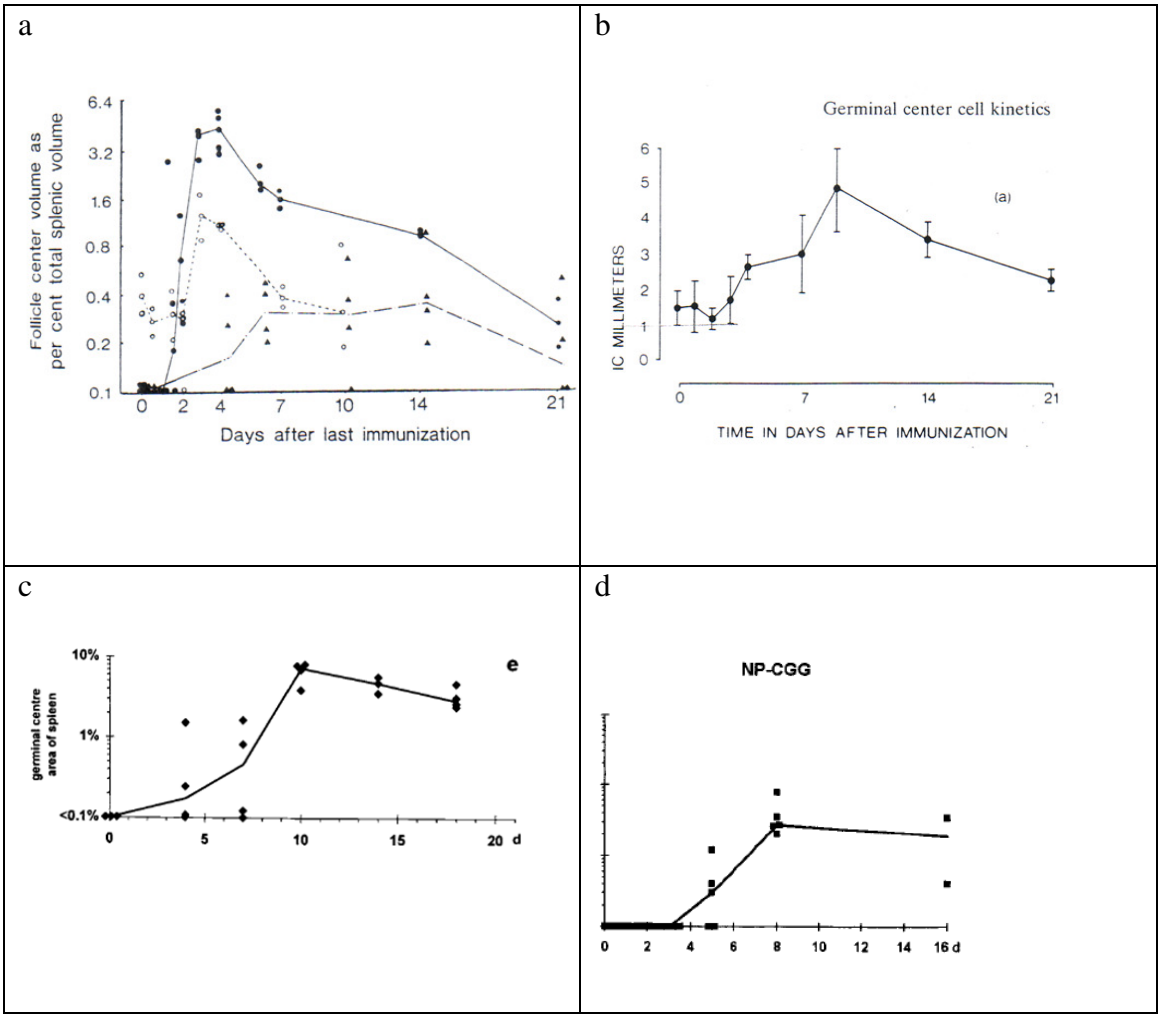

Figure 4 *:* (a) This plot is from Figure 4 in (Liu YJ et al., 1991) and shows the primary and secondary responses to T cell-dependant and T cell independent antigens. The primary response to a T cell dependant antigen is shown as triangles and the dashed lines. The plot shows the relative size of the follicle during the primary anti-hapten response in rats over a period of 21 days. (b) This plot is from Figure 3 in (Hollowood K and J., 1991) and shows the primary response to T cell dependant antigen and shows the total germinal centre splenic volume in mm^3 in mice over a period of 21 days. (c) This is the plot from Figure 2 in (Toellner et al., 1996) shows the primary response to NP-CFG. The plot shows the % germinal centre area of spleen in mice over a period of 20 days. The germinal centre response in this case is delayed, as the peak is only present around day 10. (d) The plot from Figure 8 in (Toellner et al., 1998) shows the primary immune response to NP-CGG antigen. The plot shows germinal centre area in mm^2 in mice over a period of 16 days. Note a,c,d are on a non linear scale and b is a linear scale.

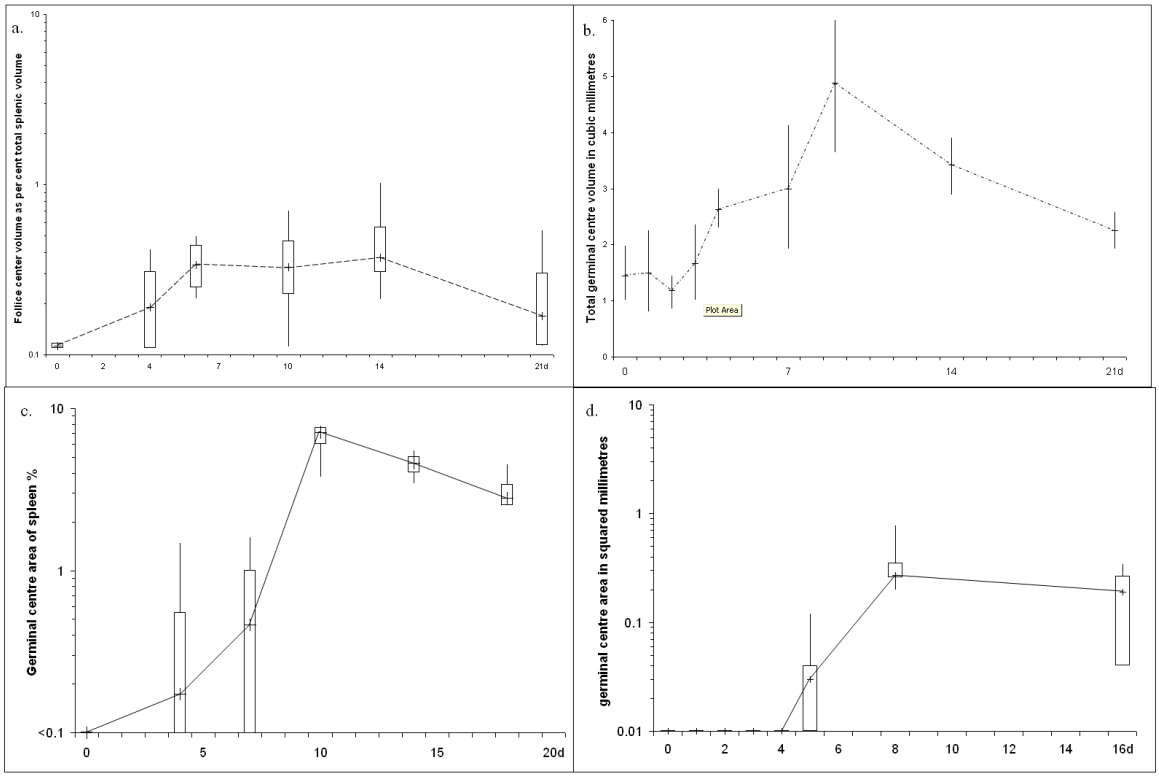

Figure 5 : (a) This boxplot is uses data from Figure 4 in (Liu YJ et al., 1991) the + indicates the median value, the vertical lines show the range of the maximum and minimum values and the boxes indicate the number of data points inside the first- and third-quartile for each of the data points. (b) This plot uses data from Figure 3 in (Hollowood K and J., 1991) and it shows the average value and the shows the primary response to T cell dependant antigen and shows the total germinal centre splenic volume in mm^3 in mice over a period of 21 days. (c) This is the plot from Figure 2 in (Toellner et al., 1996) the + indicates the median value, the vertical lines show the range of the maximum and minimum values and the boxes indicate the number of data points inside the first- and third-quartile for each of the time points. (d) This boxplot uses data from Figure 8 in (Toellner et al., 1998) the + indicates the median value, the vertical lines show the range of the maximum and minimum values and the box indicate the number of data points inside the first- and third-quartile for each of the time points. Note a,b,c,d are on a log scale.

The experimental data from the three studies showed a significant degree of variability in the sampled date ranges. This is illustrated by the use of box plots in Figure 5. As discussed above, the data in 4a were not used. In Figure 4b (Hollowood K and J., 1991) data points are not provided and it is therefore not possible to calculate the first- and third-quartile to be able

to observe the variance in the data. Note that Figures 4b-d are also plotted on a logarithmic scale with the result that the variance is far greater than initially apparent from the length of the boxes.

The radius of the germinal centre can be calculated from Figure 4d using Equation 4 because the units are in mm^2. If the germinal centre area approximates the shape of a circle, Equation 4 formula can rearranged to calculate the radius.

$$A = \pi r^2$$

Equation 4: Area of circle equation source (Kreyszig, 2005), where A is the area of the circle, r is the radius of the circle, π is a fixed constant =3.141592653.

The measurements for day 8 are 0.77, 0.35, 0.27, 0.26, 0.2 mm^2 and represent the maximum areas recorded in the results in Figure 4d. However, these results have to be adjusted to take into account the normal size of the germinal centre and it is then possible to calculate the maximum radius of the germinal centre during the germinal centre reaction. The results from day 0 (Figure 4d) represent the areas where the germinal centre reaction is not occurring and the values at this time are all 0.01 mm^2. The values for day 8 (Figure 4d) can then be adjusted by -0.01mm^2 to yield 0.76, 0.34, 0.26, 0.19 mm^2 respectively.

Figure 6a is a box plot showing the variation of the maximum germinal centre radius calculated using Equation 4. These data also suggest that a median germinal centre radius of 287μm should be used in the modelling. It is also possible to estimate the total number of germinal centre cells that can occupy this space. The average radius of the centroblast is 7.5μm (Meyer-Hermann et al., 2006, Meyer-Hermann, 2002b) and this value can be used to

calculate the area occupied by the centroblast. The resultant area calculated using Equation 4 is therefore 1.77×10^{-4} mm^2.

