GENOMIC AND MOLECULAR ASPECTS OF MATURE HUMAN B CELLS

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Abstract

This thesis covers aspects of the mature human B cell and its different guises on its way to become a fully differentiated antibody forming plasma cell (AFC), the key player in the humoral immune system. The understanding of the complex molecular mechanisms taking place in the B cell, as it first encounters antigen, enters the germinal center (GC) reaction, giving rise to memory cells and plasma cells, is not only of importance for the curious scientist, but will also benefit the understanding of B cells in disease. For instance, what is the cause of B cell lymphomas and what role does B cells play in the etiology of autoimmunity? These questions and many more will be greatly facilitated by gaining knowledge of the molecular events influencing healthy B cells. This thesis is based on four original papers (I-IV) which deals with several different aspects of the mature human B cell. In paper I we looked into the properties of CD44 signaling and its role in B cell activation. Naive B cells isolated from human tonsils and sorted by FACS, were cultured in vitro for 3 days, stimulated with anti-BCR, and anti-CD40 stimuli in condition one and with an additional anti-CD44 stimuli in condition two. B cells, sampled at 6, 24 and 72 hours from each condition were analyzed by high-density oligonucleotide microarray analysis. The results demonstrated a role for CD44 in immunomodulation and inflammation, since transcripts for molecules such as IL-6 and IL-1alpha were specifically up-regulated by CD44. In paper II, by using high-density oligonucleotide microarrays, we analyzed mantel cell lymphomas (MCL) in relation to normal mature B cell subsets comprising naive, pre-activated, germinal center and memory cell subsets. From this analysis we could conclude that the malignant transformation event of MCLs appears to be an event occurring during the transition from a primary B cell follicle to a germinal center. In paper III we looked closer into what differs between human GC centroblasts and centrocytes isolated by FACS. By using high-density oligonucleotide microarray analysis and flow cytometry we concluded that the current phenotypic description of the different GC B cell subsets is misleading and that the centrocyte population represents a heterogeneous subset of cells comprising centroblasts, centrocytes and plasmablasts. In paper IV we extended our analysis of the mature tonsillar B cell subsets from paper II and III to also include plasmacells, GC founder cells, sub-epithelial cells and GC B cells described by other means. The microarray analysis grouped cells into three main categories: proliferating, non-proliferating and plasma cells. Cell type specific transcription programs were characterized.

Key words: B-cell, human, germinal center, centroblast, centrocyte, mantel cell lymphoma, microarray

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"In photography, the smallest thing can become a big subject, an insignificant human detail can become a leitmotiv. We see and make seen as a witness to the world around us; the event, in its natural activity, generates an organic rhythm of forms... Our eye must constantly measure, evaluate. We alter our perspective by a slight bending of the knees; we convey the chance meeting of lines by a simple shifting of our heads a thousandth of an inch"

Henri Cartier-Bresson

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Lastly to my dearest Lena, thank you for all your love and for being so patient with me!

Contents

Original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numeral (I-IV). All published material is reproduced with permission from the publishers.

I Högerkorp CM, Bilke S, Breslin T, Ingvarsson S, Borrebaeck CAK.
CD44-stimulated human B cells express transcripts specifically involved in immunomodulation and inflammation as analyzed by DNA microarrays.
Blood. 2003 Mar 15;101(6):2307-13.

II Ek S, Högerkorp CM, Dictor M, Ehinger M, Borrebaeck CAK.
Mantle cell lymphomas express a distinct genetic signature affecting lymphocyte trafficking and growth regulation as compared with subpopulations of normal human B cells.
Cancer Res. 2002 Aug 1;62(15):4398-405.

III Högerkorp CM and Borrebaeck CAK.
The human $CD77^{-}$ germinal center B cell population represents a heterogeneous subset of cells comprising centroblasts, centrocytes and plasmablasts, prompting for a phenotypic revision.
Submitted for publication 2005

IV Högerkorp CM and Borrebaeck CAK.
Whole-genome transcriptional profiling of naïve, germinal center, memory, plasma and sub-epithelial tonsillar B Cell subsets.
Manuscript to be published

Abbreviations

AFC	Antibody forming plasma cell
APC	Antigen presenting cell
BCR	B cell-receptor
BER	Base excision repair
CDR	Complementary determining regions
CH	Heavy chain constant region
CLP	Common lymphoid progenitor cell
CSR	Class switch recombination
dsDNA	Double stranded DNA
FACS	Fluorescent activated cell sorting
FDC	Follicular dendritic cell
GC	Germinal center
HEV	High endothelial venules
HR	Homologous recombination repair
HSC	Hematopoietic stem cell
IC	Immune complex
IDC	Interdigitating dendritic cell
Igs	Immunoglobulins
LT	Lymphotoxin
MALT	Mucosa-associated lymphoid tissues
MMR	Mismatch repair
MZ	Marginal zone
NHEJ	Non-homologous end joining DNA repair
PNA	Peanut agglutinin
RAG1	Recombination activating gene protein 1
SCID	Severe combined immunodeficiency syndrome
SHM	Somatic hypermutation
SLC	Surrogate light chain
ssDNA	Single stranded DNA
TD	T cell dependent
TFH	Follicular helper T cell
TI	T cell independent
V(D)J	Variable, diversity, joining
VH	Heavy chain variable region

NB. Several gene names are represented in the text as acronyms, which may or may not have been spelled out in the text. The gene names used are in accordance with the HGNC nomenclature which is commonly used in several publicly available databases such as the National Center for Biotechnology Information (NCBI) database Entrez Gene (Maglott, Ostell et al. 2005) available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi

1. Introduction

In our everyday life we are usually not aware of that we are constantly fighting a battle against a harsh and hostile environment, which surrounds us. Although this assertion seems somewhat exaggerated, it is in fact a reality, and a reality that is ever more perceptible when your immune system is at fault. For example, individuals suffering from severe combined immune deficiency syndrome (SCID) die at young age if they are not treated by bone marrow transplantation. These individuals lack several essential parts of a competent immune system and are therefore unprotected against virus, bacteria and other pathogens. Consequently, a simple infection will be fatal to these individuals (Buckley 2004).

Our defense against exogenous pathogens requires a wide range of different cells and secreted molecules, all crucial components of our immune system. Based on function the immune system can be divided into several different branches. At the first level we talk about innate and adaptive immunity, of which the latter is further subdivided into humoral and cellular mediated immunity (Figure 1).

The innate immune system includes the most basal components of immune protection, such as the physical barriers of the skin, the mucosal surfaces of the respiratory and gastrointestinal passages as well as the chemical barrier of the gastric juice. The all-familiar fever is another example of the more basal innate protection measures. Innate immunity also comprises a cellular and molecular defense, where the actions of macrophages, natural killer (NK) cells, granulocytes etc. together with components of the complement system obliterates pathogens. This is mainly achieved by phagocytosis or cytotoxic killing and is regulated by a vast amount of, so called, pattern-recognition receptors, recognizing evolutionary conserved pathogen-associated molecular patterns (PAMPs), which can be lipopolysaccharides, unmethylated CpG DNA or double stranded RNA from the pathogenic microbe (Medzhitov 2001; Janeway and Medzhitov 2002). The innate immune system thus constitutes the first line of defense to invading pathogens.

The adaptive immune system, on the other hand, handles more complex tasks, where it is required to establish an immune response not limited by conserved PAMPs. The basis for the adaptive immune system is the generation of highly specific antigen receptors within T and B cells (known also as T and B lymphocytes). Since this immune response has to adapt to the specific target, it is not as rapid as the innate immune system. Nonetheless, the clear benefit with the adaptive immune system is also the establishment of an acquired immunological memory towards the antigen, and upon a second immunological challenge this immunological memory mobilize a fast and highly efficient response.

The adaptive immune system can further be subdivided into a cellular and humoral part. The cellular immunity involves various antigen-specific T cell effector functions, ranging from CD8+ cytotoxic T cells, targeting intracellular pathogens such as virus and intracellular bacteria, to CD4+ T cells, which will, depending on the type of antigen, differentiate to a number of various effector subsets, with a primary role to secrete cytokines such as IFNγ (secreted by TH1), IL-4 (secreted by TH2) or IL-21 (secreted by TFH). The cellular immunity is also involved in cell mediated immuno-regulatory functions mediated by the regulatory T cells, secreting IL-10. The humoral immunity is dependent on

B cells and encompasses the secretion of antigen-specific immunoglobulins (Igs) targeting the pathogen.

Although the immune system can be clearly divided into different subsystems, they are by no means segregated in function. The shaping of both the cellular and the humoral immune response is dependent on the innate immune system, and a mutual collaboration between all these subsystems is needed for an efficient eradication of the pathogen.

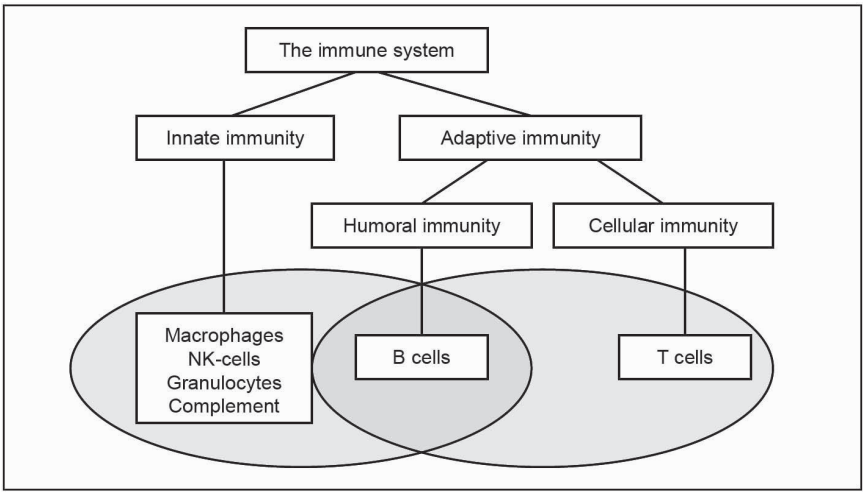

Figure 1. Conceptual outline of the immune system.

This thesis covers aspects of the mature human B cell and its different guises on its way to become a fully differentiated antibody forming plasma cell (AFC), the key player in the humoral immune system. The understanding of the complex molecular mechanisms taking place in the B cell, as it first encounters antigen, enter the germinal center (GC) reaction, giving rise to memory cells and plasma cells, is not only of importance for the curious scientist, but will also benefit the understanding of B cells in disease. For instance, what is the cause of B cell lymphomas and what role do B cells play in the etiology of autoimmunity? These questions and many more will be greatly facilitated by gaining knowledge of the molecular events influencing healthy B cells.

