Molecular definition of the germinal centre stage of B-cell differentiation

Chi Ma and Louis M. Staudt*

Metabolism Branch, Division of Clinical Sciences, National Cancer Institute, Bethesda, MD 20892, USA

Genomic-scale gene expression analysis provides views of biological processes as a whole that are difficult to obtain using traditional single-gene experimental approaches. In the case of differentiating systems, gene expression profiling can define a stage of differentiation by the characteristic expression of hundreds of genes. Using specialized DNA microarrays termed 'Lymphochips', gene expression during mature B-cell differentiation has been defined. Germinal centre B cells represent a stage of differentiation that can be defined by a gene expression signature that is not shared by other highly proliferative B-cell populations such as mitogenically activated peripheral blood B cells. The germinal centre gene expression signature is maintained to a significant degree in lymphoma cell lines derived from this stage of differentiation, demonstrating that this gene expression programme does not require ongoing interactions with other germinal centre cell types. Analysis of representative cDNA libraries prepared from resting and activated peripheral blood B cells, germinal centre centroblasts, centrocytes and tonsillar memory B cells has confirmed and extended the results of DNA microarray gene expression analysis.

Keywords: microarray; Lymphochip; germinal centre; B-cell differentiation; gene expression; genomics

1. INTRODUCTION

One important line of immunological inquiry has used lymphocyte cell surface markers to define stages of lymphocyte differentiation. Cell sorting experiments have revealed important functional changes that occur in concert with these cell surface expression changes. As new markers have been defined, more refined stages of differentiation have been proposed, making the lymphoid system one of the best studied differentiation systems in mammalian biology. However, the fact that lymphocyte differentiation stages have been subdivided repeatedly during the last decade suggests that we need a more robust definition of what 'differentiation stage' means. Often, key events in differentiation are controlled by the expression of transcription factors or signalling molecules that then activate or repress a set of target genes. Genetic ablation of these regulatory genes can cause an arrest of differentiation and is one way in which one could define a differentiation stage. This approach fails if compensatory mechanisms are invoked by the differentiating cell in the absence of the regulatory factor, leading to a pseudo-differentiation stage that never occurs in wild-type animals.

A complementary way in which differentiation stage could be defined is by gene expression profiling on a genomic scale. Using cDNA microarray technology, the mRNA levels of as many as 20 000 genes can be quantitated in parallel in a single experiment (Staudt & Brown 2000). Mathematical analysis of such data using 'clustering' algorithms can find patterns in the voluminous data set that emerges from microarray experiments

(Eisen *et al.* 1998). These algorithms group genes together that show coordinate expression across the samples studied. Often, clustered genes appear to share a biological theme and thus have been termed gene expression 'signatures' (Alizadeh *et al.* 2000). A gene expression signature can be defined either because expression of the signature correlates with a particular cell type (e.g. B-cell restricted genes) or a biological process (e.g. cellular proliferation). Stages of differentiation, as defined by cell surface markers, can have a discrete gene expression signature. Germinal centre B cells express a large set of genes in a coordinated manner that distinguish them from B cells at other stages of differentiation (Alizadeh *et al.* 2000) and this signature is the focus of this report.

A further confounding issue in the study of differentiation is provided by the interplay between cell types. Thus, signals delivered by a neighbouring cell type (or from a distance by a cytokine or hormone) may alter gene expression. Should such 'activation' events be construed as changing the differentiation stage? In some cases, extrinsic signals may transiently alter gene expression but, when the cell is removed from the signals, the cell will return to its original state. This is what is presumed to happen when lymphocytes are stimulated *in vitro* through the antigen receptor and are then 'rested down'. This assumption has yet to be tested by gene expression profiling, however. In other cases, extrinsic signals may irreversibly push a cell to a new differentiation stage, although the mechanisms underlying such irreversible changes have not been clearly elucidated. In one possible scenario, a key transcription factor might be upregulated by the extrinsic signal and subsequently this factor might maintain its own expression via an autoregulatory loop. Alternatively, a change in chromatin structure caused by

*Author for correspondence (lstauidt@box-1.nih.gov).

the extrinsic signal might become stable, leading to a change in differentiation state that is inherited by daughter cells. The phenomenon of position effect variegation that has been described in *Drosophila* and yeast provides an example of stable chromatin effects that modify gene expression (Wakimoto 1998). Most definitions of differentiation stage would incorporate some aspect of stability and irreversibility in phenotype, irrespective of the mechanisms by which this is achieved.