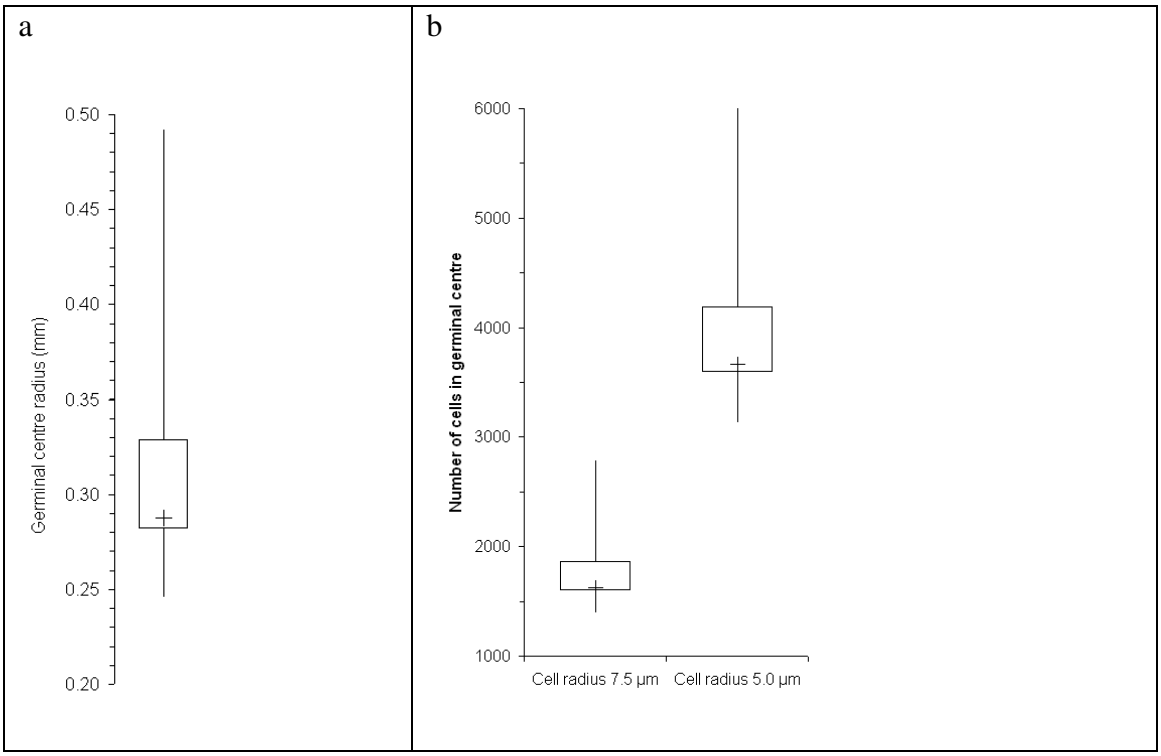

Figure 6 *:* (a) Box plot showing the calculated germinal centre radius in mm from Figure 4d day 8. (b) Box plot showing the number of cells in the germinal centre area for a given cell size. (b) Box plot showing the estimated maximum population size using the area data from day 8 in Figure 8 in (Toellner et al., 1998). There are two box plots showing the total number of cells the germinal centre can contain if the cell radius was 7.5µm or 5µm.

The expanding germinal centre consists predominantly of centroblasts (MacLennan et al., 2000) and in Figure 4d it can be assumed that this is the situation in day 8. After day 8, the germinal centre begins to decrease in size and this corresponds to the appearance of centrocytes (MacLennan, 1994). The number of centroblasts inside the germinal centre at day 8 (Figure 4d) can be estimated by dividing the area occupied by the centroblasts by the total

measured area of the germinal centre. This is illustrated in a box plot in Figure 6b for cell radii of 7.5µm and 5µm respectively. The software models use an average cell radius of 5µm and Figure 6b shows the effect that increased cell radius of 7.5µm has on the maximum number of cells that the germinal centre can hold in a two-dimensional slice.

The data in Figures 4b, c and d can only be used after they have been normalised to an appropriate scale (e.g. 0 to 1) using the min-max normalisation of (Han and Kamber, 2001). The peaks of Figures 4c and 4d also have to be shifted to correspond to the peak position in Figure 4b. This becomes necessary because the germinal centre reaction is delayed in the data sets in both Figures 4c and d. Figure 7b illustrates the resulting plot of the normalised data sets after scaling and peak-shifting. This corresponds to the characteristic germinal centre response that typically shows a rapid rise followed by a gradual decline over the period of the germinal centre reaction.

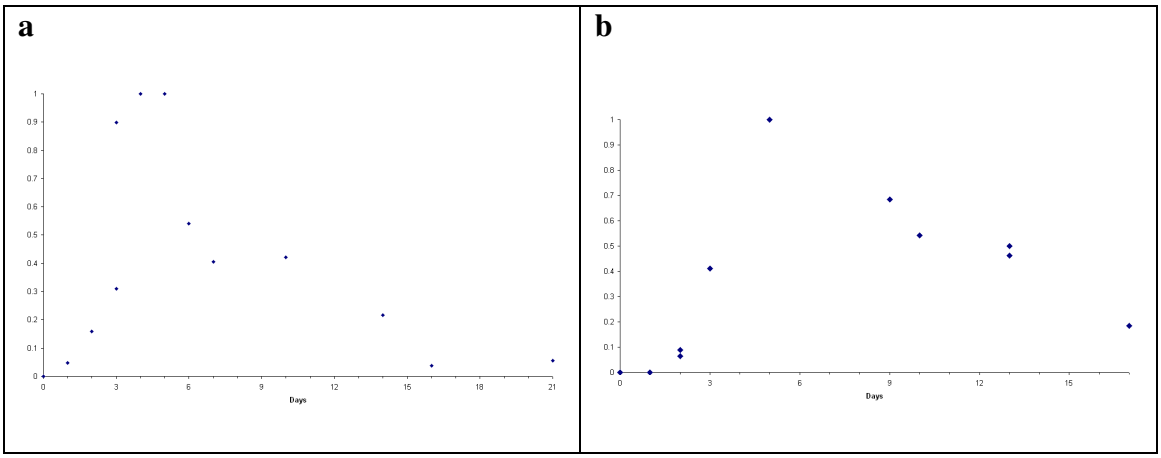

Figure 7 : (a) This plot shows the data points used in (Meyer-Hermann et al., 2006) to evaluate the germinal centre model. The data points have been normalised to a range of 0-1 using min max normalisation. (b) This plot shows the data points used in this thesis to evaluate the germinal centre simulation results. The data points have been normalised to a range of 0-1 using min max normalisation (Han and Kamber, 2001).

It is necessary to compare the simulation results with the data from Figure 7a to ensure that the population dynamics are comparable with previously published results. However, it should be noted that Figure 7b also includes data from the two new sources (Toellner et al., 1996, Toellner et al., 1998) whereas the rat data of (Liu YJ et al., 1991) have been removed.

2.3 Conclusion

Examination of the current experimental data on primary immunisation of T cell-dependent antigens in mice germinal centres shows that the published results of are not directly comparable. However, use of Equation 3 enables a comparison to be made after the magnitude of the actual numerical values have been normalised. It should, however, be noted that the simulation results will not necessarily match the actual number of experimentally determined cells in the germinal centre because the normalisation process eliminates the actual numerical range of the data. In spite of the lack of actual numerical data, the slopes of the gradients produced by the normalised data remain comparable.

The box plots in Figure 5 illustrate the extreme variations that exist in the experimental data that are not evident in the normalised comparison data plots in Figure 7. Error bars are not shown on the normalised comparison because mixing and matching similar data points from different sources yields overlapping error bars that are difficult to interpret and could be misleading. The only definite conclusion that stems from the experimental literature review is that the germinal centre exhibits an initial small size, but after a short period it increases rapidly in size to a maximum after which it rapidly reverts back to its initial size.

CHAPTER 3 -DEVELOPING THE GERMINAL CENTRE MODEL

3.1 Introduction

The literature review of the germinal centre population dynamics in chapter 2 was used to evaluate the biologically relevant results for use in the modelling process. The actual framework for creating the germinal centre model is defined in this chapter and examines the way in which the germinal centre is structured. This includes the definition of the lattice shape required to represent the germinal centre, the modelling of the movement algorithm for the B cells and the definition of the B cell model parameters.

3.2 Definition of the Germinal Centre Lattice Shape

In modelling it is simpler to create fixed geometric structure for data handling purposes because it is easier to process an equidimensional data array than a non-uniform, dynamic data structure (Holzner, 2001). In representing the structure of the germinal centre, it is assumed that a single germinal centre can be contained within a larger cube. This cube is subdivided into smaller cubes having a fixed lattice size. A fixed lattice size of 10μm was selected to reflect the average size of the B cells contained in the germinal centre (Meyer-Hermann et al., 2006). This lattice can then be used to determine whether a cell is located inside or outside the germinal centre. This approach allows the germinal centre to be defined as any type of structure that is able to fit inside the larger cube. The advantage of this approach is that it

allows the germinal centre structure function to be updated without the need to modify any other parts of the computer program code.

In the developed model, the coordinates of the germinal centre (x, y and z) are defined by the formula of a sphere (Equation 5) and the initial starting locations of the centroblasts, T cells and follicular dendritic cells are specified. However, instead of starting the cells in the same position each time the program is run, it is more appropriate to define a region within the cube where the cells can be added. These regions can then be used to define the areas in which the follicular dendritic cells, centroblasts and the T cells are located in the lattice. The placement of these regions is illustrated in Figure 8.

$$x = c \times width \times \cos(\alpha) \times \sin(\beta)$$
$$y = c \times height \times \sin(\alpha) \times \sin(\beta)$$
$$z = c \times breadth \times \cos(\beta)$$

Equation 5: Formula of a Sphere (Kreyszig, 2005), where x, y and z are the calculated coordinates of the sphere. α is an angle and is varied from ($0 \rightarrow 2\pi$) radians. β is an angle and is varied from ($0 \rightarrow \pi$) radians. π is a numerical constant with the value equal to 3.141592653. c, is varied in the range of ($0 \rightarrow R_{GC}$) where R_{GC} is the radius of the germinal centre in μm. The breadth is equal to R_{GC}. The height and width are equal to $K*R_{GC}$, where K is a constant equal to 0.7. By using a value of 0.7 it creates an ovoid shape that is similar to shape of the germinal centre. Note setting K to equal to 1.0 will creates a spherical germinal centre.

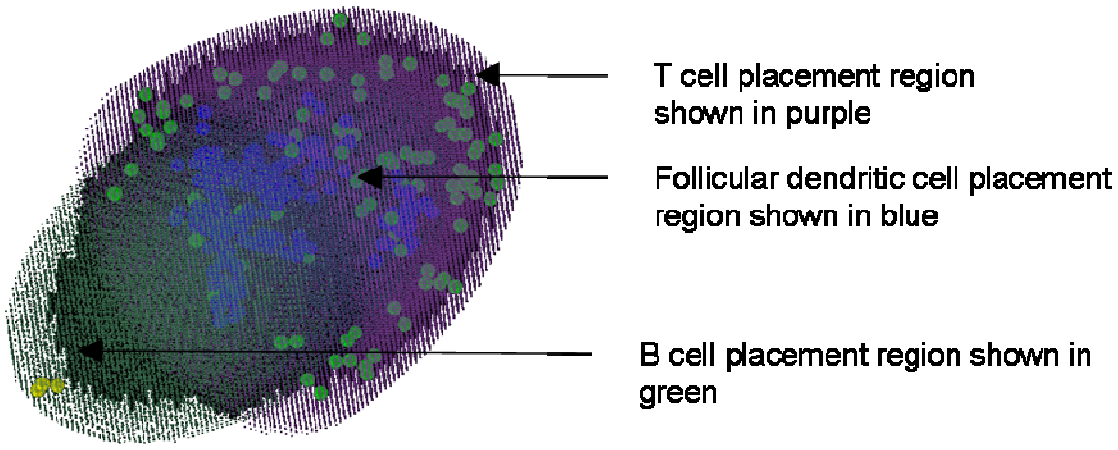

Figure 8 : A three dimensional view illustrating the germinal centre. Inside the germinal centre there are colour-coded spheres that represent the different cell types, with the centroblasts in yellow, T cells in green and the follicular dendritic cells in blue. The centroblasts are randomly placed inside the B cell placement region shown in green. The T cells are randomly placed inside the T cell placement region shown in purple. The follicular dendritic cells are randomly placed inside the follicular dendritic cell placement region shown in blue. The space outside the shaded regions is defined as a physical barrier to constrain the centroblasts and centrocytes inside the germinal centre.

In Figure 8 the stromal cell chemotactic signal is located at the base of the germinal centre at the point where the yellow centroblasts are located. The follicular dendritic cells represent the locations from which the follicular dendritic cell chemotactic signal originates. The T cell coordinates are the sources for the T cell chemotactic signal. The follicular dendritic cell placement region is calculated using Equation 5 where β varies from ($\pi R_{FDC} \rightarrow \pi$) radians and R_{FDC} is the radius of the follicular dendritic cell placement region. The B cell placement region is calculated using Equation 5 where β varies from ($0 \rightarrow \pi R_B$) radians, where R_B is the radius of the B cell placement region. The T cell placement region is calculated using Equation 5 where c is varied from ($0 \rightarrow R_T$) and R_T is the radius of the T cell placement region.