In the following chapters I will briefly go through some of the decisive events in B cell development, covering both antigen-independent and dependent phases as well as some more detailed aspects of the somatic hypermutation (SHM) and class switch recombination (CSR) events occurring in the GC. Some views on malignant transformation of B cells will also be given, although, this has not been a major focus of the works in the thesis.

2. B cell development

The pioneering work by Edward Jenner in the late 18th century, exploring the principles behind vaccination and the use of cowpox inoculations to protect against smallpox, is recognized as one of the most significant breakthroughs in modern medicine. This discovery was the dawn of the field of immunology and had bearing on the whole medical community at the time (Jenner became an honored fellow of the Swedish Medical Society in 1813). Jenner's discovery illustrated how acquired immunity could be exploited for the benefit of our health, a concept which was further explored by Louis Pasteur in the 1880s, who also inaugurated the term *vaccine* for prophylactic inoculations of attenuated virus. The first evidence of what we today know of as humoral immunity, and which is taken advantaged of at vaccinations, was presented by Emil von Behring and Shibasaburo Kitasato who demonstrated that non-cellular serum components, designated antitoxins, taken from immunized animals confer immunity to unimmunized animals (Behring and Kitasato 1890; Behring and Kitasato 1991). Today we know that these "antitoxins" are antigen-specific Igs; generally known as antibodies. These antibodies, as Frank Macfarlane Burnet proposed in his clonal selection theory (Burnet 1959), are derived from discrete B cell clones and represents the outcome of one unique antigen-specific B cell, which has responded to antigen. Thus, the key player in the humoral immunity is the B cell, giving rise to antibodies.

2.1 Antigen independent B cell development

The early B cell developmental pathway has for the most part been explored in mice, because of limitations in appropriate and biologically relevant experimental systems for human B cell development (Kondo, Wagers et al. 2003). Like all cells in the adult hematopoietic system, B cells are derived from multipotent hematopoietic stem cells (HSC), residing in the bone marrow. As the HSC differentiates it gives rise to different lineage-committed progenitor cells, which for lymphocytes is represented by the common lymphocyte progenitor (CLP) (Kondo, Weissman et al. 1997). In humans this precursor cell is phenotypically characterized as Lin-CD34+CD38+CD10+ (Galy, Travis et al. 1995). From the CLP stage the B cell enters an antigen independent differentiation pathway, which takes place in the bone marrow. Contributions to the understanding of early B cell development has been made in mice, by the groups of Hardy (Hardy, Carmack et al. 1991) and Rolink and Melchers (ten Boekel, Melchers et al. 1995). Since these groups have delineated the different developmental stages somewhat differently, this has created some confusion in terms of nomenclature (Hardy vs. Basel nomenclature). Nevertheless, the general feature of both models describes the same thing. However, the human system has been delineated according to the Basel nomenclature (Ghia, ten Boekel et al. 1996).

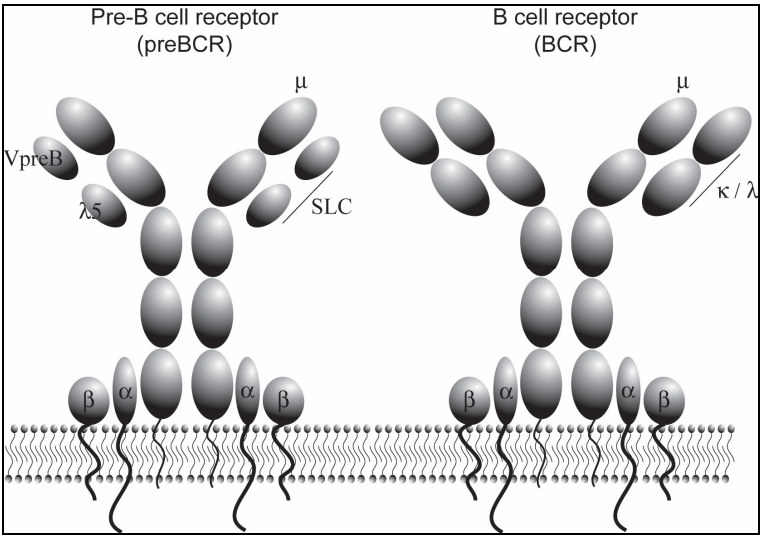

Figure 2. Schematic description of the pre-BCR and the BCR.

The antigen independent developmental pathway takes the B cell from the CLP stage to the mature B cell stage through a tightly regulated chain of complex events that primarily targets the immunoglobulin genes. These events are regulated by a number of transcription factors and DNA editing enzymes, which includes the recombination activating gene (RAG) proteins 1 and 2, interleukin 7 (IL-7), the B cell lineage-specific activator protein (BSAP), as well as epigenetic regulation such as DNA methylation and histone acetylation / methylation (Bergman and Cedar 2004; Corcoran 2005).

At the pro-B cell stage, which succeeds the CLP, rearrangements of the V(D)J Ig heavy chain gene segments takes place. These rearrangements are mediated by the RAG1 and RAG2 proteins which recombines the Ig heavy chain D (diversity) and J (joining) gene segments prior to the V (variable) gene segment, and result in a rearranged heavy μ chain (Tonegawa 1983). As a heavy μ chain is productively rearranged, a heavy μ chain polypeptide, combined with a surrogate light chain (SLC), which comprises a λ5 and VpreB polypeptide, will form the pre-BCR (Fig 2). However, if the first attempt to create a functional pre-BCR fails, rearrangements of the second allele will be carried out.

The pre-B cell stage is reached when a functional pre-BCR is formed and expressed on the surface. At this point signals imparted by the pre-BCR (Bannish, Fuentes-Panana et al. 2001) (Fuentes-Panana, Bannish et al. 2004) results in an extensive proliferation and a clonal expansion of the pre-B cell. This expansion is carried on until the number of pre-BCRs, due to cell division, is too low to induce sufficient signals promoting further proliferation (Melchers 2005). As the cell starts to proliferate the accessibility of the Ig genes are reduced by epigenetic regulation (Johnson, Angelin-Duclos et al. 2003); (Chowdhury and Sen 2003) and the RAG proteins are down regulated (Grawunder, Leu et

al. 1995; Galler, Mundt et al. 2004), which together leads to a shut-down of the rearrangements machinery. The consequence of this feedback inhibition is the preservation of the rearranged heavy chain in the cell, since further rearrangements on the second chromosomal allele is prevented, a phenomenon referred to as "allelic exclusion".

The importance of the SLC in the pre-BCR signal induction is demonstrated in experimental mice deficient in either λ5 or VpreB or both (Rolink and Melchers 1993; Mundt, Licence et al. 2001; Shimizu, Mundt et al. 2002). These mice develop mature B cells, but at reduced numbers, which shows that the SLC is necessary for the expansion of the pre-B cell. The exact function of the SLC and the SLC mediated signaling is still elusive but it is likely to interact with components in its surroundings. Stromal cell components such as Galectin-1 and heparan sulfate has been found to interact with λ5 (Gauthier, Rossi et al. 2002; Bradl, Wittmann et al. 2003); λ5 has also been found to establish homotypic interactions (Ohnishi and Melchers 2003).

The immature B cell stage is reached as the B cell expresses both a rearranged heavy chain as well as light chain, creating a functional BCR on the surface. For this to happen, the pre-B cell needs to re-enter the process of RAG mediated recombination. At this time the rearrangement machinery instead targets the light chain V and J genes. Now several options for the creation of the light chain are offered, since both the κ light chain encoding genes (on chromosome 2) as well as the λ light chain encoding genes (on chromosome 22) can be used. It appears that κ gene usage precedes λ in this process (Brauninger, Goossens et al. 2001), however, the details governing this are not fully understood (Liang, Hsu et al. 2004).

In the subsequent transition, leading to a mature B cell, the immature B cell will go through a selection process, which, first of all, negatively selects for self-reactivity, but also positively selects for signaling competence. This will happen as a productive heavy and light chain is rearranged and expressed on the surface (Fig 2). The outcome of this selection process will be a shaping of the B cell repertoire (Gu, Tarlinton et al. 1991; de Wildt, van Venrooij et al. 1999).

It is important that the mature B cell is not only negatively selected for self-reactivity, but also positively selected for signaling competence. Tonic signaling through the BCR complex is crucial for the survival of the mature B cell (Lam, Kuhn et al. 1997; Bannish, Fuentes-Panana et al. 2001; Kraus, Alimzhanov et al. 2004) and it is thought that this signaling competence is established through the positive selection of the immature B cell. It is not clear how these tonic signals are mediated or selected for, but it has been postulated that the signal-competent BCR has a low level of self-reactivity, mediating a constitutive signal as the B cell interacts with its surroundings (Wang and Clarke 2003; Wang and Clarke 2004). Another suggestion is that the BCR complex mediates a constitutive basal and ligand independent signal on its own (Fuentes-Panana, Bannish et al. 2004).

However, if signaling through the BCR exceeds or falls below a certain threshold, receptor editing will take place (Gay, Saunders et al. 1993; Tiegs, Russell et al. 1993; Shivtiel, Leider et al. 2002; Keren, Diamant et al. 2004; Halverson, Torres et al. 2004). Thus, a secondary gene rearrangement of the κ or λ locus will be executed (and/or possibly also VH replacements (Zhang, Burrows et al. 2004).Thus, the rationale for this secondary editing process is to generate a receptor that can be positively selected. Although, if all the receptor editing possibilities are exhausted, the B cell will eventually be

deleted (i.e. clonal deletion). In fact, a majority of the immature B cells show self reactivity to various self antigens, including nuclear antigens, dsDNA, ssDNA, immunoglobulins, insulin and bacterial polysaccharides and therefore need to be edited and/or deleted (Wardemann, Yurasov et al. 2003; Meffre, Schaefer et al. 2004). As a result, a majority of the cells are lost in this transition (Levine, Haberman et al. 2000).

Taken together, the antigen independent phase of the B cell development is to generate a signaling competent but non-self reactive BCR, which has a unique set of heavy and light chain variants that renders an exclusive antigen specificity.

2.2 Secondary lymphoid organs and basic micro-architecture

In order to understand the antigen dependent B cell development, it is necessary to have some knowledge of the basic anatomy of the secondary lymphoid organs. Even though the general structural composition between the different lymphoid organs differs to a great degree, several factors prevails.