The germinal centre reaction is an exquisite example of the interplay of extrinsic signals and differentiation (reviewed in Kelsoe 1996; MacLennan 1994). In response to an antigenic stimulus, naive B cells and T-helper cells collaborate to induce differentiation of both cell types. The B cells adopt one of two fates. During the first few days following antigenic challenge, some antigen-specific B cells differentiate within the periarteriolar sheath into antibody-secreting plasmablasts and plasma cells. These cells provide the bulk of the serum antibody during the first week. Alternatively, the B cell migrates into the follicular area towards follicular dendritic cells and establishes a new microenvironment, the germinal centre. Here, in concert with antigen-specific germinal centre T cells and follicular dendritic cells, B cells mutate their immunoglobulin genes. This specialized hypermutation machinery is activated within centroblasts, the highly proliferating cells of the germinal centre dark zone. Centroblasts give rise to less proliferative centrocytes, which are presumed to engage in a process of negative selection, whereby B cells are eliminated that have lost the ability to bind antigen as a consequence of somatic mutation.

Following multiple rounds of mutation and selection, centrocytes terminally differentiate into memory B cells and plasma cells. A variety of memory B-cell types have been defined. A tonsillar B-cell population that is depleted of naive B cells, germinal centre B cells and plasma cells has been shown to be enriched for functional memory B cells (Liu *et al.* 1995). A B-cell population in the peripheral blood that is CD27⁺ is enriched in cells that have undergone immunoglobulin somatic hypermutation and are presumed to be memory B cells (Klein *et al.* 1998). Splenic CD27⁺, CD148⁺ B cells may represent a similar memory B-cell population (Tangye *et al.* 1998).

Interestingly, no combination of *in vitro* conditions has been devised that mimics the differentiation of naive B cells into cells with the full germinal centre phenotype. By definition, signalling through the B-cell antigen receptor is required for germinal centre B-cell differentiation. A further required signal is delivered by CD40 as a result of interactions with CD40 ligand on activated T-helper cells (Castigli *et al.* 1994; Kawabe *et al.* 1994). Attempts to create germinal centre B cells *in vitro* from naive B cells have often involved cross-linking the B-cell receptor and CD40, together with a variety of other stimuli (Forman & Pure 1991; Galibert *et al.* 1996; Qin *et al.* 1998; Wheeler & Gordon 1996). To date, these conditions have failed to generate bona fide germinal centre B cells, as judged by expression of known germinal centre B-cell markers. In particular, such conditions do not upregulate a highly specific germinal centre marker, *BCL-6* (Allman *et al.* 1996). *BCL-6* is a proto-oncogene

that is translocated frequently in human non-Hodgkin's lymphomas and is required for germinal centre formation (Dent *et al.* 1997; Fukuda *et al.* 1997; Ye *et al.* 1997). *BCL-6* protein expression is upregulated in germinal centre B cells compared with peripheral blood B cells (Allman *et al.* 1996). However, *in vitro* stimulation of peripheral blood B cells by anti-IgM and CD40 ligand actually extinguishes *BCL-6* mRNA and protein expression (Allman *et al.* 1996). Thus, despite the fact that B-cell receptor and CD40 signalling events are clearly required to initiate germinal centre differentiation, there are still key signalling events that direct germinal centre differentiation *in vivo* that remain to be elucidated.

2. THE GERMINAL CENTRE B-CELL GENE EXPRESSION SIGNATURE

Genomic-scale gene expression profiling has been used to define more fully the germinal centre phenotype with molecular precision (Alizadeh *et al.* 2000). The major tool enabling this effort has been the Lymphochip DNA microarray, a specialized microarray that currently contains over 18 500 cDNAs representing human genes relevant to the study of normal and pathological immune responses (Alizadeh *et al.* 1999). Roughly 15 000 clones on the Lymphochip represent novel genes of unknown function that were identified during high throughput sequencing of cDNA clones from a variety of B lymphocyte cDNA libraries. 12 000 of the Lymphochip cDNAs were chosen from a germinal centre B-cell cDNA library, whereas the remainder of the novel clones came from libraries of various B-cell malignancies. 3500 array elements on the Lymphochip represent 'named' genes for which a full length cDNA has been sequenced and a function has been proposed. Thus, the Lymphochip has two essential uses: expression analysis of the named genes on the array provides immediate insight into the biology of the cell type being studied, whereas differences in expression of the novel genes provide robust gene expression signatures of different cell populations.