3.3 Generation of the Germinal Centre Structure

The germinal centre structure can be generated automatically by the use of a function that requires the input of appropriate variables. For example, the radius of the germinal centre; radius of the centroblast placement region; radius of the follicular dendritic cell region and the T cell placement region. This approach is flexible and allows a germinal centre of any size to be generated automatically. It should also be possible to define the germinal centre structure using a digitised image of a microscopic section. This approach is beyond the scope of the current study, but would be important in testing the effect of complicated anatomical structures that restrict or control the movements of the B cells in the tissue (Suzuki et al., 2009).

3.4 Defining the Lattice

Each B cell stores the status of the adjacent lattice spaces (i.e. empty, occupied or cell-type) and this information is then used to determine the direction in which the B cell is able to move. This is achieved by using a global array and a local array to store the positional information in the lattice. The global array holds all the information about the status of the cell types at a particular time in the simulation. The local array only stores information about the adjacent lattice spaces surrounding each B cell. The B cell can only move inside the space of the lattice to ensure that they remain inside the germinal centre space.

3.5 Modelling the Chemotactic Signals that Influence B Cell Migration

The migration of B cells inside the germinal centre is driven by three chemotactic signals. A chemotactic signal is a localised molecule that is secreted by active cells into the surrounding tissue and targets a specific cell type having a matching receptor. It is thought that the target cells will then begin to migrate towards the sources of these signals (Kindt et al., 2007). The centroblasts are attracted to a signal secreted by stromal cells located in the base of the germinal centre (Suzuki et al., 2009). The centrocytes are, in turn, attracted to a signal secreted by the follicular dendritic cells that enables them to migrate efficiently towards the follicular dendritic cells (Suzuki et al., 2009). However, it is predicted that the centrocytes that successfully receive the survival signal from the follicular dendritic cells will migrate towards the T cells in a directed motion (Suzuki et al., 2009). In the current model it is assumed that the T cell secretes a signal that is received by the centrocytes that have passed the selection metric that determines whether they have successfully captured the antigen. The selected centrocytes will then migrate towards the T cell.

The three chemotactic signals can be modelled using a standard technique referred to as the *finite difference method* (Press et al., 1992). This method uses previously published parameters that are appropriate for the germinal centre (Meyer-Hermann et al., 2006) and enables the representation of chemotactic signal values in the lattice space that can be used in the movement algorithm for the B cells.

3.6 Modelling B Cell Movement

This section outlines the various problems that need to be addressed when trying to model movement of the B cells in the germinal centre. The movement algorithm first captures the position of all the different cell types in the lattice. The next step then determines which B cell moves in the lattice and this position is stored in a new array. This process is then repeated until all of the B cells have moved. This new array is then copied back and updates the starting lattice. However, before each B cell can move, the status of the adjacent lattice spaces of the B cell are stored in the local array. The lattice that stores the current positions of all the cell types is, in turn, progressively updated for each incremental time step during the simulation.

The B cell that is about to move can be a centroblast, centrocyte, selected centrocyte or output cell. The centrocytes are the only cells in the germinal centre model that can have a direct interaction with a T cell or a follicular dendritic cell. The term "selected centrocyte" is used in this model to denote those centrocytes that successfully pass the selection metric. This selection metric represents the physical process by which the immunoglobulin molecule on the centrocyte displaces the antigen from the antibodies on the immune complexes that are located on the surface of the follicular dendritic cell. The term "output cell" is used in this model to denote either a plasma blast or a memory B cell. All of the modelled B cells can move with a random and directed motion in the germinal centre (Suzuki et al., 2009, Allen et al., 2007a). The movement algorithm is also required to complete a number of tasks before the B cell can move as discussed in the following sections.

3.7 Checking whether B Cell is in Contact with a T Cell or a Follicular Dendritic cell

The first stage is to evaluate whether the B cell is in contact with either a T cell or a follicular dendritic cell after which the following series of rules are applied:

- **If** the B cell is a centrocyte and is in contact with a follicular dendritic cell **then** the selection metric is executed to determine whether the centrocyte becomes selected.
- **If** the B cell is a selected centrocyte and is in contact with a T cell **then the** selected centrocyte will become either an output cell or a centroblast.

If any of these conditions are true, then the B cell does not move. For any other conditions, the movement algorithm continues.

3.8 Modelling B Cell Movement Random Direction

The next stage of the movement algorithm checks the adjacent lattice spaces to determine whether they are occupied or not. If the adjacent space is free, it is added to a list of available sites to which the B cell can move. The site to which the B cell is moved is selected randomly. This process is illustrated in Figure 9.

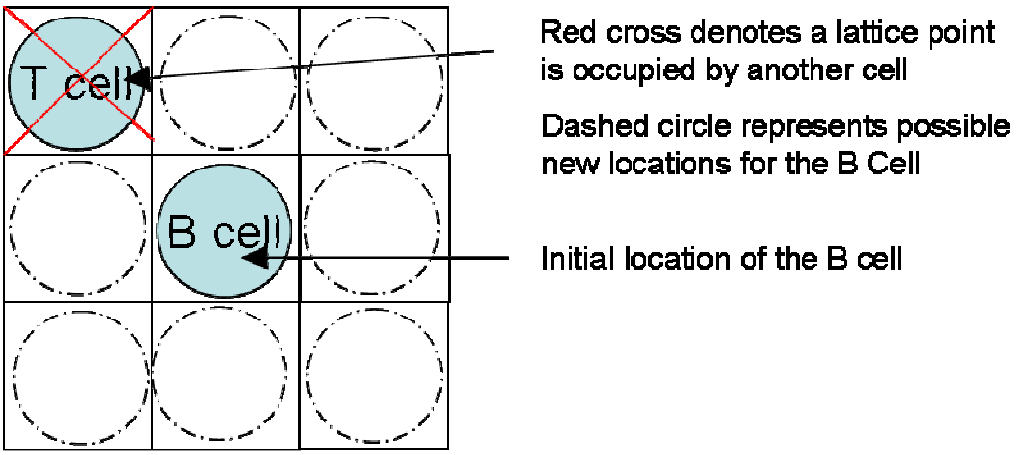

Figure 9 **:** This figure illustrates a lattice space in which a B cell is able to move into the adjacent lattice points denoted by the dashed circles. The B cell is in the centre lattice point denoted with the solid circle. The lattice point with the red cross is a position that has been removed from the list of possible directions because it is occupied by a T cell denoted by a solid circle. The remaining possible directions are listed and the T cell lattice point is removed. This leaves eight possible lattice points to which the B cell can move. A lattice point is randomly selected from this list and becomes the new position for the B cell.

3.9 Modelling the Directed Movement of the Centroblasts and Centrocytes

The movement algorithm calculates the new direction of the B cell in the lattice using the concentration values generated by the finite difference method (Press et al., 1992). This is achieved by using a global signal array that holds the values for the three chemotactic signals in the germinal centre and a local signal array to store the concentration information in the lattice. The dimensions of the global signal array map directly to dimensions of the global array that stores the positional information for all the cell types in the germinal centre. The global signal array holds all the information about the concentration of each of the chemotactic signals (stromal cell, follicular dendritic cell and the T cell) at a particular time in the simulation. The local signal array only stores the information about the adjacent lattice spaces surrounding each B cell.

After the movement algorithm has calculated the random motion direction, the movement algorithm then reuses the generated list of possible directions. The B cell will then have three concentration values for each free adjacent lattice point as follows depending on whether the B cell is a centrocyte, centroblast or selected centrocyte:

- **If** the B cell is a centrocyte **then** use concentration values from the follicular dendritic cell chemotactic signal for the free adjacent lattice points.
- **If** the B cell is a centroblast **then** use concentration values from the stromal cell chemotactic signal for the free adjacent lattice points.
- **If** the B cell is a selected centrocyte **then** use concentration values from the T cell chemotactic signal for the free adjacent lattice points.

The movement algorithm now selects lattice position and moves the B cell to this point according to the following rules:

- **If** one free lattice point has the highest value **then** the B cell moves to this point.
- **If** more than one free lattice point has the same maximum value **then** one of these points is randomly selected and the B cell is moved into that position.
- **If** all of the free lattice point values are equal **then** randomly select a new direction from the list of available lattice points.
- **If** the selected direction is the same the previous iteration value **then** randomly select a new direction from the list of available lattice points excluding the previously selected direction.
- **If** no selection is available **then** do not move the B cell.

The basis for establishing these rules derives from the behaviour of the directed motion of the cells. If only the maximum concentration is selected, it could unintentionally result in a bias

towards a particular direction that is not necessarily valid. This form of bias is eliminated by the use of random directions under appropriate conditions in the rules.

The algorithm can be applied to both two-dimensional and three-dimensional environments in which there is an increase in possible number of directions that the B cells can move.

3.10 Checking for B Cells Moving to the Same Location

The concentrations that the B cells use to determine their new location are only updated once every incremental time step. The actual B cell positions are also are updated only once every incremental time step using a synchronous approach. This creates a situation in which more than one B cell could potentially move into the same lattice point because the local array information is only updated at the start of the incremental time step whereas the updated global array is only updated after all the B cells have completed their movements. This potential conflict situation is resolved by only confirming the B cell lattice positions after all the new lattice positions of the B cells have been calculated. This is achieved by applying the following rules to each B cell:

- **If** the new lattice position in the global array is empty **then** update the B cell lattice position and empty the old lattice position.
- **If** the new lattice position is occupied **then**
    1. If the B cell at the new lattice position is to moving to the current B cell position then swap the two B cell lattice positions.
    2. Else the B cell remains in the current lattice position

These rules deal with the situations that can arise when two cells try to move to the same lattice position or when two B cells are moving in opposite directions.

A situation might conceivably arise in which the simulation favours the B cell that moves first because the collision check for this B cell would always be run first. The order in which the B cells are updated is therefore always randomised when the collision check is undertaken. Consequently, every B cell has an equal chance of being updated before the new lattice positions of the remaining B cells are confirmed.

3.11 Modelling the Centroblast

The centroblast can move, divide and differentiate into a centrocyte. The movement of the centroblasts has been discussed in the previous section. The centroblast is attracted to the stromal chemotactic signal and will try to move towards the source of this chemotactic signal. The division rate of the centroblast is controlled by a parameter referred to as the centroblast division rate. A division rate of once every 10 hours was used in the current model to correspond with the conditions modelled by Meyer-Hermann et al., (2006) to permit direct comparison of the results.

In the model, a centroblast can only divide if there is a free space adjacent to it in the lattice. This is to ensure that the newly created centroblasts only occupy free spaces in the lattice. In nature, when a centroblast divides it splits into two daughter centroblasts (Kindt et al., 2007) and this process is simulated in the model by creating a duplicate copy of the dividing centroblast that occupies the same lattice space as the dividing centroblast. These two daughter centroblasts are distinguished in the model by referring to them as the

"dividing centroblast" and the "copied centroblast". The dividing centroblast then moves to the new lattice position, calculated by the movement algorithm and the "copied centroblast' does not move and remains in the same position in the lattice.

In the model, a centroblast is selected to divide using the centroblast division rate. Centroblast that are selected to divide are flagged to indicate that they will divide at some point in the future. The next time that this centroblast moves, it will divide and its division flag will be cleared. The centroblasts can, however, only divide a limited number of times, estimated to be a maximum of 8 (Turner et al., 2008, Tangye and Hodgkin, 2004). In the model, each time a centroblast divides, its associated division counter is incremented for both the copied and dividing centroblasts. Once the centroblast reaches the maximum division limit (12), it is unable to divide any further.

However, the germinal centre is estimated to start with three centroblasts (MacLennan, 1994) and if the division rate was limited to 8 as suggested by Turner et al. (2008), this would yield a maximum population of $3x2^8$=768 centroblasts in the germinal centre. This is lower than the value of $10x10^4$ estimated in Hollowood K and J (1991) and Liu YJ et al (1991) for the peak germinal centre population. In the current model, a maximum division number of 12 was chosen because it generates a population of $3x2^{12}$=12,288 centroblasts that is similar to the population size suggested by Hollowood K and J (1991) and Liu YJ et al (1991).