The secondary lymphoid organs function to monitor the input of antigen of the host. This is achieved by their location at strategic points throughout the body, where antigen is likely to pass, e.g, in or around any surface exposed to the exterior environment. For instance mucosal-associated lymphoid tissues (MALT) line most of the mucosal surfaces in the body, including the upper respiratory tract, the bronchial tubes, the small intestine (ileum) and the urogenital tract, all likely routes of entry for pathogens. Also, both mucosal surfaces and the skin house a specialized type of dendritic cells (Langerhans cells), which collects antigen at these surfaces and delivers it to a nearby draining lymph node. MALT and lymph nodes are furthermore interconnected via a network of lymphatic vasculature enabling intercommunication between these lymphoid tissues. Lymph nodes, in total counting to about 500-600, are regularly located all over the body, progressing from a central location in every direction, like a string of pearls.

Although, a majority of all pathogens will be handled by the MALT and the lymph node associated immunity, the processing of blood borne antigens is carried out by the spleen. As the blood is continuously filtered through the spleen, antigens from various parts of the body are trapped there. The spleen is therefore involved in the orchestration of the systemic immune response.

The unifying features of these lymphoid tissues (i.e. MALT, lymph nodes and spleen) is that they all are sites for antigen dependent B cell development, promoting terminal differentiation of B cells into AFCs upon antigen challenge. In order to establish a milieu facilitating an antigen response in these tissues, some fundamental components are required, which include B cell follicles, housing B cells and follicular dendritic cells (FDC), and secondly, T cell zones containing T cells and interdigitating dendritic cells (IDC).

For the most primitive B cell follicle formation to occur, an interaction needs to take place between B cells and follicular dendritic cells (FDC) (Ngo, Korner et al. 1999). This interaction is founded upon a presence of lymphotoxin (LT) $\alpha 1\beta 2$ and the B cell attracting chemokine 1 (CXCL13/BCA1/BLC). Mice deficient in LTα, LTβ, or LTβ receptor (LTβR) are devoid of secondary lymphoid structures (De Togni, Goellner et al. 1994; Alimzhanov, Kuprash et al. 1997; Koni, Sacca et al. 1997; Futterer, Mink et al. 1998) and CXCL13- or CXCL13 receptor (CXCR5/BLR1)-deficient mice display disrupted secondary

lymphoid structures (Forster, Mattis et al. 1996; Ansel, Ngo et al. 2000). It has been demonstrated that signaling via the LTβR on FDCs is required to induce expression of CXCL13, which will attract mature CXCR5+ B cells (Ngo, Korner et al. 1999). Intriguingly, however, CXCL13- or CXCR5-deficient mice lack FDCs (Ansel, Ngo et al. 2000) indicating that without a presence of CXCR5+ B cells no FDCs will develop. Indeed, B cells have been found to be an important source of LTα1β2 (Fu, Huang et al. 1998; Ansel, Ngo et al. 2000; Tumanov, Kuprash et al. 2002; Tumanov, Kuprash et al. 2004) and therefore instrumental in the first steps of the follicular structure formation. As the B cells and the FDCs have been brought together a positive feedback loop will facilitate further development of the follicular structure, such that CXCL13 augments the LTα1β2 expression of B cells, which in turn augments CXCL13 expression of FDC etc. (Ansel, Ngo et al. 2000). However, since CXCL13 expression is dependent of LTα1β2 and the LTα1β2 presence is dependent of CXCL13 what is the founding source? The so-called lymphoid tissue inducer cells (LTIC) have been found to provide an initial source of LTα1β2 needed for the formation of lymph nodes, Payer's patches and nasal associated lymphoid tissues in mice (Mebius, Rennert et al. 1997; Finke, Acha-Orbea et al. 2002). Thus the actions of an originating LTIC will instigate the formation of the B cell follicular structure. However, LTα1β2 is not fully indispensable for the formation of mesenteric lymph nodes. For these structures to form, LIGHT, another LTβR ligand has proven to play a role (Scheu, Alferink et al. 2002). Similarly, TRANCE and TNF-α are also reported to influence lymph node and Peyer's patch formation respectively (Kim, Mebius et al. 2000; Tumanov, Kuprash et al. 2004).
In summary an originating source of CXCL13 seems to be a most crucial factor for B cell follicular formation. This is also illustrated by the lymphoid neogenesis seen when CXCL13 is expressed at ectopic sites (Luther, Lopez et al. 2000; Shi, Hayashida et al. 2001; Carlsen, Baekkevold et al. 2004), which is a phenomenon occurring in several autoimmune conditions (Shi, Hayashida et al. 2001; Salomonsson, Larsson et al. 2002; Carlsen, Baekkevold et al. 2004).

Although, the follicular formation is largely dependent on the positive feedback mechanism between the B cell and the FDC, the entry of the B cell into the lymphoid organ is mediated by other factors. In CXCR5-deficient mice, B cells do not form follicles, but are still able to enter the lymphoid organs (Forster, Mattis et al. 1996). B cell entry to Payer's patches has been shown to involve both CXCR4-CXCL12 and CCR7-CCL19/CCL21 (Okada, Ngo et al. 2002), whereas entry to the spleen has been shown not to be dependent on CCR7 (Forster, Schubel et al. 1999). This is explained by the fact that B cells enter to the peripheral lymphoid organs (lymph nodes and MALT) through the follicular post-capillary high endothelial venules (HEV) (Okada, Ngo et al. 2002), whereas in the spleen the B cell entry is via the marginal sinus. The endothelial cells lining the HEV has been shown to express CCL21 (Gunn, Tangemann et al. 1998) and it has also been shown that CXCL12 and CCL19, produced by lymphoid tissue stromal cells, is present in the HEV lumen (Luther, Lopez et al. 2000 Okada, Ngo et al. 2002). Thus, it is clear that the B cell entry into lymph nodes and MALT is dependent on CXCR4-CXCL12 and CCR7-CCL19/CCL21, whereas homing to the spleen does not show this dependence.

Adjacent to the B cell follicles are intervening T cell-rich areas, so-called T cell zones, which are composed of antigen-presenting cells (APC) and T cells. The establishment of

the T cell zones is dependent primarily on the CCR7-CCL19/CCL21 receptor ligand interactions (Forster, Schubel et al. 1999). Naïve T cells are attracted to this zone by CCL21 and CCL19 produced by the HEV and the T cell-zone-specific stromal cells (Luther, Lopez et al. 2000). In contrast, the circulating memory T cells (i.e. central memory, T_{CM}) as well as APCs, such as IDC and Langerhans cells, enters the lymph node via afferent lymphatics (Forster, Schubel et al. 1999). In the T cell zone, APCs will present antigen to the T cell. As this happens the T cell becomes activated, starts to proliferate and differentiates into various effector T cells (Sallusto, Lenig et al. 1999).

2.3 Antigen dependent mature B cell development

2.3.1 Immunoregulators on the mature B cell

As the B cell becomes a mature B cell, several factors involved in regulation of the immune response are needed. Therefore, during the transition between the immature and the mature B cell, several components associated with the mature B cell functionality are upregulated. Apart from the chemokine receptors CXCR5 and CCR7 (Bowman, Campbell et al. 2000), surface expression of the δ heavy chain (IgD) together with IgM signifies the mature B cell. Up until now, the B cell has not seen any exogenous antigen and is therefore considered antigen inexperienced or "naïve". From this stage antigen recognition will determine the fate of the B cell.

Since the expression of IgD is essentially limited to the naïve B cell subsets, it is not unlikely that the coexpression of this second BCR, adds functionality facilitating the initial antigen recognition. Although not formally established, it has, however, been shown that IgD, possibly due to its higher segmental flexibility, binds antigen with low epitope density (Loset, Roux et al. 2004). In contrast, IgM is shown to have a higher functional affinity for antigens with high epitope density. Based on this, it has been suggested that IgD facilitates immune responses to antigens with low epitope density, so-called paucivalent antigens (Pure and Vitetta 1980), which typically requires T cell help. This would imply that IgD plays a role in the T cell dependent (TD) immune response (Cambier, Ligler et al. 1978), contributing with additional valency and increased antigen avidity. In transgenic mouse models, lacking IgD, it was found that, although the TD immune response was largely uncompromised, affinity maturation was delayed and ineffective (Roes and Rajewsky 1993) and that $IgD^{-/-}$ cells seemed to compensate the IgD loss by expressing three times more IgM (Nitschke, Kosco et al. 1993). Additionally, in the heterozygous mutant mice, B cells using the functional δ chain gene, were enriched in the periphery and were preferentially recruited to a TD response (Roes and Rajewsky 1993). Along with this, BCR affinity to antigen is shown to be decisive for the TD immune response (Dal Porto, Haberman et al. 2002; Shih, Meffre et al. 2002). High affinity BCRs have a selective advantage over low affinity in the TD generation of germinal centers. In this context, the presence of IgD is likely to support the TD immune response by an added antigen avidity and also to facilitate the immune response to TD antigens with low epitope density (Dal Porto, Haberman et al. 1998; Dal Porto, Haberman et al. 2002). In contrast, the T cell independent (TI) immune

response does not demonstrate this affinity requirement to antigen (Shih, Roederer et al. 2002) owing to the higher epitope density on typical TI antigens.

Parallel with the acquisition of the mature B cell functionality, several other components in the regulation of the immune response are needed. Since the mature B cell, upon antigen challenge, generates immunoglobulin secreting effector cells, it is important that the BCR signaling is regulated, in order to avoid propagation of autorectivity. Already in the bone marrow positive regulation of the BCR signal transduction is established, as the B cell express CD19 and CD45. The mature B cell has, in addition to CD19 and CD45, negative regulators such as CD22 and the FcγRIIB. The cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM) of CD22 and FcγRIIB is modulating the signal transduction from the mature BCR. Whereas the ligands of the FcγRIIB (being the only Fcγ receptor on B cells) consist of switched Igs (Heyman 2000), CD22 binds glycosylated sialic acid residues expressed on various leucocytes and inflamed endothelial cells, which may have implications in discriminating "self" (Lanoue, Batista et al. 2002). Importantly, as an immune response has developed, FcγRIIB will take part in a negative feedback loop, inhibiting further recruitment of mature B cells into antibody production. Thus, loss of these receptors would affect the immune response and tolerance to self-antigens, potentially leading to autoimmunity (O'Keefe, Williams et al. 1999; Lanoue, Batista et al. 2002; McGaha, Sorrentino et al. 2005; Pritchard and Smith 2003). However, antibody mediated negative regulation is also accomplished by soluble antibodies which mask the immunogenic epitopes on the antigen, thereby hindering further B cell activation (Heyman 2000).