However, the germinal centre is estimated to start with three centroblasts (MacLennan, 1994) and if the division rate was limited to 8 as suggested by (Turner et al., 2008) Tangye and Hodgkin (2004), this would yield a maximum population of $3x2^8$=3,072 centroblasts in the

germinal centre. This is very close to the lower estimate of the value at $1x10^3$ – $10x10^4$ estimated in (Hollowood K and J., 1991, Liu YJ et al., 1991) for the peak germinal centre population. In the current model, a maximum division number of 12 was chosen because it generates a population of $3x2^{12}$=12,288 centroblasts that is similar to the population size suggested by Hollowood K and J (1991) and by Liu YJ et al., (1991). The division counter is therefore reset when the selected centrocyte becomes a centroblast after interaction with the T cell. This ensures that the recycled centroblasts that are created in the simulation are able to divide a maximum of 12 times.

Somatic hypermutation occurs at division therefore, the copied centroblast will have its antibody mutated when it is created. This is to ensure that one of the two daughter centroblasts will have the mutated antibody when the predicted mutation rate of the centroblasts at division is 0.5 (Iber and Maini, 2002). This can be interpreted as the dividing cell keeping the original copy of the antibody and the copied centroblast receiving the mutated antibody. The timing of the onset of somatic hypermutation as well as the onset of centroblast differentiation in the germinal centre is not currently known (Bergthorsdottir et al., 2001). The differentiation of centroblasts into centrocytes has been modelled by (Iber and Maini, 2002) who models this using a signal that initiates the onset of differentiation after 96hrs to correspond to the peak at day 4. Beyond this time point centroblasts are able to differentiate into centrocytes with a probability of once every 6 hours (Meyer-Hermann, 2002b). Similarly, this 6-hour differentiation rate is used in the current model.

3.12 Modelling the Centrocyte

A centrocyte has a maximum life span of between 6 and 12 hours (Schwickert et al., 2007, Hollowood K and J., 1991, Liu YJ et al., 1991, Suzuki et al., 2009), but (Suzuki et al., 2009, Schwickert et al., 2007), during which the centrocyte has to successfully migrate to a follicular dendritic cell and pass the selection metric to stop apoptosis. In the current model, a value of 6.5 hours is used to match the value used by Meyer-Hermann (2006). If the centrocyte apoptoses, it is removed from the simulation. The selection metric goal is to recreate the process whereby the centrocyte mutated B cell receptor displaces the antigen in the immune complexes on the follicular dendritic cell (Suzuki et al., 2009).

3.13 Centrocyte selection metric

The current simulation model uses following three selection metrics:

1 The selection metric from (Meyer-Hermann, 2002b), referred to as the "Static" selection metric.

2 A selection metric based on the average affinity of the output cells, referred to as the "Average" selection metric.

3 A selection metric based on the maximum affinity of the output cells, referred to as the "Maximum" selection metric.

The B cells in the current model all have an associated affinity that is calculated using the approach by Meyer-Hermann et al, (2001). This affinity value is used to determine whether the centrocyte will be positively selected by the follicular dendritic cell and receive the survival signal. The model allows for three different antibody selection mechanisms to be

tested. One is the classical method proposed by (Meyer-Hermann and Beyer, 2004) based on the concept of static selection criteria that depend only on the affinity of the antibody to be tested (item 1 above). The other two mechanisms (items 2 and 3 above) use the current population of output cells to set the binding affinity of the antibodies in the immune complexes on the follicular dendritic cells. This binding affinity value is then used as a threshold. Only those centrocytes that have an antibody affinity higher than that of the threshold will be selected. The calculated mean and maximum values of the antibody affinities of output cells are then used as the two separate threshold metrics. These two threshold values (average and mean selection metrics) are updated every 24 hours. The 24-hour delay is to replicate the estimated time required for the plasma blasts to start secreting the soluble antigen into the surrounding tissue. This approach aims to replicate the situation in which high affinity antibody plasma blasts that are created in the germinal centre become plasma cell that secrete large volumes of soluble antibodies in the surrounding tissue. These antibodies then capture the antigen in the immune complexes on the follicular dendritic cells.

In the current model, if the centrocyte successfully passes the selection metric, it will become the selected centrocyte and migrate towards the T cell. When the selected centrocyte is in contact with the T cell, a check is made to see whether the selected centrocyte has become an output cell or was recycled into a centroblast. The probability for selected centrocyte recycling is set at 80% and is based on the simulation results from (Meyer-Hermann, 2002b). In the current model, if the selected centrocyte becomes an output cell, its details are recorded and it is then removed from the simulation. If the selected centrocyte is set to recycle, it will become a centroblast.

3.14 Modelling Follicular Dendritic Cells

The function of the follicular dendritic cell in the germinal centre is to provide a large surface area in which the centrocytes attempt to bind the antigen in the immune complexes. The follicular dendritic cells therefore have to provide a large number of binding sites for the centrocytes. In the current model, the follicular dendritic cell occupies a space in the lattice. In the 3-D lattice array, each follicular dendritic cell is in contact with a total of 26 adjacent lattice points, four of which are on the vertices and 22 are on the sides.

In our model, ten follicular dendritic cells are included to ensure there is an adequate number of binding sites because this number of follicular dendritic cells will provide the germinal centre with 26x10=260 possible lattice binding sites. The current model also simplifies the representation of the antibody antigen complexes present on the follicular dendritic cell by assuming that the complexes are equally distributed around the follicular dendritic cell. In the models that implement a static selection mechanism, the affinity of the antibodies on the follicular dendritic cell exerts no influence on the selection process. This depends only on the probability of binding as computed by Equation 1 (Meyer-Hermann et al., 2001). The average and maximum selection metric sets the follicular dendritic cell to store the current antibody affinity threshold that is determined by the current output cell population.

3.15 Implementation Specifics

The current model is coded using C++ (Holzner, 2001) and was compiled to run on the Birmingham Biosciences High Performance Computer Cluster computer cluster. The random number generator used in the program is that of Matsumoto and Nishimura (1998) instead of

the generic rand() function in the C++ math library (Holzner, 2001). This random number generator requires a starting seed integer number, to generate a random sequence of numbers. Typically, this seed value is set using the current time on the computer running the simulation. However, in using the cluster to run multiple simulations at the same time the seed used will be potentially the same. Instead of using this approach a list of 1,000 random numbers that has been sourced from (Haahr, 2006) is used as the seed value. The source code is provided in the back page of the thesis in a plastic holder. However, some additional development may be required to run the program on other platforms. It also includes the R script to perform the analysis and the bash scripts to automate the running of the germinal centre simulations for use on Birmingham Biosciences High Performance Computer Cluster.

3.16 Conclusion

This chapter has outlined the conditions required for the development of the germinal centre model as well as the approaches that were followed by Meyer-Hermann (2006) in their implementation. The model now needs to be evaluated to establish whether it reproduces their results and whether the proposed selection metric improves the quality of the binding affinity of the output cells. The following chapter examines the parameters used by Meyer-Hermann (2006 and 2002b) and compares the results obtained using static selection method of Meyer-Hermann (2001) and the average and maximum selection methods developed for the current model.

CHAPTER 4 -RESULTS FROM THE GERMINAL CENTRE MODEL

In the previous chapter the key features and model parameters were outlined. In this chapter the model is evaluated by testing its behaviour using parameters taken from the literature (Table 1 with the main source Meyer-Hermann et al, 2006). The behaviour of the simulated germinal centre was analysed using diagnostic plots representing the variables of interest (i.e. population dynamics of the cells in the germinal centre, population dynamics of the output cells, population dynamics of the dark and light zone, population dynamics of the centroblasts and centrocytes, antibody affinity dynamics of the centroblasts, antibody affinity of the dynamics of the output cells, speed of centroblasts in the germinal centre, speed of the centrocytes in the germinal centre, number of divisions the centroblasts undergo and the number of times the centrocytes recycle back into centroblasts).

The objective of this analysis is two-fold:

1 To assess whether the current implementation of the germinal centre model is compatible with the previously published parameters optimized for other existing models.

2 Establish whether the alternative average and maximum selection metrics proposed in the previous chapter improve the overall affinity maturation of the output cells in the germinal centre.

In these simulation runs, only the selection metric was changed with the other model parameters using the conditions defined in the previous chapter. These conditions remained

unchanged for the ten repeated runs for each selection metric. The simulations are repeated to ensure the results are not by chance. The population dynamics use the data from Figures 7a and 7b. These two plots use the experimental datasets from the literature where the Figure 7a uses the data from Michael Hermann (2006) and Figure 7b uses the dataset created in chapter 2 that includes data from the two new sources (Toellner et al., 1996, Toellner et al., 1998).

The results from this comparison are summarised in Table 2 and show that the implemented model does not produce a good fit with the comparison metric published by Michael Hermann (2006) because all the values are >0.3. However, in chapter 2 of this thesis, Figures 4a-d show the existence of very large variations in the experimental results for the germinal population centre dynamics. The use of this quantitative comparison using Equation 3 (Meyer-Hermann et al., 2006) may therefore be misleading because of this high degree of data variability and the relatively small number of data points. The following analysis assumes that the model at least reproduces the observed population dynamics and the analysis of the different selection metrics was therefore continued.

Table 2 – Germinal centre simulation results comparing selection metric

Selection Mechanism	Static	Average	Maximum
Fit with Figure 7a dataset using Equation 3	0.389	0.423	0.533
Fit with Figure 7b dataset using Equation 3	0.383	0.432	0.552

4.1 Quantitative Description of the Germinal Centre Simulations

This section presents various graphical representations of the germinal centre simulation that facilitate the comparison of the results of the static, average and maximum selection metrics. In all cases, the experimental data derived from Figure 7a (Meyer-Hermann, 2006) are referred to as Experimental Results 1 (Exp 1) with those from Figure 7b (Toellner et al., 1996, Toellner et al., 1998) being referred to as Experimental Results 2 (Exp2).

The results of the germinal centre simulation produces output files that are then used to generate plots by reading them into a statistical analysis program called R (Team, 2008). R has the ability to automate the analysis of multiple datasets and generate summary it uses a text based input that can be automated using a script file and this has been included on the attached DVD. The resultant plots are analysed in the following section with emphasis on the comparison of each selected metric.

4.2 The Germinal Centre Population Plot

Figure 10 represents a log-normal plot of the total number of cells inside the germinal centre against time during the germinal centre reaction for the ten repeat simulations. Note that the population increases markedly in the first 96 hours at which time the centroblast-to-centrocyte differentiation signal is received. Thereafter the number of centroblasts decreases at the expense of the centrocytes. At a later stage the population decrease is halted and stabilise for approximately 10 hours, after which it again starts to increase, but at a slower rate. This further increase indicates that the selected centrocytes are starting to recycle back into centroblasts that repopulate the germinal centre. However, centroblasts will continue to

differentiate into centrocytes at a fixed rate, again slowing the rate of increase. Note that the average population number continues to increase slightly beyond 192 hours indicating that equilibrium is not attained. Additional information on the nature of these inflection points for the three selection metrics (static, average and maximum) is summarised in Figure 11.

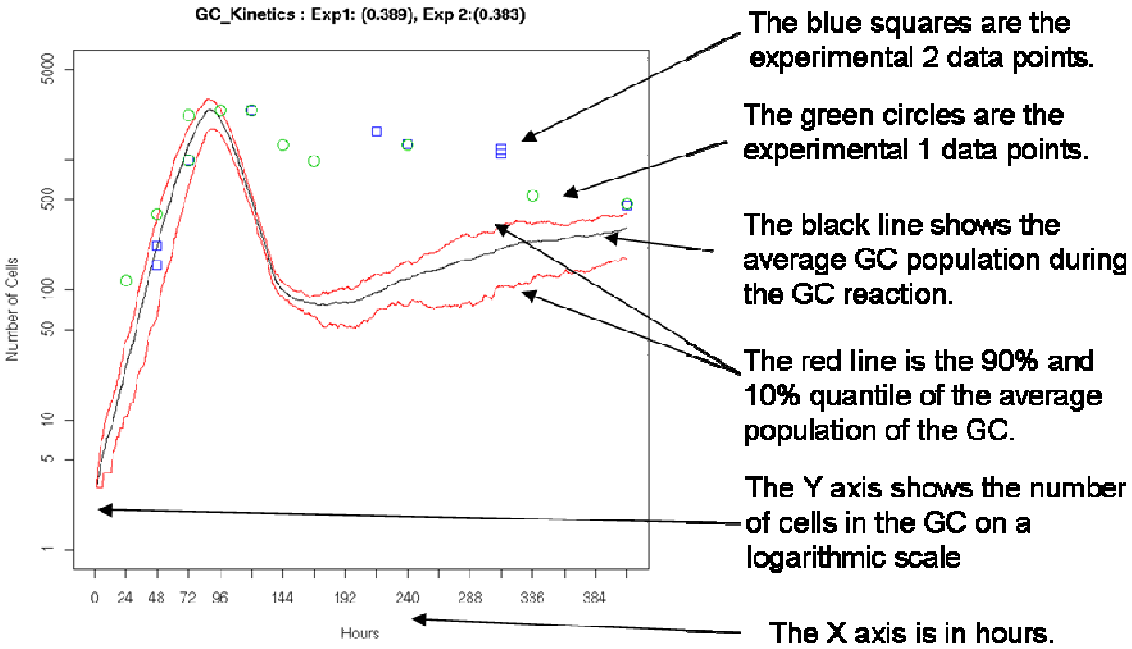

Figure 10: A log-normal plot showing the average population dynamics of the germinal centre for the static model. The circles and squares show the experimental data points. The 10% and 90% quantile of the number of cells are shown in red. The average population number is shown in black. The Exp 1 and Exp 2 values show the resultant value from Equation 3 the data from Figure 6a and 6b.

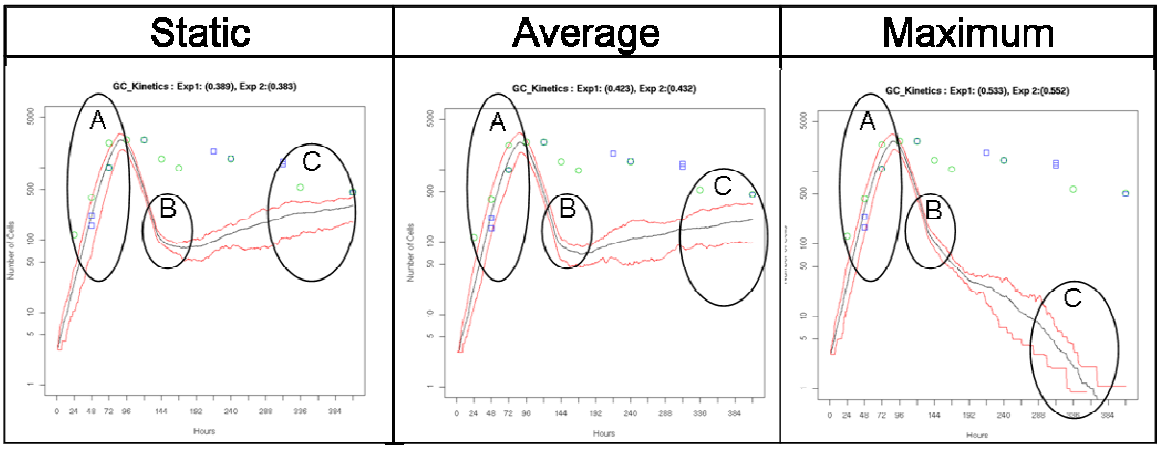

Figure 11: A summary of the average log-normal population dynamics for the static, average and maximum selection metrics. Circle A shows the rapid expansion of the centroblasts that fill the germinal centre space. Circle B shows the rapid drop in population size as the centroblasts begin to differentiate into centrocytes. Then circle C for static and average selection metrics has the population stabilised with a high degree of variation. The maximum results for circle C shows the germinal centre declining as the antibodies have reached the maximum affinity threshold and the germinal centre declines rapidly.

Figure 11 compares the population dynamic for the static, average and maximum selection metrics. The static and average selection metrics produce very similar dynamics whereas the maximum selection metric yields a different result. All three simulations initially show a very similar rapid rate of increase in the total number of cells (circled areas A). This is because only centroblast division occurs at this stage in the germinal centre reaction thus matching the observed experimental behaviour. The total population of cells then starts to decline rapidly (circled areas B). This indicates that the centroblasts have started to differentiate into centrocytes, thus reducing the number of centroblasts available for division. This could be explored further by investigating the effect of reducing the time taken for the centrocytes to be successfully selected and recycled back into a centroblasts, but was beyond the scope of the current study. Note that after this point (circled areas C) the static and average selection metrics for the total population increase whereas that of the maximum selection metric decreases.

The decline in the population generated by the maximum selection metric results the threshold value being higher than the current antibody affinity values for the centrocytes. Consequently, no centrocytes can be selected and the total population goes into terminal decline. This behaviour is highlighted more clearly in the later individual centroblast and centrocyte population plots.

4.3 Population Dynamics of the Output Cells

The current model, records the affinity value and the time at which the output cell leaves the germinal centre. Figure 12 shows the average number of output cells that are created in the germinal centre. These results cannot be actually verified because the total number of output cells does not reflect the situation in the biological germinal centre. The output cells are a sub-population of pre-plasma blasts and memory B cells and as the pre-plasma blasts mature, they secrete large numbers of higher affinity antibodies. This results in outputs cell having the potential to create large number of new B cells from a relatively low number of output cells.

Examination of Figure 12 shows that only output cells are present at approximately 136 hours. This is a consequence of the first 96 hours during which no centrocytes are present to undergo selection to become output cells. After 96 hours the centroblasts are able to differentiate into centrocytes. However, there is then a further delay that is associated with the time it takes centrocytes to undergo selection before becoming an output cell are responsible for large delay. After this point there is a steady increase in output cells.

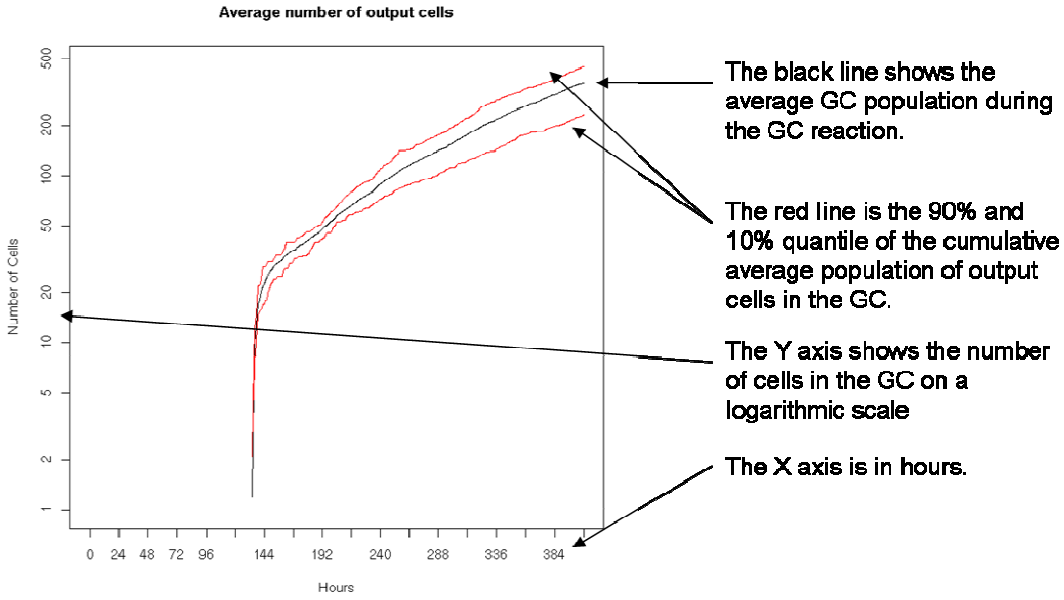

Figure 12: A log-normal cumulative frequency plot illustrating the average number of output cells from the static model. The black line shows the average number of output cells. The red lines show the 10% and 90% quantile of the average number of output cells.

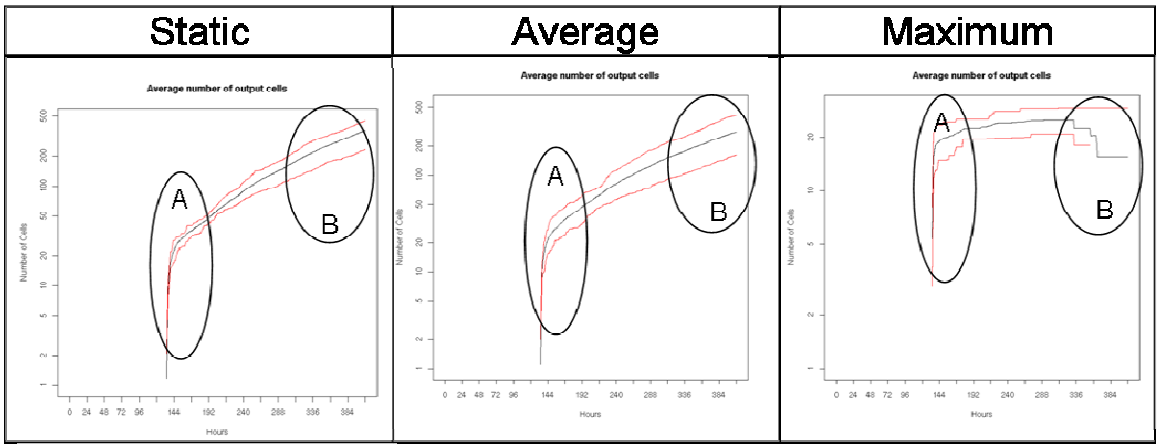

Figure 13: Summary of the number of output cells for the static, average and maximum simulation runs.

A comparison of the three different output cell populations in Figure 13 again shows that the static and average selection metrics have similar dynamics as shown in circled areas A. However, the maximum metric yields fewer output cells than the static and average selection metrics. The maximum metric in circled area B in (Figure 12) is an artefact as resulting from situations in which some of the repeated simulation run have not data for these time points. This results in the calculation an incorrect average at these time points.

The continued disparity between the results for both the static and average selection metric and the maximum selection metric is still evident because the maximum selection metric germinal centre terminates before the static and average selection metric models. This is because no more centroblasts are present to sustain the germinal centre reaction.

4.4 Population Dynamics of the Dark and Light Zones

The following section examines the behaviour of the cells in the different compartments of the germinal centre. The dark and light zones used in the static, average and maximum selection metrics are of equal volume because their lengths are set at the radius of the germinal centre. Figure 14 shows the log-normal plot of the total number of cells in the dark and light zones. The dark zone corresponds to the region in Figure 1 that should be occupied largely by centroblasts whereas the light zone corresponds to the region that should be occupied largely by centrocytes.