2.3.2 B Cell Activation

B cell activation can, according to a model by Nicole Baumgarth (Baumgarth 2000), be divided into a two-phase process where phase one comprises a TI, antigen-specific activation leading to a primary B cell expansion and the generation of secreted IgMs. The second phase involves a TD formation of GCs. The initial trigger in the TD immune response is indeed TI and involves the formation of an antigen/BCR synapse (Batista, Iber et al. 2001). This synapse formation is facilitated by collaboration between complement factors (Carroll 2004), supplied by the innate immune system, and polyclonal immunoglobulins, such as pre-existing natural antibodies, IgM and IgG (Boes, Prodeus et al. 1998; Boes, Esau et al. 1998; Ehrenstein, O'Keefe et al. 1998; Hannum, Haberman et al. 2000). At this point in the antigen dependent development the nature of the antigen will influence the subsequent differentiation. Evidence is implicating that the antigen induced BCR signal strength determines whether the B cell will commit to a follicular TD differentiation pathway or embark on a marginal zone (MZ) pathway (Wen, Brill-Dashoff et al. 2005; Pillai 2005).

FDCs captures particulate antigen in the form of immune complexes (IC) that have been recognized by pre-existing immunoglobulins and complement (Klaus and Humphrey 1977; Aydar, Sukumar et al. 2005). Present on both FDCs and B cells are the complement receptors CR1 (CD35) and CR2 (CD21), which bind and retain the ICs and thereby facilitating the antigen presentation to the B cell. In collaboration with the CD19 co-receptor, CD21 also assists B cell activation by lowering the BCR signal transduction

threshold as the B cell recognizes antigen (Carter and Fearon 1992). Without CD21 the required BCR signal strength can not be established for a TD response, which results in a differentiation towards MZ B cells (Cariappa, Tang et al. 2001). Antigen presented to the B cell is internalized and subject to a proteolytic degradation in endosomes. The degraded antigen peptide is then complexed to MHC class II and displayed on the surface of the B cell for a subsequent antigen presentation to the T cell (Lanzavecchia 1985; Clark, Massenburg et al. 2004). Even for these processes to occur both CD21 and CD35 have shown to be of importance (Croix, Ahearn et al. 1996; Ahearn, Fischer et al. 1996; Molina, Holers et al. 1996; Fischer, Goerg et al. 1998). Apart from these receptors and co-receptors several other molecules, such as CD72 and CD81 are also implicated in these initial events affecting the TD response (Bishop and Hostager 2001).

2.3.3 The T cell-independent immune response

As alluded to previously both BCR signal-strength (Cariappa, Tang et al. 2001) and affinity to the antigen (Dal Porto, Haberman et al. 2002; Shih, Meffre et al. 2002; Shih, Roederer et al. 2002) is decisive in directing the immune response to a TD pathway. Consequently, the nature of the antigen plays a critical role in these instances. Generally it is known that protein-antigens give rise to TD responses whereas lipopolysaccharides and polysaccharides stimulate TI type 1 (TI-1), and type 2 (TI-2) responses, respectively. Characteristic for the TD response is the formation of a GC reaction and the generation of isotype switched, affinity matured antibodies. The TI responses, on the other hand, generates an immune response without T cell help, which through an extrafollicular expansion phase gives rise to AFC (MacLennan, Toellner et al. 2003). However, a series of experiments have challenged this paradigm. For instance in experimental mice, transient GCs were induced to a TI-2 antigen in absence of T cells (de Vinuesa, Cook et al. 2000; Toellner, Jenkinson et al. 2002) and it has also been proposed that memory cells respond to polysaccharide-antigens, giving rise to high affinity antibodies (Vinuesa, Sze et al. 2003). Furthermore, CD40L-deficient mice are able to produce antigen-specific antibodies of all isotypes except IgE in response to a TI antigen (Renshaw, Fanslow et al. 1994). Together, these studies illustrates that it may be difficult to draw clear-cut conclusions as to the different roles of TD and TI antigens. Nevertheless, it is clear that T cell help gives rise to an enhanced immune response and perhaps it could be conceived that, for antigens not requiring T cell help, B cells will enter a "default" TI pathway, directing them to an extrafollicular response and a subsequent entry to the MZ, as suggested by several studies (Ehrenstein, O'Keefe et al. 1998; Dammers, de Boer et al. 1999; Cariappa, Tang et al. 2001; Wen, Brill-Dashoff et al. 2005). Although, the MZ primarily is a phenomenon of the spleen, cells with phenotypes similar to MZ B cells have been described in lymph nodes, Payers's patches and also in the sub-epithelial areas of the tonsil (MacLennan, Toellner et al. 2003).

2.3.4 The T cell-dependent immune response

The encounter of antigen directing the B cell to a TD immune response instructs the B cell to seek T cell help. What exactly determines these instructive events is unclear but it is

known that the B cell will migrate towards the T zone (Garside, Ingulli et al. 1998) by exhibiting an increased responsiveness to the T zone-specific CCR7 ligands CCL19 and CCL21 (Reif, Ekland et al. 2002; Okada, Miller et al. 2005) paralleled by a RGS1-mediated desensitization of CXCR4 and CXCR5 (Reif and Cyster 2000; Moratz, Hayman et al. 2004; Han, Moratz et al. 2005). It has been established that cognate, i.e., specific, B and T cell interactions can last up to an hour whereas non-cognate interactions are short lived (Okada, Miller et al. 2005). These B-T "conjugates" move around extensively and this movement is dictated by the B cell seeking more T cell interactions (Okada, Miller et al. 2005).

A consequence of the B-T cognate interaction is the delivery of a CD154-CD40 signal which is the key signature in the TD immune response (Han, Hathcock et al. 1995). This signal will authorize the antigen specific B cell to enter a clonal proliferation program; initiating a GC reaction (Han, Hathcock et al. 1995). It could be conceived that a B cell that establish more than one B-T cognate interaction will be favored in the process of forming a GC reaction, since there is a direct correspondence between CD40 ligand density and signal strength, and consequently the induction of DNA synthesis (Pound, Challa et al. 1999) (Haswell, Glennie et al. 2001). Perhaps this is the selective event that ensures that only a very few B cells give rise to GCs and are allowed to go through clonal expansion. Several studies have shown that GCs have an oligoclonal source of fewer than five ligand selected B cells (Kroese, Wubbena et al. 1987; Liu, Zhang et al. 1991; Jacob, Kassir et al. 1991; Jacob, Kelsoe et al. 1991; Kuppers, Zhao et al. 1993) and that there is a positive selection of high affinity, but yet unmutated B cells (McHeyzer-Williams, McLean et al. 1993; Dal Porto, Haberman et al. 1998; Shih, Meffre et al. 2002). B cells unable to establish this multivalent interaction will on the other hand still proliferate and contribute to the primary immune response (McHeyzer-Williams, McLean et al. 1993).

The cognate B-T interaction further results in a change in the responsiveness to the follicle-specific CXCL13. Similarly to the directed migration towards the T zone after antigen encounter, the return to the follicle may also be modulated by RGS factors. Although, not formally established, it is likely that this modulation is mediated by RGS3. RGS3 has been found to inhibit chemotaxis to CXCL12 and CCL19 in a murine B cell line (Reif and Cyster 2000). In paper I we found that RGS3 expression is induced in human naïve B cells by *in vitro*-stimulation through BCR, CD40 and CD44. However, the responsiveness to the CCL19/CCL21 is also reduced as a cause of the down regulation of CCR7 on B cells, which are entering the follicle. CCR7 transcriptional down regulation is a clear expressional signature of GC B cells, which was evident in all our expressional studies so far (Paper II, Paper III and Paper IV).

Another consequence of the cognate B-T interaction, apart from the CD40-CD40L mediated proliferation signal imparted on the B cell, is follicular helper T cell recruitment. Cognate signals, largely governed by the CD40-CD40L, CD80/86-CD28 and ICOSL-ICOS interactions, will turn the antigen-primed T cell to a follicular helper T cell (T_{FH}), which confer to the T cell a CXCL13-responsiveness, by upregulating CXCR5. The T_{FH} will thus, along with the B cell, home to the follicle, where it will entertain B-T interactions supporting B cell survival, GC formation and Ig secretion (Andersson, Dahlenborg et al. 1996; Breitfeld, Ohl et al. 2000; Schaerli, Willimann et al. 2000; Akiba, Takeda et al. 2005; Kim, Lim et al. 2005). These events are further assisted by several secreted T cell-derived

soluble factors, such as IL-2, IL-4, IL-5, IL-6, IL-10, IL-13 and IL-21 (Johansson-Lindbom, Ingvarsson et al. 2003; Ebert, Horn et al. 2004; Chtanova, Tangye et al. 2004) which all play distinctive roles in the GC reaction and Ig secretion.

2.3.5 The germinal center

The founding descriptions of the germinal center dates back to anatomical studies performed by Flemming in 1885 (Flemming 1885). From observations that highly mitotic areas in lymph nodes were actively giving rise to lymphocytes, these areas where named "Keimzentrum" , i.e., germinal center, which is referring to the notion that these centers were the sites where lymphocytes originated from. Later, following the issuing of Macfarlane Burnet's clonal selection theory of acquired immunity (Burnet 1959), a renewed interest was spurred as to the origin of the antibody forming plasma cell, first discovered by Astrid Fagraeus in 1948 (Fagraeus 1948). In a series of studies on antibody production, utilizing fluorescein conjugated antigen-specific antibodies, Coons and colleagues concluded that antibody production could be coupled to lymphocytes (Coons, Leduc et al. 1955) and that a primary response is characterized by only few antibody containing cells, whereas the secondary response generates hundreds (Leduc, Coons et al. 1955; White, Coons et al. 1955). They could also conclude that areas of antibody-containing plasma cells are found at sites remote from the point of injection of antigen (White, Coons et al. 1955). Ward et al. (Ward, Johnson et al. 1959) found that the predominant histological changes associated with antibody formation are confined to the follicular centers of the splenic white pulp and are coupled to an "explosive mitotic activity". They also proposed that plasma cells arise only following secondary or intense antigenic stimulation. In two consecutive studies Nossal and Mäkelä (Nossal and Makela 1962; Makela and Nossal 1962), using autoradiography presented a model for clonal expansion following antigenic stimuli, where it was proposed that a single antigen stimulus causes a burst of clonal expansion and differentiation to the plasma cell. They also suggested that immunological memory depends on the persistence of a continuously dividing stem line of lymphocytes which are reactive to further antigenic stimulation at all times. The cell division kinetics of these studies demonstrated that the mitotic cells completed a cell cycle every 8 to 12 hours. In following kinetic studies (Fliedner, Kesse et al. 1964; Hanna 1964) doubling times of 5-7 hours were found. These studies also described a high degree of cell death in the GC and a histological classification of a densely populated dark-staining zone (dark zone) and a thinly populated light-staining zone (light zone) of the GC. The designation centroblasts and centrocytes for the respective dark and light zone residing cells, stems from the lymphoma meeting in Kiel 1974 (Lubarsch, Henke et al. 1978).