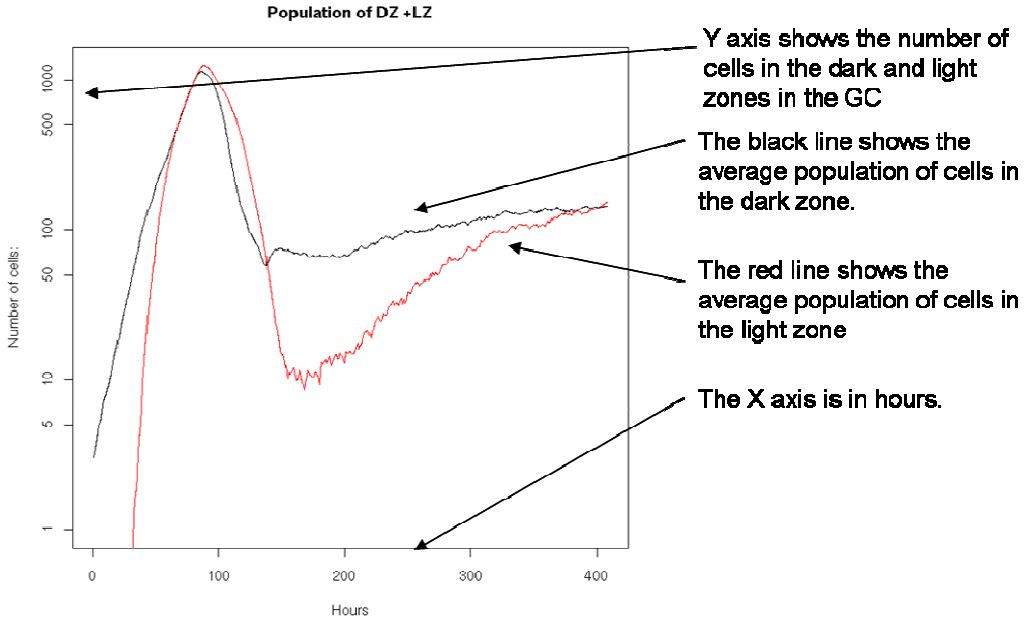

Figure 14: A log-normal plot illustrating the average population of B cells inside the dark and light zone regions inside the germinal centre for the static model. The black line shows the average population of the dark zone. The red line shows the average population of the light zone.

Examination of Figure 14 shows that the dark zone population increases steadily up to 96 hours at which time the signal to commence differentiation is activated. After this point the population in the dark zone decreases rapidly. It is only after approximately 144 hours that the population in the dark zone stabilises and again starts to increase. The population of the light zone behaves differently in that it only begins to increase after approximately 48 hours. This then continues and the centroblast population increases to fill the entire germinal centre, reaching a maximum at 144 hours. Thereafter the population decreases rapidly showing that the centrocytes are either dying, being selected to become output cells or being recycled into centroblasts. After a further 10 hours, the population of centrocytes entering the light zone again begins to increase because of selected centrocytes being recycled back into centroblasts and then differentiating back into centrocytes.

Figure 15 illustrates similar plots to Figure 14, but in this case the results for the static, average and maximum selection metrics are shown. As before, the results for the static and average selection metrics yield a common response (circled areas A and B). One difference, however, is that the static selection method yields an equal population for the dark and light zones (circled area C).

The maximum selection metric results diverge around 144 hours after which both the dark and light zone populations decline rapidly after 150 hours confirming the previous conclusion that the germinal centre is in terminal decline.

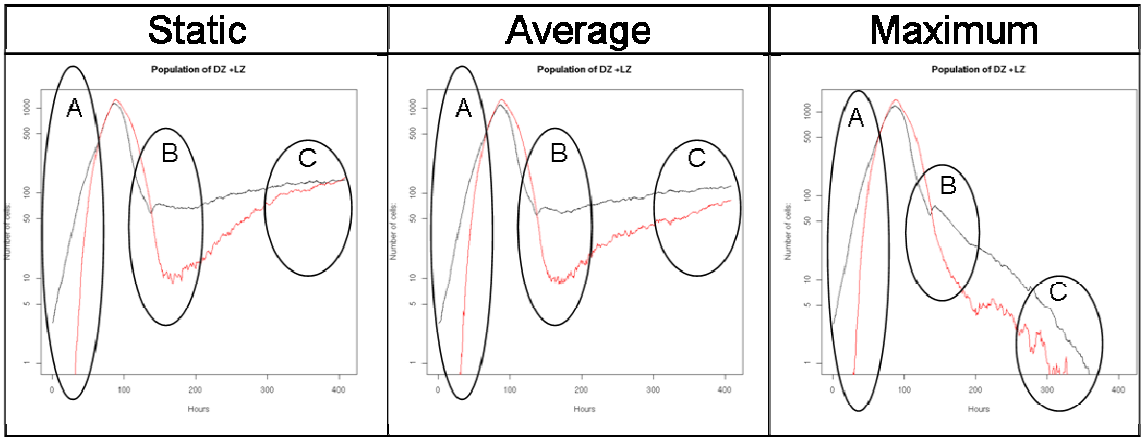

Figure 15: A log-normal plot illustrating the average population of B cells inside the dark and light zone regions inside the germinal centre for the static, average and maximum selection metrics. The black line shows the average population of the dark zone. The red line shows the average population of the light zone.

4.5 Population Dynamics of the Centroblasts and Centrocytes

Figure 16 summarises the dynamics of the centroblast and centrocyte cell populations during the germinal centre reaction. As in the previous plots, the centroblast population increases rapidly for as long as the start of differentiation signal is not active. The population reaches a maximum after which it declines rapidly as centroblasts differentiate into centrocytes. The centrocyte population begins to increase at this point, the centrocyte population reaches a maximum after which the centrocyte population begins to decline. This decline is caused by the removal of centrocytes that are not selected are then removed. The centroblast population stabilises at approximately 144 hours indicating that the selected centrocytes are beginning to be recycled back into centroblasts.

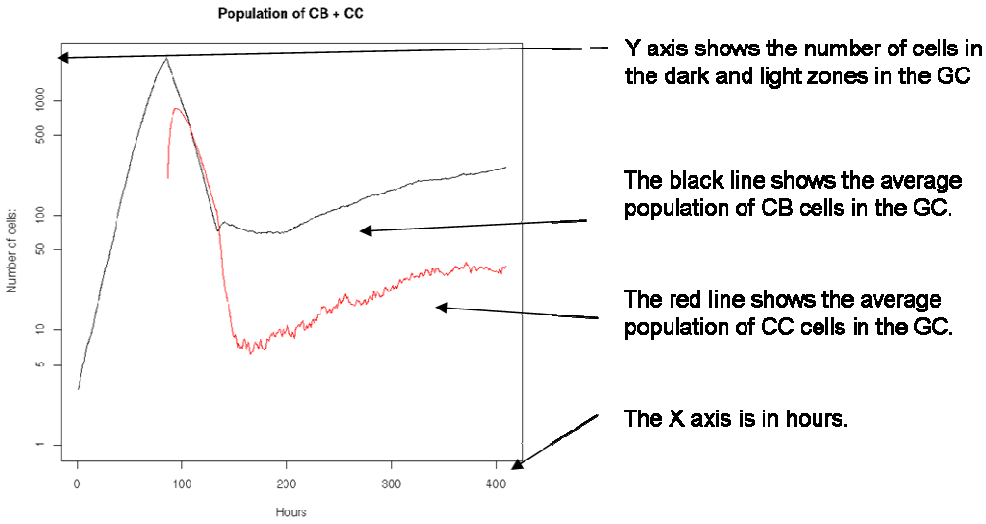

Figure 16: A log-normal plot illustrating the centroblast and centrocyte populations inside the germinal centre for the static model. The black line shows the average population of centroblasts (CB). The red line shows the average population of the centrocytes (CC).

The results for the grouped plot (Figure 17) again show that the static and average metrics yield similar results (circled areas A, B and C), namely and initial rapid increase to a maximum, followed by a rapid decrease that again changes to a gradual increase. In contrast, the maximum selection metric model shows a rapid decline(circled areas B and C).

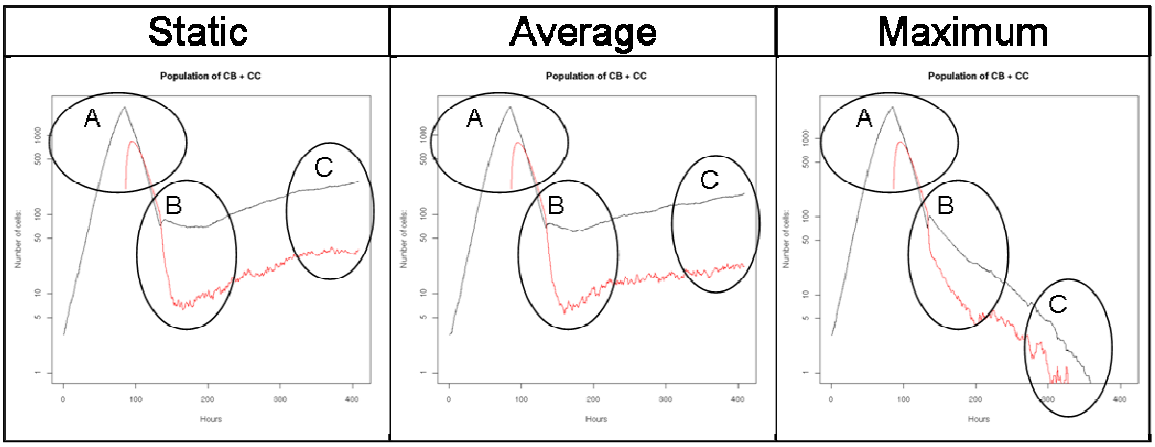

Figure 17: A log-normal plot illustrating the centroblast and centrocyte populations inside the germinal centre for the static, average and maximum models. The black line shows the average population of centroblasts (CB). The red line shows the average population of the centrocytes (CC).

4.6 Antibody Affinity Dynamics of the Centroblasts in the Germinal Centre

This section examines the antibody affinity dynamics of the centroblasts in the germinal centre. Figure 18 shows a series of box plots that illustrate the distribution of affinity values. The aim of this is to determine what effect changing the selection metrics might have on the behaviour of the germinal centre. The day 4 and day 6 box plots show similar results, but at day 12 (circled Area A) subtle differences in the maximum affinity value are evident. In this case, the maximum selection metric shows the presence of two high affinity outliers, whereas

the static selection metric has one and the average selection metric has none. However, the situation is reversed for the static and average selection metric at day 12 and day 15 (circled area B) because the average selection metric exhibits four high affinity outliers whereas the static selection metric shows the presence of two outliers. Unfortunately, by day 15 (360 hours) the maximum germinal centre is in decline and this is shown by the absence of any high affinity outliers. Another notable observation is that at day 12, the upper quartile of the maximum selection metric contains more centroblasts relative to the other two static and average selection metrics.

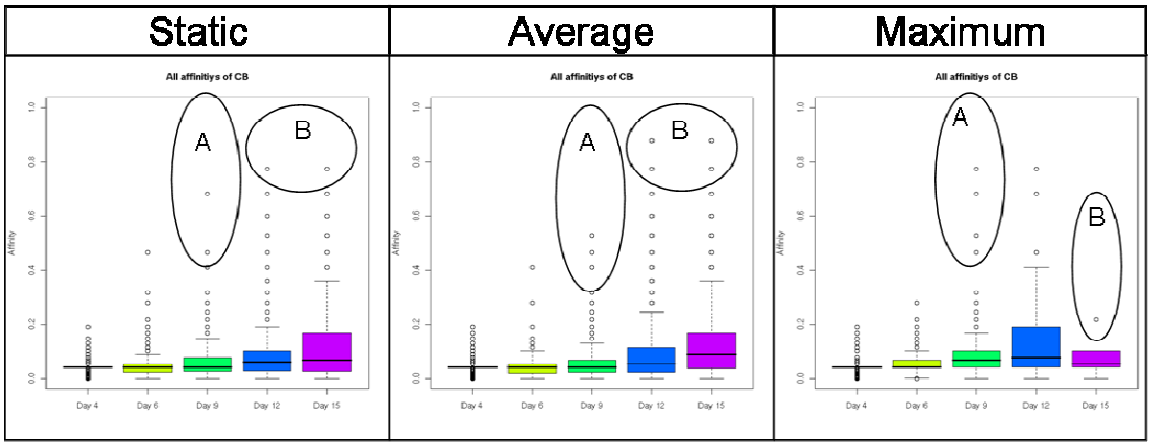

Figure 18: A box plot of the centroblasts antibody affinity value for the static, average and maximum selection models for Day 4, Day 6, Day 12 and Day 15.

4.7 Antibody Affinity Dynamics of the Output Cells

This section examines the antibody affinity dynamics of the output cells in the germinal centre. Figure 19 shows a series of box plots that illustrates the distribution of affinity values. The aim of this is to determine what effect changing the selection metrics might have on the behaviour of the germinal centre. The day 4 and day 6 box plots show similar results, but at day 9 (circled Area A) subtle differences in the maximum affinity value are apparent. In this

case, the maximum selection metric clearly shows that the average output affinity is very high. However, it is also possible that only a few output cells are present on this day because the box plot does not show any significant range. At day 9 the static selection metric now exhibits the lowest average population distribution when compared with the average selection metric that shows a higher average value (circled area A). At day 12 and day 15 (circled area B) the average value for the maximum selection metric is lower indicating that some output cells are still being generated. However, in general, the static and average selection metrics box plots show that the average method is slightly higher than the static method when the outliers are taken into consideration. Limited differences exist between the affinity dynamic of the static and average select metrics. However, the maximum selection metric is very different because far fewer output cells are available to produce accurate box plots.

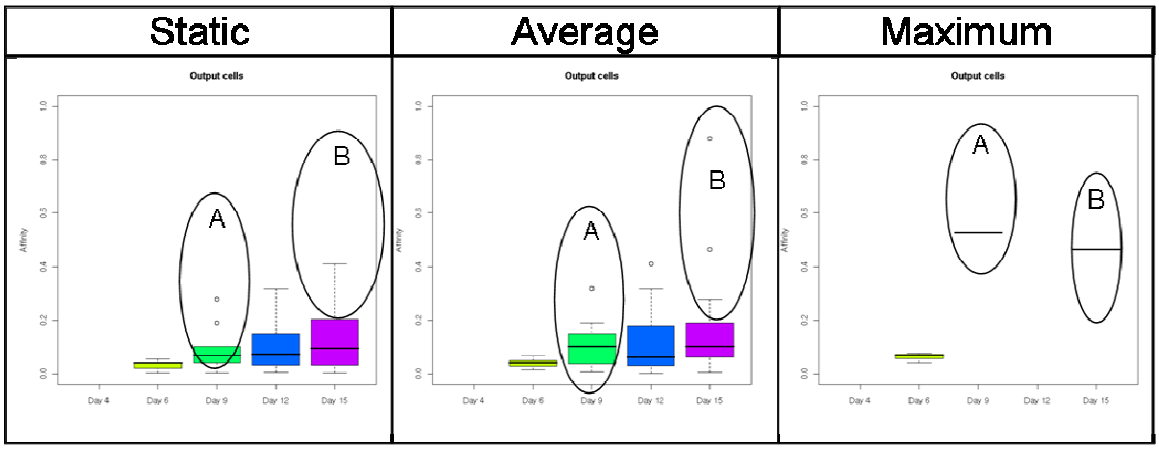

Figure 19: A box plot of the output cells antibody affinity value for the static, average and maximum selection models for Day 4, Day 6, Day 12 and Day 15.

4.8 Relative Speeds of the Centroblasts in the Germinal Centre

The simulation records the number of times a B cell moves in the germinal centre as shown in Figure 20. The model stores the total number of movements and the time period for which the B cell is a centroblast. This information can then be used to calculate the speed of the B cells because the distance that the centroblast has moved as well as the time that the cell exists as a centroblast have been recorded. The centroblast is configured to move 0.2 times within the permitted 10µm/min. The speed of the majority of cells is close to the 2µm/min average speed. A few cells move faster than this and a few cells appear to not move. This suggests that there is some resistance, that effects movement in the germinal centre. The observed average speeds for the B cells *in situ* are only representative for the specific periods during which they are observed in the germinal reaction. These results show the average speed for the whole simulated period.

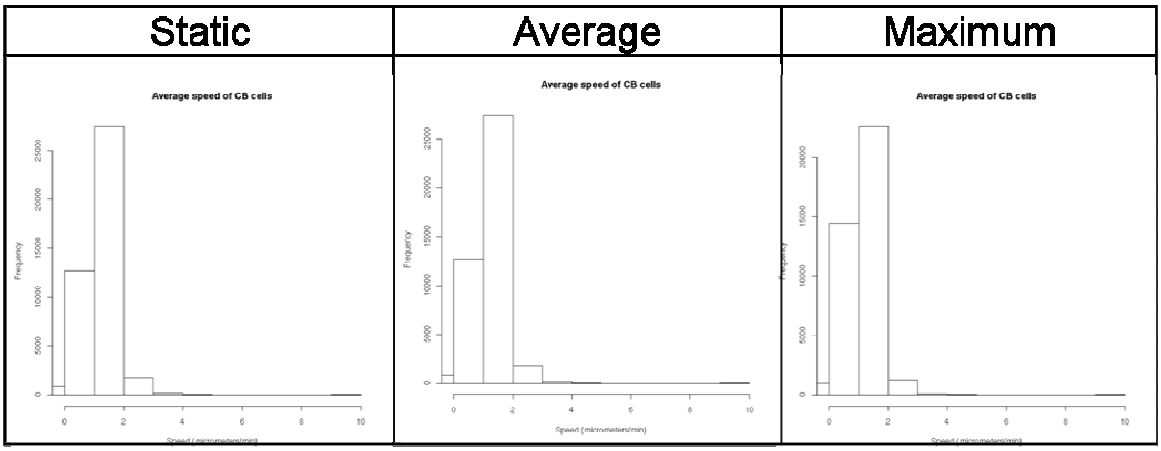

Figure 20: Comparison of the histograms of the average centroblast speed in the static, average and maximum selection metrics. The frequency of the centroblast that move at these specific average speeds and the number ranges between zero and 25000. The x axis ranges from 0 to 10µm/min.

4.9 Relative Speeds of the Centrocytes in the Germinal Centre

The centrocytes have been observed to have an average speed of 5μm/min (Suzuki et al., 2009). The majority of centrocytes shown in Figure 21 move at an average speed of approximately 3.5μm/min. This figure, however, includes the time that the centroblasts are immobile whilst in contact with the follicular dendritic and T cells. This will have the effect of reducing the recorded average speed. The program does not include an option not to count the time that the centrocyte is in contact with the follicular dendritic cell and T cells. The addition of this step was beyond the scope of the current program development. The histograms in Figure 21 show that the centroblasts are highly mobile and are able to reach speeds close to their average experimentally observed speed. The speed profiles are also similar across all the selection metric models. The centrocytes in the static selection metric are slower than both the average and maximum selection metric even though there are fewer centroblasts in the maximum selection metric. The speed of the centrocyte may be linked to other dynamics of the germinal centre that have not yet been observed.

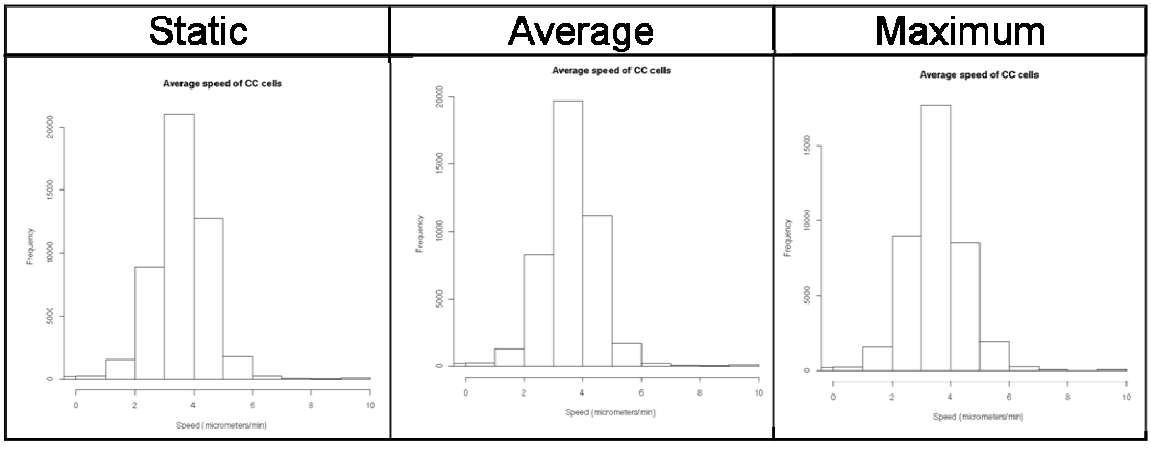

Figure 21: Comparison of the histograms of the average centrocyte speed in the static, average and maximum selection metrics. The frequency shows the number of centrocyte that move at these specific average speeds and the number is in ranges of zero to 25000. The x axis ranges from 0 to 10μm/min.

4.10 Results from the Cell Division and Recycle Counter

Figure 22 shows the number of divisions that the centroblasts have undergone during the germinal centre reaction. When the selected centrocyte becomes a recycled centroblast, the division counter is incremented. This results in an observed division rate that is larger than 12. This enables the recycled cells in the histogram to be filtered otherwise the graph would only show a number of division between 0 and 12. The recycled centroblasts can also undergo a further potential 12 divisions. However, the number of times a selected centrocyte can be recycled has been set to five times to ensure that the selected centrocytes do not become cells are not stuck in a continual loop of differentiation, selection and recycling.

The results from the static selection metric (Figure 22) show a maximum frequency of division of approximately 12, indicating that the initial centroblasts are able to divide a full 12 times before the onset of differentiation. The drop in frequency after this point suggests that only a small proportion of the selected centrocytes are recycled and are then able to undergo further rounds of division and mutation. The results also show that in the maximum selection metric, the number of divisions after the first round of selection is negligible in comparison with that of the static and average selection metrics.

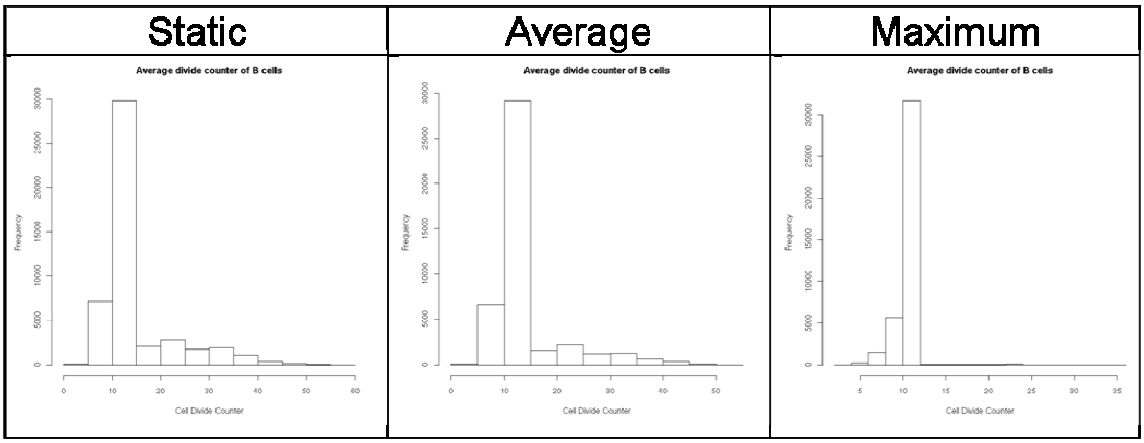

Figure 22: Comparison of the histograms of the divide counter of the germinal cells in the static, average and maximum selection metrics simulations.

The recycled counter histogram (Figure 23) shows that the centrocytes are restricted to recycling up to five times. Each selection metric shows that the majority of centroblasts do not pass the first round of selection because they have a recycled counter of zero. This indicates that a large proportion of centrocytes either die or become output cells. The only notable difference is that the maximum selection metric only has centrocytes that do not recycle more than once whereas the static and average selection metrics both have centrocytes that recycle the full five times that are allowed before becoming an output cell.