The current understanding of the functional properties of the GC is, first of all, that the GC environment facilitates extensive proliferation, with doubling times of 5-7 hours. This proliferation is mainly driven by the CD40-CD40 ligand signals, although a key regulator, the transcriptional repressor BCL-6 is crucial for the formation and the maintenance of the GC reaction (Dent, Shaffer et al. 1997; Ye, Cattoretti et al. 1997; Fukuda, Yoshida et al. 1997). Secondly the outcome of the GC reaction is primarily AFCs, secreting affinity matured and isotype switched immunoglobulins. The mechanism behind the generation of

affinity matured immunoglobulins is governed by somatic point mutations, which targets the complementary determining regions (CDR) within the V(D)J segments of the immunoglobulin genes. This process is antigen-driven and highly dependent on positive selection of antigen-specific clones; non-selected cells will be deleted (Han, Zheng et al. 1995). Affinity maturation, which involves SHM and the subsequent selection process, has been shown to target amino acids in the periphery of the antigen binding site and thus preserving antigen specificity (Tomlinson, Walter et al. 1996; Ramirez-Benitez and Almagro 2001) It appears that this process is also implicated in reducing poly- and self-reactivity. Poly- and self-reactivity have been associated with long CDRH3 and is evidently a feature of the immature repertoire (Ichiyoshi and Casali 1994; Wardemann, Yurasov et al. 2003). It has been observed that the GC reaction results in shorter CDR3 (McHeyzer-Williams, McLean et al. 1993), thereby evading the formation of high affinity self reactive antibodies. Immunoglobulin genes are furthermore subject to class switch recombination (CSR) which is believed to also take place in the GC. This process implicates DNA recombination events that juxtapose recombined H-chain V(D)J segments with different constant region (CH) gene segments, resulting in a switch in immunoglobulin isotype, e.g., from IgM to IgG1. Isotype switching is influenced by external factors such as IL-4 or IL-21 (Ozaki, Spolski et al. 2002). Although it has been proposed that SMH and CSR can be functionally associated to the dark zone and the light zone, respectively, (Liu, Arpin et al. 1996) it must be pointed out that both SHM and CSR occurs early after the cognate B-T interaction and before a formal establishment of the GC (Levy, Malipiero et al. 1989; Berek, Berger et al. 1991; McHeyzer-Williams, McLean et al. 1993; Jumper, Splawski et al. 1994; Toellner, Gulbranson-Judge et al. 1996). It is therefore difficult to assign specific functionalities to different regions of the GC. This was also our conclusion in paper III were we investigated the transcriptional difference between centroblasts and centrocytes based on the current phenotypic characteristics for these subsets (paper III). Nevertheless, it seems like the genetic events occurring in the GC are tightly linked to proliferation, since CSR and antibody formation have been demonstrated to be dependent on cell division (Hodgkin, Lee et al. 1996; Hasbold, Lyons et al. 1998; Tangye, Avery et al. 2003; Hasbold, Corcoran et al. 2004). Furthermore, the enzyme implicated in both CSR and SHM, namely activation-induced cytidine deaminase (AID) (Muramatsu, Kinoshita et al. 2000) has lately been demonstrated to have a cell division-linked activity (Rush, Liu et al. 2005), which indicates that proliferation is a prerequisite for both CSR and SHM as was suggested by Gearhart and Bogenhagen in already in 1983 (Gearhart and Bogenhagen 1983). In our studies (Paper III and IV) it is evident that AID and other factors involved in SHM and CSR are upregulated in the B cell populations that are actively cycling. Thus, since the dark zone is enriched in hyperproliferating centroblasts it is likely that both SHM and CSR are most active here, whereas the selection process takes place in the dense FDC networks of the light zone (Hardie, Johnson et al. 1993).

2.3.6 Terminal differentiation

The terminal differentiation to AFC is an event following the positive selection process and likely to be initiated in the light zone. Dramatic changes to the cellular differentiation program needs to take place in order to convert the centrocyte to an AFC. The B-

lymphocyte-induced maturation protein 1 (BLIMP1) is the master regulator of the plasma cell differentiation program. BLIMP1 shuts down both the GC program, by repressing BCL6, as well as the B cell program, by repressing the B-cell-lineage-specific activator protein (BSAP) gene PAX5. The repression of PAX5 will in turn alleviate the BSAP-mediated repression of the X-box binding protein 1 (XBP1), setting off the plasma cell differentiation program and the subsequent secretion of immunoglobulins by AFCs (Shapiro-Shelef and Calame 2005). A number of external factors have been shown to positively regulate the induction of the plasma cell program. Among these have IL-2, IL-10 and CD27/CD70 interactions been demonstrated to drive plasma cell formation (Agematsu, Kobata et al. 1995) (Agematsu, Nagumo et al. 1998), IL-21 to induce BLIMP1 (Ozaki, Spolski et al. 2004) and IL-6 to activate the CDK inhibitors $p18^{INK4c}$ and $p21^{WAF1/CIP1}$ (Morse, Chen et al. 1997).

The memory cell is enigmatic. Its importance in long term protection was clearly demonstrated recently, when it was shown that stable antibody responses could be maintained up to 75 years after vaccination to smallpox (Hammarlund, Lewis et al. 2003). However, there are, so far no compelling evidence demonstrating the origin of the memory cell. It has been shown that memory cells carry mutated Ig genes (Pascual, Liu et al. 1994) and that the establishment of the memory compartment is dependent on CD40-CD40 ligand signalling (Gray, Dullforce et al. 1994; Foy, Laman et al. 1994). It is also established that memory cells, which give rise to high affinity immune responses are IgD^- (Herzenberg, Black et al. 1980) and are found in the PNA^- population (Coico, Bhogal et al. 1983). Rajewsky and colleagues concluded that a high affinity memory compartment is established already after a primary response (Weiss and Rajewsky 1990) and that it is constituted by quiescent cells that make up the founders of the secondary response (Schittek and Rajewsky 1990) (Weiss and Rajewsky 1990) and that it is furthermore independent of antigen for its long-term persistence (Vieira and Rajewsky 1990; Maruyama, Lam et al. 2000), corroborated also by others (Karrer, Lopez-Macias et al. 2000). The long term persistence of memory cells may however be facilitated by polyclonal stimuli mediated by Toll-like receptors (Bernasconi, Traggiai et al. 2002; Bernasconi, Onai et al. 2003). Phenotypic characteristics of the memory cells include expression of CD80/86 (Liu, Barthelemy et al. 1995), CD27 (Klein, Rajewsky et al. 1998) (Avery, Ellyard et al. 2005) and the Fc-receptor homologue 4 (FcRH4) (Ehrhardt, Hsu et al. 2005). Memory cells are found in the splenic MZ (Dunn-Walters, Isaacson et al. 1995), in peripheral blood (Klein, Rajewsky et al. 1998) and in the sub-epithelial areas of the tonsil (Liu, Barthelemy et al. 1995). Based on this data it is difficult to draw any conclusions as to whether the memory cell is at the end of a GC reaction or not. Most data favors a diversion of a memory cell precursor somewhere just before the initiation of the GC reaction. Indeed, when comparing the potential between GC B cells and GC founder cells to *in vitro*-generate memory cells, we (Månsson 2004) found that memory cells are more readily generated from the GC founder population than from the GC B cell population. In these cultures GC B cells were dependent on BCR- and CD40-stimuli for survival and entered apoptosis within 24 hours if the CD40-stimuli was omitted. In contrast, the GC founder cells were found to revert to a memory cell phenotype, i.e., CD20+CD38- (Arpin, Dechanet et al. 1995) and to still be viable at 120 hours in culture, only relying on the BCR stimuli. With

the additional CD40 stimuli a GC B cell phenotype was generated from these GC founders (Månsson 2004). It was also found that memory cells and naïve B cells were the best responders to CD40 mediated DNA synthesis, when compared to sorted fractions of GC B cells, GC founders and plasma cells. Together these results indicates that the GC founder population is at a bifurcation which could either direct the cell into a the GC reaction or, in the absence of CD40 stimuli, revert to a memory cell.

3. Somatic hypermutation and class switch recombination

3.1 Somatic hypermutation

The mechanisms governing the SHM process is still not well understood. Although, the current idea is that targeted lesions in DNA generates point mutations through an error prone repair mechanism, as proposed by Brenner and Milstein in 1966 (Brenner and Milstein 1966). The typical mutations occurring in the productively mutated IgV genes are mainly point mutations generated by frequent nucleotide substitutions and preferentially purine transitions (i.e., purine to purine) (Neuberger and Milstein 1995; Dorner, Foster et al. 1998). These point mutations occurs at a rate of 10^{-3}-10^{-4} per base per cell generation and are directed to the complementary determining regions (CDR) within the rearranged V(D)J segments of the heavy and light chain genes (Storb 1996). It has further been shown that the mutation machinery especially favours mutational "hotspots", where the consensus sequence motif RGYW (Purine, Guanine, Pyrimidine, Adenine/Thymidine) or its complement sequence WRCY are more frequently mutated (Rogozin and Kolchanov 1992; Dorner, Foster et al. 1998). It is also known that C and G mutate more frequent than A and T (Dorner, Foster et al. 1998). The SHM machinery has been shown to be dependent on active transcription and it is necessary that the target gene has a transcriptionally active promotor along with an enhancer element (Fukita, Jacobs et al. 1998; Papavasiliou and Schatz 2002).

A central part in SHM is played by the activation-induced cytidine deaminase (AID). This enzyme, discovered in its association to CSR (Muramatsu, Sankaranand et al. 1999) is indispensable to all genetic changes shaping the secondary response (Muramatsu, Kinoshita et al. 2000; Revy, Muto et al. 2000). The AID function in these processes is not known, but a model outlining the AID induced genetic alterations in SHM, CSR and gene conversion has been proposed (Petersen-Mahrt, Harris et al. 2002). In this model the AID deaminates cytosines at the mutation hotspots, converting them to the deaminated version namely uracil. The occurrence of uracil in the DNA triggers the base excision repair pathway (BER) and more specifically, the uracil-DNA glycosylase (UNG) mediated base excision. This process generates an abasic site which is excised by the multifunctional apurinic/apyrimidinic endonuclease (APEX1) and further repaired through a short patch repair mechanism involving DNA polymerase beta (POLB), XRCC1 and DNA ligase 3 (LIG3), which reinserts the cytosines. However, alternative routes in the processing of the deaminated cytosines are proposed to instead give rise to mutations at theses sites. For instance if the uracil were to serve template for replication this would produce a C:G to T:A transition and if the abasic site were to enter replication, the outcome would be both transitions and transversions. These two events could thus account for the mutations that targets C and G. This model has gained further support since it has been demonstrated that AID, *in vitro*, mediates a transcription dependent and strand biased deamination of cytidines on ssDNA (Chaudhuri, Tian et al. 2003; Bransteitter, Pham et al. 2003; Dickerson, Market

et al. 2003; Ramiro, Stavropoulos et al. 2003; Sohail, Klapacz et al. 2003), and that this process also targets mutational hotspots (Pham, Bransteitter et al. 2003). Furthermore, the impact of UNG on SHM and CSR has been shown both *in vitro* (Petersen-Mahrt, Harris et al. 2002; Di Noia and Neuberger 2002) and *in vivo* (Rada, Williams et al. 2002; Imai, Slupphaug et al. 2003).

A phenomenon observed in relation to SHM and CSR is the occurrence of single- (SSB) and double- (DSB) strand DNA breaks (Wuerffel, Du et al. 1997; Lo, Ching et al. 1997; Sale and Neuberger 1998; Bross, Fukita et al. 2000; Papavasiliou and Schatz 2000; Kong and Maizels 2001). The implication of these breaks in SHM and CSR has been a matter of controversy. It has been shown that the DSBs are preferentially found at RGYW motifs and that their occurrence depends on transcription (Bross, Fukita et al. 2000). However, AID is seemingly having no role in this break formation (Bross, Muramatsu et al. 2002) (Papavasiliou and Schatz 2002). It has been further demonstrated that AID acts downstream of the process generating the DSB (Bross, Muramatsu et al. 2002; Papavasiliou and Schatz 2002) and upstream of the DSB repair mechanism (Petersen, Casellas et al. 2001). In line with these observations Paolo Casali and colleagues (Zan, Wu et al. 2003) presented an alternative mechanism for the AID dependent SMH, where it is proposed that AID is involved in the generation of resected DSBs. Apparently, resected DSBs is a unique feature of B cells undergoing SHM (Zan, Wu et al. 2003). In this model, the resected DSB acts as substrates for an error-prone repair pathway which is involving the homologous recombination (HR) repair proteins Rad51 and Rad52 along with error-prone translesion DNA polymerases.

Three error-prone DNA polymerases have been implicated in SHM: Pol zeta (REV3L), Pol eta (POLH) and Pol iota (POLI). Polymerase zeta has been reported to be expressed in human tonsilar GC B cells (Zan, Komori et al. 2001) as well as murine PNA^+ splenic B cells (Zeng, Winter et al. 2001). The Pol zeta expression has also been shown to be regulated by BCR engagement (Zan, Komori et al. 2001) and is affecting SHM when inactivated (Zan, Komori et al. 2001; Diaz, Verkoczy et al. 2001). The role of the two DNA polymerase Y family members Pol eta and iota in SHM is controversial. Whereas the Pol eta is upregulated in PNA^+ murine B cells (Zeng, Winter et al. 2001), human tonsilar GC B cells have been reported to down regulate this enzyme (Zan, Komori et al. 2001), and although xeroderma pigmentosum patients, which have Pol eta defects show altered mutational patterns affecting A and T (Zeng, Winter et al. 2001; Yavuz, Yavuz et al. 2002) this enzyme has been concluded not to be requisite for SHM (Yavuz, Yavuz et al. 2002).

In Paper III we looked into the transcriptional regulation of all the components that have been implicated in the SHM and CSR processes so far. In this analysis it was clear that AID and UNG were specifically upregulated in the GC B cell subsets. However, this regulation did not apply to some of the other proposed factors, such as Rad51 and Rad52 or any of the Y family polymerases. In contrast, we found that the A family, polymerase theta (POLQ) had a significant upregulation in the GC subsets. This finding was highly interesting since this polymerase had been demonstrated to be a DNA-dependent ATPase with polymerase activity on nicked dsDNA (Seki, Marini et al. 2003). It is also proposed to function in DNA repair (Shima, Munroe et al. 2004) with a possible error prone translesion polymerase activity, with the ability to incorporate G opposite T (Seki, Masutani et al. 2004). It has also been found to bypass apurinic (AP) sites more efficiently than any other

known polymerase. Moreover, Pol theta is unique in that it carries out both the insertion and the extension reaction when incorporating bases opposite of an AP site. For this, Pol theta follows an "anti-C rule", poorly incorporating C, but preferentially A opposite an AP site (Seki, Masutani et al. 2004). Together these properties thus make Pol theta an attractive candidate for the processing of AP sites, which, for example, are generated by the UNG mediated base excision. Not only that, the seemingly less targeted and strand biased A:T mutations occurring in SHM (Milstein, Neuberger et al. 1998; Spencer, Dunn et al. 1999; Neuberger, Di Noia et al. 2005) could also potentially result from the low fidelity and error prone polymerase activity of Pol theta. Interestingly, two recent studies have demonstrated that Pol theta, indeed, is a major constituent of the SHM machinery. When disrupting the Pol theta gene in mice a greater than 80% decrease in mutations could be seen (Zan, Shima et al. 2005), although primarily affecting C:G mutations (Masuda, Ouchida et al. 2005). Thus, among the error-prone polymerases, Pol theta has the greatest impact on SHM and it is preferentially implicated in C:G pairs.

For mutations at A:T pairs, the mismatch repair (MMR) pathway is the likely contributor. As outlined by Michael Neuberger and colleagues (Petersen-Mahrt, Harris et al. 2002; Di Noia and Neuberger 2002; Rada, Williams et al. 2002) A:T biased mutations in the SHM process are believed to be generated by a post replication recognition of the U:G mismatch and, hence, processed by the MMR pathway. Several studies have investigated the role of the MMR pathway in SHM and the results have been interpreted in differnt ways (Wood 1998; Reynaud, Bertocci et al. 1999). Although, a significant C:G bias is evident in the mutation-pattern of the Ig genes in mice, which are deficient in the MMR components MutS homologue 2 (MSH2) and MSH6 (Rada, Ehrenstein et al. 1998; Jacobs, Fukita et al. 1998; Phung, Winter et al. 1998; Wiesendanger, Kneitz et al. 2000), it is argued that these may be secondary effects from a proliferative defect of the B cells (Vora, Tumas-Brundage et al. 1999; Frey, Bertocci et al. 1998). However, the altered mutation pattern in MSH2- and MSH6-deficient mice is not manifest in mice deficient in some of the other components of the MMR pathway. This is true for MLH1 (Phung, Winter et al. 1999; Kim, Bozek et al. 1999), PMS2 (Winter, Phung et al. 1998; Kim, Bozek et al. 1999) and MSH3 (Wiesendanger, Kneitz et al. 2000). This functional difference may be reflected in the expression pattern of these molecules in the GC B cell subsets, as seen in our analysis (Paper III). It was clear that only the MSH2 and MSH6 genes were significantly upregulated in these B cell subsets. Thus the representation of the MMR pathway in our analysis does confirm the lack of involvement by MMR components other than MSH2 and MSH6. However, another component of the MMR pathway that also, alike MSH2 and MSH6 is upregulated in the GC B cell subsets (Paper III) is the human MutH homologue EXO1. This exonuclease functionally interacts with MSH2 but also with MSH3 and MLH1 (Schmutte, Sadoff et al. 2001). Interestingly, also EXO1 has now been proven to play a role in SHM, affecting the mutation pattern in the same way as MSH2 and MSH6 when disrupted (Bardwell, Woo et al. 2004).

In summary, the mechanism governing the SHM process is evidently implicating AID, UNG, DNA Pol theta and the several MMR factors. In the model presented by Sven Petersen-Mahrt (Petersen-Mahrt, Harris et al. 2002) all these factors can be accounted for.

Thus, in this model AID deaminates cytosines at mutation hotspots which results in an U:G mispair, from here UNG and Pol theta generates transitions and transverions at this site. The alternative route implicates the U:G mispair recognition by MMR leading to mutations at A:T sites (Rada, Di Noia et al. 2004), although, the details around this mechanism is still unclear (Neuberger, Di Noia et al. 2005).

In paper III, we also observed an induction of transcripts for several factors involved in the processing of the mutagen 7,8-dihydro-8-oxoguanine (8-oxoG), which is generated during metabolism. Since gene expression for the 8-oxo-dGTPase MTH1 has been found to correlate with the occurrence of 8-oxoG (Meyer, Fiala et al. 2000), the increased expression of transcripts such as the DNA glycosylases OGG1, HFPG1 and HFPG2 probably reflects the same thing. The role of OGG1 in GC B cells and SHM has previously been discussed (Kuo and Sklar 1997) and addressed experimentally (Winter, Phung et al. 2003) concluding that it does not participate in SHM, at least not in mice (Winter, Phung et al. 2003). However, the role of 8-oxoG itself in SHM has never been assessed. 8-oxoG is a common modification occurring in DNA as a result of oxygen free radicals from the cellular metabolism. This oxidized product can be incorporated opposite adenines or cytosines during replication, and give rise to A:T to C:G or G:C to T:A transversions if unrepaired. If the guanine, instead, is oxidized *in situ* within the DNA, a G:C to T:A transversion will occur (Sekiguchi and Tsuzuki 2002). So to what extent is the presence of 8-oxoG influencing the mutational events occurring in the GC B cells? 8-oxoG is a significant contributor to the mutator phenotype of MMR deficient cells (Colussi, Parlanti et al. 2002) and it is known that, among the MMR members, especially MSH2 and MSH6 are activated by 8-oxoG lesions (Mazurek, Berardini et al. 2002). They have also been found to participate in the post-replication repair of 8-oxoG base pairs, which are not recognized by OGG1 (Colussi, Parlanti et al. 2002). Additionally, since the bifunctional glycosylases OGG1 and HFPG1, at least, are known to produce single strand breaks and nucleotide gaps as intermediates in the repair process of 8-oxoG changes (Wilson, Sofinowski et al. 2003), what role does 8-oxoG and these glycosylases play in the break formation associated with SHM (Papavasiliou and Schatz 2002; Bross, Muramatsu et al. 2002) and CSR (Wuerffel, Du et al. 1997); which evidently is not mediated by neither AID (Papavasiliou and Schatz 2002; Bross, Muramatsu et al. 2002; Unniraman, Zhou et al. 2004) nor UNG (Begum, Kinoshita et al. 2004) or only seen in the IgV genes (Zan, Wu et al. 2003)? The effects of 8-oxoG on the genetic changes occurring in the GC B cells clearly needs to be further investigated.