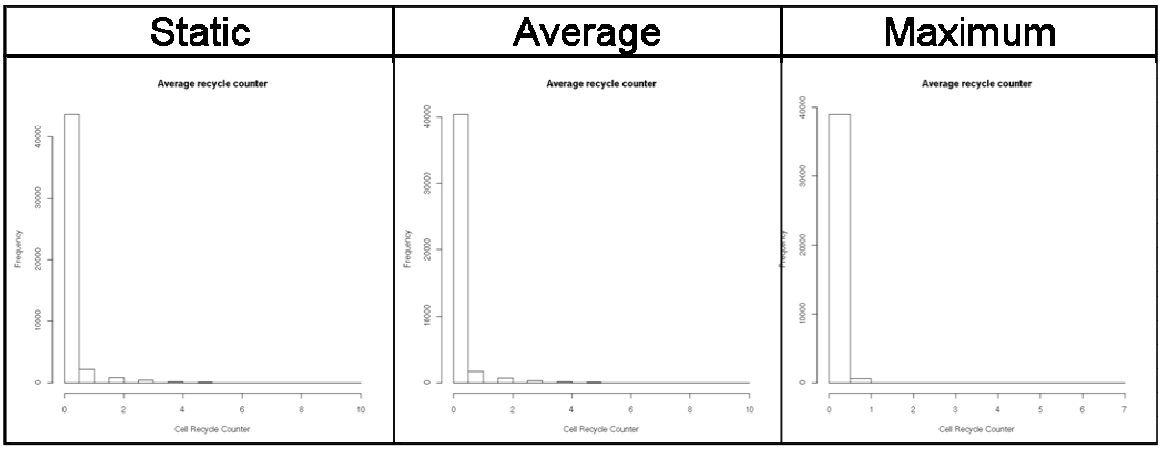

Figure 23: Comparison of the histograms of the recycle counter for the B cells in the germinal centre.

4.11 Conclusion

This chapter has focused on and examination of the structure and behaviour of the B cells in the germinal centre model. This shows that the model is able to reproduce germinal centre dynamics that are similar to those of the experimental data. It also shows how a lattice-based approach can be used to model the germinal centre. The approach that has been outlined so far can be adapted to model other systems in which the movement of B cells performs an important role. Figure 24 shows a sequence of screen shots of the germinal centre simulation program at various stages of the reaction.

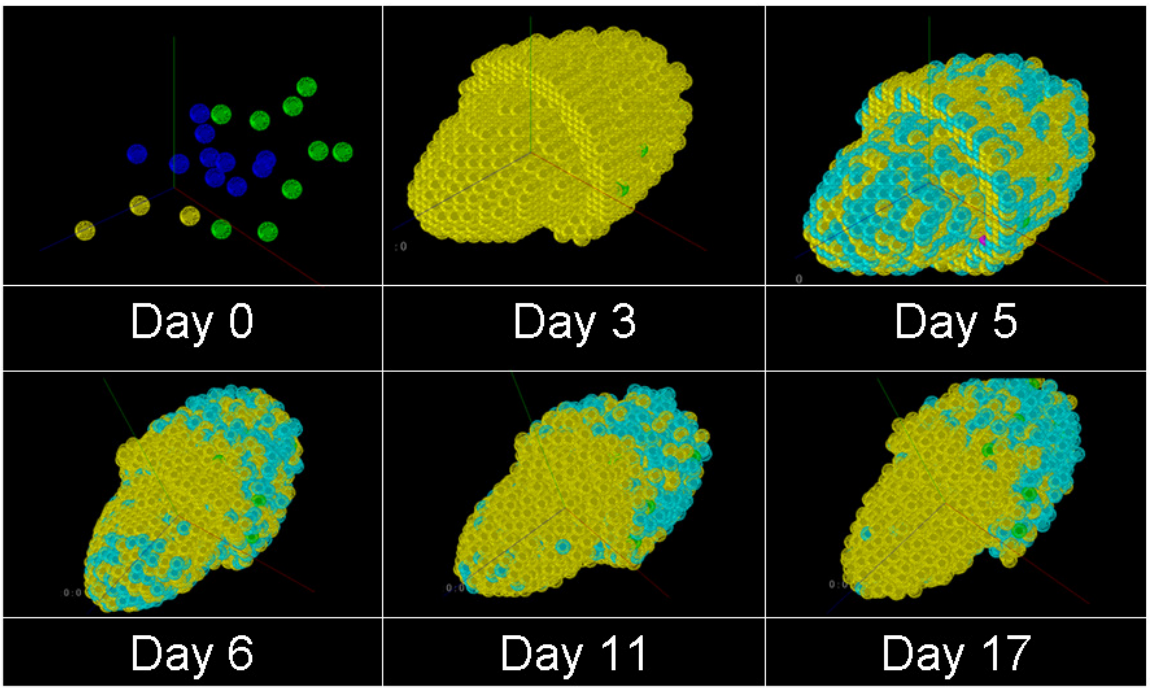

Figure 24: Screen shots of the germinal centre on different days. The yellow cells are the centroblasts, cyan is the centrocytes, blue is the follicular dendritic cell and green are the T cells. Note that from Day 6 a dark zone and light zone can be observed.

Subsequently, a broad range of parameters was tested to identify optimal parameters using the fitness function (Equation 3) to guide the selection of parameters before undertaking a detailed analysis of the source population dynamics. This analysis of the source data indicates that it might not be valid to use the fitness equation (Equation 3) to drive the selection metric. Consequently, these results are omitted. Further analysis, including the extraction of more data to fit the model, was beyond the scope the current thesis.

CHAPTER 5 -CONCLUSION

The results have shown that additional experimental work is required to generate additional data to optimise the germinal centre model. The current study has also raised the possibility that optimisation of the parameters using Equation 3 to determine the optimum parameters is not valid. This is because the limited number of data points and the wide variation between the experimental data sources available for this study suggest that they are not an appropriate metric against which to compare the simulation results. The germinal centre parameters used in this model obviously need to be optimised when appropriate data become available in the future.

The results shown in the previous chapters have validated the lattice-based modelling approach used to model the germinal centre. The simulation tool that was developed is therefore able to represent a system in which B cells can follow chemotactic gradients, divide and interact with other cell types.

Currently there are few relevant experimental data available that can be used to test whether the developed model is biologically reasonable. These are data on population dynamics that have been acquired in animal models of primary immunization (mouse and rat) and more recent data describing the migratory dynamics of individual cells in the germinal centre. The current model fits the available dynamics using parameter values that are within a reasonable biological range and suggest that a shorter contact time between centrocytes and follicular

dendritic cells may be more realistic. These results are in accordance with recent experimental evidence from two-photon microscopy studies (Schwickert et al., 2007).

The primary aim of the current project was to determine whether the dynamics of the affinity maturation of B cells changes when the selection metric is also changed. The selection method used to calculate the binding affinity of a B cell antibody is shown in Equation 1. This approach measures the distance between the current antibody and the perfect antibody and has an associated value of between zero and one. In the existing model, however, the probability of binding with the antigen remains fixed for the duration of the maturation of B cells in the germinal centre. This thesis suggests an alternative method in which feed-back from the plasma cells produced from B cells that successfully underwent affinity maturation in the germinal centre now produce large amounts of higher affinity antibodies. These higher affinity antibodies displace the initial antibodies in the antigen-antibody complexes currently located on the follicular dendritic cells. This concept was previously suggested as a mechanism for terminating the maturation of B cells in the germinal centre reaction (Iber, D. and Maini, P. K. 2002). In the current thesis two different feedback mechanisms were investigated using the average and maximum affinity value of the population of output cells in the model as a dynamic threshold that changes during the germinal centre reaction. The average model produced results similar to those of the Meyer-Hermann et al., 2006 model. In the maximum threshold model, the population dynamics changed dramatically and terminated the germinal centre reaction after reaching the highest affinity of 1.0 in Equation 1. A more realistic affinity model can probably be used to examine the interaction between the antigen and antibody of the B cell undergoing selection to become an output cell. This is because the feedback mechanism rapidly reaches the maximum possible affinity in a biologically

unrealistic time. However, the time required could be increased if the affinity model rate of improving affinity was slowed to be closer to the observed biological time scale.

The current model has only tested simple rules that are an over-simplification of the competition between the antibody on the surface of the centrocyte and the antibody in the immune complexes on the surface of the follicular dendritic cells. By considering a threshold that is computed as an average of the antibodies secreted by output cells biologically reasonable results are obtained. However, a more complex model of antibody competition should be implemented. The most obvious first step would be the development of a Michaelis Menten (Rakesh et al., 2008) based model that at least represents the formation of an antibody-antigen complex. This would validate the dynamic feedback of antibodies from the output cells because the affinities of the produced antibodies would be directly related to a more realistic selection metric that compares the interaction between antigen and antibodies that does not use a model that excludes the antigen from the selection metric.

The model has provided further evidence for the hypothesis in which the antibodies in the immune complexes are updated during the germinal centre reaction and this mechanism then drives the increase in affinity maturation of the B cells. This is realistic only in case where antibodies on the immune complex are in equilibrium with the newly produced antibodies. In an experiment examining the primary immune response in mice, our collaborators (Toellener, unpublished) have recently demonstrated that within a time frame predicted by our model, lower affinity antibodies present at the onset of the germinal centre reaction have been replaced by other antibodies (Figure 25).

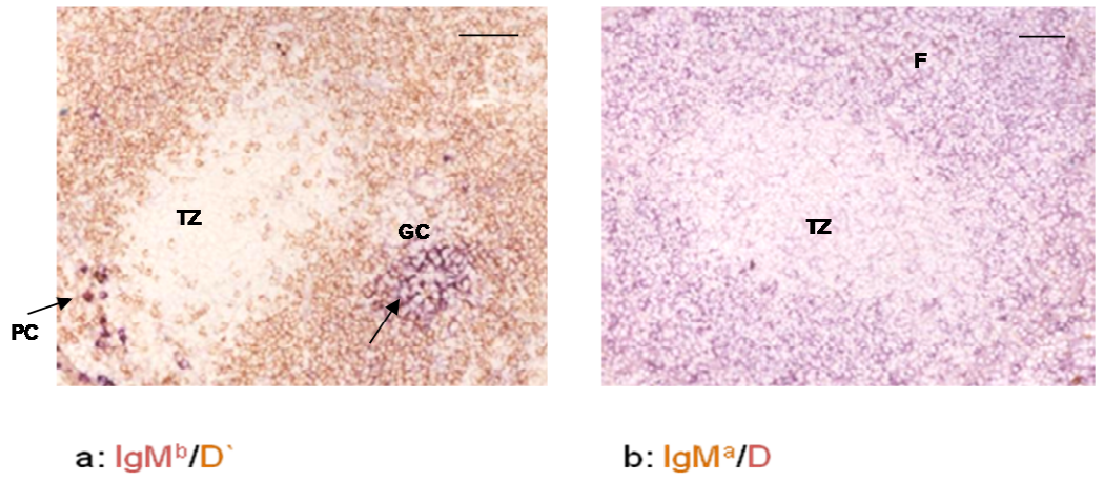

Figure 25: Replacement of antibody with newly produced antibody during a germinal centre reaction. C57BL/6 mice (IgHb) were immunized with NP-CGG plus anti-NP IgMa immune complex. Two days after appearance of germinal centres IgMa is not detectable anymore in germinal centres (Fig. 25a) and has been replaced by endogenous IgMb (Fig. 25b). Dual colour immunoenzymatic staining of frozen spleen sections. F: follicle, GC: germinal centre; TZ: T zone, PC: IgMb producing plasma cells.

Unfortunately, direct proof that the developed dynamic threshold selection model is biologically valid, is not yet available. For example, data on the affinity of cellular clones within the germinal centre is required to verify that the predicted increase in affinity commensurate with the developed model is consistent with the affinities of the real germinal B cell populations. However, Figure 25 shows that the assumption of a static selection metric that does not take into consideration a change of the antibody affinity in the immune complexes on the follicular dendritic cell, is no longer valid. This is because the starting antibodies in the immune complexes on the follicular dendritic cells are no longer present after two days post-immunisation.

In conclusion, the work described in this thesis has lead to the development of a new modelling framework for the germinal centre. This has the flexibility to model new biological systems and may be extended to address other questions related to the biology of the germinal centre.

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