3.2 Class switch recombination

The downstream processes of an AID induced lesion leading to CSR is proposed to be mediated by the non-homologous end joining (NHEJ) DSB repair system (Manis, Dudley et al. 2002). This system, which is also crucial in the recombination of V(D)J genes, facilitates the joining of the μ switch region and the target switch region and thereby generating a change in the IgH constant region usage. Since switch junctions do not contain long stretches of homology, DSB repair by the HR system is not involved in this mechanism. CSR has therefore been found to be dependent on the NHEJ mediators DNA-PKcs (PRKDC) (Rolink, Melchers et al. 1996; Bemark, Sale et al. 2000), Ku70

(G22P1) (Manis, Gu et al. 1998) and Ku80 (XRCC5) (Casellas, Nussenzweig et al. 1998). It is also likely that CSR is dependent on other factors of the NHEJ system like XRCC4 and DNA Ligase IV (LIG4) which, when defective confers a severe combined immunodeficiency (SCID) syndrome (Manis, Dudley et al. 2002). The histone protein H2AX (H2AFX) has also been proposed to play a role in the chromatin remodeling involved in the long-range recombination process of CSR (Reina-San-Martin, Difilippantonio et al. 2003). However, recent studies rather suggest that the affected CSR in H2AX deficient mice are secondary to a general DSB repair deficiency in these mice (Bassing, Suh et al. 2003; Celeste, Fernandez-Capetillo et al. 2003). H2AX may nonetheless still have a role in this process since it involves DSB formation.

It has also been speculated whether nucleases of the nucleotide excision repair (NER) pathway is involved in the break formation necessary for CSR and possibly also in SHM. In an *in vitro* study by Tian et al. (Tian and Alt 2000) it was found that XPF-ERCC1 and XPG cleave R loops formed in the transcribed S regions and that this system also may take part in the transcription dependent mutagenic process of SHM. However, the NER contribution to SHM and CSR has been evaluated by several groups (Wagner, Elvin et al. 1996) (Shen, Cheo et al. 1997; Jacobs, Fukita et al. 1998) and so far none of the investigated NER members show any involvement in either of the processes. Individuals with xeroderma pigmentosum (XP) and Cockayne syndrome (CS) do also have normal mutation frequencies in the immunoglobulin genes.

4. B cell malignancies

The highly complex maturation process of the B cell, is also implicated in the undesirable, but rare events that can result in the transformation of a healthy B cell to a cancer cell. These events gives rise to a number of different B cell malignancies, summarized in table 1. Several factors, such as the DNA targeted V(D)J gene rearrangements, SHM and CSR, but also the high-proliferative expansion phases occurring in the bone marrow and the secondary lymphoid tissues, can potentially give rise to genetic changes leading to a malignant transformation of the B cell. Characteristic for several lymphomas is a chromosomal translocation involving an Ig locus and a proto-oncogene. These translocations are detrimental since the proto-oncogene will come under the control of the Ig locus. The implication is that the proto-oncogene becomes deregulated and is expressed in the totally wrong context. This phenomenon gets even more complicated when the translocated gene, is a gene that in some way is involved in the regulation of the life and death of the cell, which is the case for proto-oncogenes.

The events leading to these oncogenic changes involves, primarily the recombination machinery, active during the V(D)J rearrangements that occurs at the pre-B cell developmental stages in the bone marrow. This is for instance the case for follicular lymphomas (FL), where the region joining the IgH locus and the BCL2 gene carries characteristic features of a V(D)J rearrangement process (Jager, Bocskor et al. 2000) (Marculescu, Le et al. 2002). It is also likely that this process is involved in the translocation of the BCL1 gene CCND (coding for cyclin D), which is the hallmark of mantel cell lymphoma (MCL) (Welzel, Le et al. 2001). However, the cause of these events is not all that clear and other DNA break forming mechanisms may also be implicated (Jager, Bocskor et al. 2000; Marculescu, Le et al. 2002; Welzel, Le et al. 2001). Thus, the genetic alteration events occurring at later stages of the B cell development, such as SHM and CSR, are also likely to influence the malignant transformation (Goossens, Klein et al. 1998; Bemark and Neuberger 2003). In fact, translocations with characteristic breakpoints near or within the rearranged V(D)J genes, indicates that the translocation occurred during the SHM process, especially also if the V region is mutated (Kuppers 2005). Likewise, translocations with characteristic breakpoints in the CH switch regions indicates a CSR mediated mechanism (Kuppers 2005). Another event that is also likely to have an effect on the malignant transformation is the occurrence of somatic mutations in non-Ig genes. It has been demonstrated that genes coding for BCL6 and CD95 can be mutated as a consequence of a normal GC reaction (Shen, Peters et al. 1998; Pasqualucci, Migliazza et al. 1998; Muschen, Re et al. 2000), but in transformed cells these non-targeted mutations are more extensive and targets other genes (Peng, Du et al. 1999; Pasqualucci, Neumeister et al. 2001).

Although transformed lymphoma cells are burdened by oncogenic translocations that targets the Ig genes, most lymphomas still seem to depend on the BCR for survival (Kuppers 2005). In fact, some lymphomagenesis seem even to be antigen-driven, as is suggested for MCL and CLL (Walsh, Thorselius et al. 2003; Tobin, Thunberg et al. 2003)

Table 1. Human B cell lymphomas (adapted from (Kuppers 2005))

Lymphoma	Chromosomal translocations	Tumor-suppressor gene mutations	Proposed cellular origin
Mantel-cell lymphoma (MCL)	CCND-IgH	ATM, TP53	CD5+ Mantel-zone B cell
Chronic lymphocytic leukemia (CLL)	-	ATM, TP53	Memory, Naïve or MZ B cell
Follicular lymphoma (FL)	BCL2-IgH	-	GC B cell
Diffuse large B cell lymphoma (DLBCL)	BCL6-various BCL2-IgH MYC-IgH MYC-IgL	CD95, ATM, TP53	GC or post-GC B cell
MALT lymphoma	API2–MALT1 BCL10-IgH MALT1-IgH FOXP1-IgH	CD95	MZ B cell
Multiple myeloma (MM)	CCND1-IgH FGFR3-IgH MAF-IgH	CD95	Plasma cell

Additional components in the microenvironment have also shown to be of importance for lymphomagenesis (Hussell, Isaacson et al. 1996; Dave, Wright et al. 2004). FL, for example, seem to have a dependency of CD40 signaling for survival (Johnson, Watt et al. 1993; Ghia, Boussiotis et al. 1998), whereas MCL has recently been shown to acquire survival mechanisms dependent on normal immuno-regulatory circuits (Ek, Björk et al 2005, in press). Together this illustrates that several features of the non-malignant B cell are retained in the malignant cell and that these features are critical for the fate of the transformed cell. It is important to keep this in mind when trying to understand the malignant B cell behavior and to delineate their cellular origin. Thus, since most of the oncogenic translocations in lymphoma are associated with Ig genes, it can be foreseen that the onset of the tumor outgrowth is linked to events in the B cell development that requires Ig gene transcription. Therefore, both TD and TI antigen-activation may be founding events in this process, and that the resulting tumor phenotype is dictated by the translocated proto-oncogene. Whether or not this implies a "freezing" of a certain differentiation stage, at which the transforming event occurred, is unclear. Although, several studies have used this as a basis for the delineation of the cellular origin of the malignant cell (Shaffer, Rosenwald et al. 2002). Additionally, since several lymphomas have mutated Ig genes it could be conceived that many of these originates from the GC reaction (Kuppers 2005). This has for instance been suggested for MCL, which have been found to have mutated Ig genes (Du, Diss et al. 1997). In paper II we explored the transcriptional profile of MCL, comparing them to non-malignant mature B cells, comprising subsets of naïve, GC and memory B cells. In this study we found that the MCLs had an expressional profile that could be associated more with the pre-activated non-GC B cell subsets than with the GC B cell subsets. It was further exemplified by the regulation of CCR7, which was upregulated, and CXCR5, which was downregulated, that the transforming event had occurred during the initial phases of antigen-activation. Nevertheless, an oncogenic translocation of the MCL character could very well be introduced during the GC reaction as suggested (Kuppers 2005), this would then explain a mutated and clonaly related phenotype.

Taken together, it is becoming increasingly clear that several aspects of the normal B cell development needs to be considered when trying to understand the malignant B cell differentiation (Kuppers 2005).

Concluding remarks

A common theme for the works in this thesis has been the use of fluorescent activated cell sorting (FACS) techniques to isolate several different human mature B cell subsets, which have subsequently been analyzed by means of high-density oligonucleotide microarrays. Some brief methodological considerations regarding the techniques used, needs to be made.

FACS has always been tightly linked to studies in B cell biology. Leonard and Leonore Herzenberg pioneered this field in the late sixties and have since then been instrumental in the further development of the technique and its implementation in immunology and many other fields of biology. The first instrument to be built was founded on the principles around the particle sorting instrumentation originally used in the atomic-bomb testing program, in Los Alamos, NM (Herzenberg 2004). This first instrument was intended for the immunological research carried out at the Herzenberg's and Weissman's lab, at Stanford University, CA. The first paper using the technique is a Science study from 1969, which demonstrated how the new cell-sorting method could be used to enrich antibody forming cells from mouse spleens (Hulett, Bonner et al. 1969). The Herzenbergs then further added two-color (1983), three-color (1984) and eventually eleven-color (2001) and now seventeen-color (2004) immuno-fluorescence capacity to the instruments (Herzenberg 2004) (Perfetto, Chattopadhyay et al. 2004).

In the studies in this thesis, I have utilized several different types of instruments, ranging from the low pressure FACSVantage to the new digital high-speed multiparameter FACSAria sorters. Although, the newer instruments are sorting several times faster than the old versions, it is amazing that it still takes all night to isolate the material. However, the first study (paper I) only implicated one B cell population (naïve B cells), isolated by three-color flow cytometry. In the second and third study (paper II and III) we sorted five different B cell populations still not using more than three colors. In the last study (paper IV) we sorted nine different B cell populations of which several never have been characterized before. For this study we used as many as seven colors on a modified FACSDiva.

It stands clear that the increasing amount of colors added to these sorting- and analysis-regimens will benefit our understanding of biology. The more character-markers that can be used at the same time, the more detailed the information is, that is gained around each cell population studied. For instance, in paper III we found that the $CD77^-$ GC B cell population represented a heterogeneous subset of cells consisting of both centroblasts, centrocytes and plasmablasts. This study was based on the current phenotypic characteristics for human centroblasts and centrocytes, and we concluded that the GC B cell phenotype needs to be revised. In paper IV we discriminated GC B cells differently, thereby getting more representative subsets, however, still not indicating any clear-cut functional dichotomy between centroblasts and centrocytes, as based on the transcriptional profile. However, in this study we identified the plasmablast population that was retained within the centrocyte population in paper III. Indeed, this population was both IgD^- and $CD38^+$ but, importantly, it was also IgM^+. Thus, by not using IgM as a discriminator in paper III, the IgM^+ plasmablast subset was included in the centrocyte subset, which clearly

showed up in the transcription analysis. This illustrates that it is difficult to associate an absolute phenotype to a cell, since there will always be another marker, which distinguish a different biological activity.

One problem with the use of many colors in flow cytometry, is that the multidimensionality of the data makes it non-intuitive and hard to comprehend since we in our minds are limited to three dimensions only. Based on this, we keep on running our multicolor sorts and analysis using the same rationale we've used since two-color flow cytometry. By this, we are missing out on a lot of relevant biological information hidden within the data. Therefore, the next piece in the puzzle must be to find a rational way of analyzing multidimensional flow cytometry data. I personally think that we need to stop visually interpret the data and instead apply robust statistical analysis algorithms. For this, I think we can learn a lot from the strategies used in microarray data analysis.

We have in all the studies, paper I-IV, used the Affymetrix GeneChip technology. This technology was developed during the 1990s (Chee, Yang et al. 1996); originally envisaged conceptually by Fodor et al. in 1991 (Fodor, Read et al. 1991). In contrast to the two-color, spotted, cDNA-technology, developed at the Brown-lab at Stanford University (Schena, Shalon et al. 1995), the Affymetrix technology make use of a photolithography technique when fabricating their GeneChips, which consists of several hundred thousands probes of *in situ*-synthesized 25-mer oligonucleotides. In paper I the HuGeneFL GeneChip (app 5,600 genes) was used, in paper II: HG-U95A (app. 10,000 genes); in paper III: HG-U133A (app. 14,500 genes) and in paper IV the HG-U133 Plus 2.0 (app 38,500 genes) was used.

Paper I is the outcome of one of the first microarray studies carried out at the department. In this paper we ran a transcriptional profiling analysis of B cells that had been cultured *in vitro*. From this study we learned a lot about the possibilities and limitations of microarray analysis. For example, the data-sets in this first study (paper I) were very "noisy". We came to the conclusion that several underlying parameters caused this. First of all, it is likely that the individual "pre-excision" activation-status of the fresh biological samples greatly affects the outcome of the analysis, especially when culturing the cells *in vitro*. This would therefore introduce a great variance in the data-sets. Furthermore, in this particular analysis we had two well characterized basal signals (anti-BCR and anti-CD40) upon which, we added an unknown signal (anti-CD44), which was not as potent as either of the other signals. For that reason the anti-CD44 signal seemed to be masked, to some degree, by the two other signals. Our solution was to apply Bayesian statistics to the data-analysis. In this way we could extract the most probable behavior of the genes in the data-set. In contrast, with all the experience from paper I-III, paper IV came out as a schoolbook example. In this analysis all biological replicates were processed together, in an attempt to minimize the effects from technical inconsistencies. Also, the fresh material was processed immediately coming out of the FACS. We further, applied a robust data pre-processing strategy, before the analysis of the material, resulting in very high correlation coefficients between replicates. What these studies pinpoints is that microarray analysis can be a useful tool when applied to clear and well defined biological contexts.

Populärvetenskaplig sammanfattning

Den här avhandlingen omfattar fyra olika arbeten som handlar om immunförsvarets B-celler. B-celler är en typ av blodceller som tillhör de vita blodkropparna och som finns i blod och i lymfkörtlar. B-cellernas främsta funktion är att producera antikroppar när vi blir sjuka, smittade av virus eller av bakterier. Antikroppar är sinnrikt konstruerade proteiner som har förmågan att binda till de virus eller bakterier som smittat oss. Då antikropparna har bundit till viruset eller bakterien startas en reaktion som får till följd att viruset eller bakterien dödas av kroppens immunförsvar.

B-cellerna har sitt ursprung ur benmärgen, som finns i alla kroppens ben. I benmärgen finns s.k. blodbildande stamceller som kan ge upphov till blodets alla olika celler, från röda blodkroppar till vita blodkroppar. Ur dessa blodbildande stamceller bildas hela tiden nya B-celler. Innan dessa nybildade B-celler blir s.k. mogna B-celler som kan cirkulera runt i blodet och till lymfkörtlar, måste de genomgå en mognadsprocess i benmärgen. Under denna mognadsprocess kommer delar av B-cellens arvsmassa, dvs DNAt, att ”blandas om” genom s.k. rekombination. De delar av DNAt som genomgår denna rekombination består av de gener (DNA kod) som ska användas när B-cellen ska bilda antikroppar då vi blir sjuka. Tanken med denna rekombinationsprocess är att blanda om i antikroppsgenens DNA-kod så att en B-cells antikroppsgener skiljer sig helt och hållet från alla andra B-cellers antikroppsgener. Om vi i kroppen har flera miljarder B-celler innebär det att det finns flera miljarder B-celler med en uppsättning helt unika antikroppsgener. När denna rekombinationsprocess är färdig kommer B-cellen att tillverka antikroppar med utgångspunkt från de omblandade antikroppsgenerna. Först börjar den att tillverka antikroppar som sitter på utsidan av B-cellen. Dessa antikroppar blir en del av vad vi kallar B-cells-receptorn. B-cells-receptorns funktion är att binda in till allt som är okänt som t.ex. ett virus eller en bakterie. Eftersom antkroppsgenerna blandas om helt slumpmässigt innebär det att när en bakterie infekterar oss så lär det finnas åtminstone någon eller några av dessa miljarder B-celler som har en B-cells-receptor som kan binda till den okända bakterien. När B-cellen har en B-cell-receptor på utsidan är den en mogen B-cell. Först nu kan den cirkulera runt i blodet och till lymfkörtlar.

I de arbeten som presenteras i denna avhandling har vi tittat på vad som händer med den mogna B-cellen efter att den lämnat benmärgen.

I det första arbetet tittade vi närmare på det som händer med en mogen B-cell efter att den bundit ett s.k. antigen, t.ex. en bakterie. När detta har skett kommer B-cellen att behöva hjälp från en T-cell, en annan av immunförsvarets celler. Denna hjälp leder till att B-cellen kan bilda ett s.k. germinalt center där den snabbt börjar växa och dela på sig för att skapa fler av sig själv. Detta sker i lymfkörtlarna. Vi studerade vad som händer i B-cellen efter att den interagerat med T-cellen, och mer specifikt undrade vi vad en receptor som heter CD44 gör på B-cellen. Vi kom fram till att CD44 aktiverar flera olika molekyler som kan kopplas till s.k. immunomodulering och inflammation. Detta kan tänkas påskynda bildandet av ett germinal center.

I det andra arbetet studerade vi en typ av B-cells-cancer s.k. mantelcellslymfom (MCL) som egentligen är mogna B-celler som är sjuka. I den här studien tittade vi på dessa MCL-celler i relation till friska B-celler för att försöka lista ut när MCL-B-cellen blir sjuk. Vi kom

fram till att det antagligen var så att MCL-B-cellen hade blivit sjuk efter det att den träffat på ett antigen. Det som då gjort att cellen, istället för att reagera som en vanlig B-cell, utvecklats till en sjuk MCL-B-cell beror på ett fel som uppstått under rekombinationsprocessen i benmärgen. Felet är att en annan gen har smugit sig in, intill antikroppsgenen. När den felaktiga genen blir aktiverad efter att B-cellen träffat på ett antigen kommer allt gå snett och ge upphov till B-cellslymfom.

I det tredje arbetet tittade vi närmare på olika utmognadsformer av den mogna B-cellen. Dessa utmognadsformer finns i lymfkörtlarna men inte i blodet. Det är viktigt att förstå mer kring dessa olika B-cellsformer eftersom de kan ge upphov till flera andra typer av B-cellslymfom som t.ex. follikulärt lymfom och diffust storcelligt lymfom. Det visade sig i våra studier att en av dess B-cellsformer, den s.k. centrocyten inte betedde sig som man tidigare trott. Vi kunde visa att centrocyten, till skillnad från vad man tidigare trott, var en högst aktiv cell som var mycket lik den s.k. centroblasten, vilken är ett föregångsstadie till centrocyten i utmognadsprocessen.

I det fjärde arbetet tittade vi djupare in i andra mogna B-cellsformer, totalt nio stycken, av vilka flera tidigare aldrig varit studerade. Vi kunde i våra analyser se att man kan dela in dessa B-cellsformer i tre grupper. Den första gruppen utgörs av s.k. naiva och minnesceller, den andra gruppen innefattar centroblaster och centrocyter och i den tredje gruppen återfinns de s.k. plasma cellerna, dvs. de celler som tillverkar antikroppar som utsöndras till blodet vid en infektion.

I alla dessa arbeten har två huvudsakliga tekniker använts. För att specifikt kunna plocka ut och särskilja B-cellerna från varandra har en teknik som heter fluorescens-aktiverad cellsorteing (FACS) använts. Med denna teknik kan vi särskilja B-cellerna, som beroende på vilken utmognadsform de befinner sig i, kan märkas in med olika fluorecerande molekyler. Dessa inmärkta B-celler känns igen på sitt unika ”fingeravtryck” av fluorescens och med hjälp av detta ”fingeravtryck” kan vi instruera FACS-maskinen att sortera ut den ena typen av B-cell till ett provrör och den andra typen av B-cell till ett annat provrör. Totalt kan maskinen sortera ut celler till fyra olika rör samtidigt och den kan utföra denna sortering med en hastighet på upp till 20 000 celler i sekunden.

Den andra tekniken som använts är en typ av analys som mäter vilka och hur många av cellernas totalt ca 30 000 olika gener som är aktiva i ett visst skede. Tekniken kallas på svenska för mikromatrisanalys eller populärt genchipanalys. Med denna teknik kan vi se att de gener som är aktiva i en naiv cell skiljer sig helt från de gener som är aktiva i en centroblastcell. Så i våra arbeten har de celler som vi sorterat ut med hjälp av FACS analyserats med genchipanalys. På detta sätt har vi kunnat få en mycket detaljerad bild av de olika B-cellsformerna och vad som skiljer dem åt.

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