To identify genes preferentially expressed in germinal centre B cells, a variety of mature B cell populations were prepared (figure 1) and compared using Lymphochip gene expression profiling (Alizadeh *et al.* 2000). Figure 2 depicts the relative expression of 86 genes (represented by 107 array elements) in resting peripheral blood B cells, *in vitro* activated peripheral blood B cells, germinal centre B cells and four haematopoietic cell lines (data used for this analysis are publicly available at <http://lmpp.nih.gov/lymphoma>). Genes were selected that were more highly in the germinal centre B-cell populations (either total germinal centre B cells or purified centroblasts) than in any of the resting or activated peripheral blood B-cell samples. Since germinal centre centroblasts are more highly proliferative than these other B-cell populations, this selection criterion did not exclude genes that are generally upregulated during cellular proliferation. Indeed, several hundred genes represented on the Lymphochip were found to be cell-cycle regulated (Alizadeh *et al.* 2000). To exclude such proliferation genes from the present analysis, the gene list was further trimmed by requiring genes to be expressed at least threefold more highly in germinal centre cells than in a highly proliferating myeloid cell line,

Figure 1. Purification scheme for human B-cell subpopulations. mRNA prepared from each of the isolated B-cell populations was used to generate directional cDNA libraries and for gene expression analysis using Lymphochip DNA microarrays.

U937. A hierarchical clustering algorithm (Eisen *et al.* 1998) was used to order the resulting gene list such that genes that were correlated in their expression across this set of samples were placed next to each other in a tabular display (figure 2). In this display, relative gene expression is depicted according to a grey intensity scale, with white indicating higher expression and black indicating lower expression. The clustering algorithm grouped multiple array elements representing the same human gene together in most cases, demonstrating the reproducibility of the gene expression measurements. The position of named genes is indicated in figure 2; all other rows represent novel genes on the Lymphochip that were expressed at elevated levels in germinal centre B cells.

This analysis identified a number of genes already reported to be preferentially expressed in germinal centre B cells, including the cell surface proteins CD10 and CD38. *BCL-6* mRNA was clearly more highly expressed at the germinal centre stage of differentiation, although the high expression of *BCL-6* protein in germinal centre B cells is largely due to post-transcriptional regulatory mechanisms (Allman *et al.* 1996). *A-myb* is a transcription factor highly expressed in centroblasts that can cause follicular hyperplasia when overexpressed in transgenic mice (DeRocco *et al.* 1997; Golay *et al.* 1998). Another gene upregulated in germinal centre B cells, *OGG1*, encodes a DNA repair protein that recognizes and excises oxidated guanosine residues (Bruner *et al.* 2000; Kuo & Sklar 1997). Interestingly, another DNA repair gene, *FA-A* (Fanconi Anemia Complementation Group A), was also highly expressed in germinal centre B cells suggesting that these cells may coordinately induce DNA repair genes in a coordinated way in order to cope with challenges to genome integrity that may result from the rapid proliferation rate of centroblasts.

Genes involved in B-cell receptor signalling were found to be coordinately upregulated in germinal centre B cells. The B-cell receptor beta chain gene, *CD79B*, was increased in expression at this stage of differentiation as were the genes for the cell surface proteins CD21, CD81 and CD22 which modulate B-cell receptor signalling. A

role for complement binding and the recognition of immune complexes by CD21 in the germinal centre has been suggested previously (reviewed in Fearon & Carroll 2000). Interestingly, genes encoding intracellular signalling molecules involved in B-cell receptor signalling were also upregulated in germinal centre B cells. *BLNK* is a necessary adapter protein that is required to induce calcium signals following B-cell receptor signalling (reviewed in Kurosaki & Tsukada 2000). Phospholipase $\text{C}\gamma 2$ was also upregulated in germinal centre B cells, which is notable because *BLNK* interacts with phospholipase $\text{C}\gamma 2$ during B-cell receptor signalling. Another adapter protein, *APS*, is tyrosine phosphorylated following B-cell receptor signalling, but its function has not been clearly defined (Yokouchi *et al.* 1997). Finally, it is noteworthy that the *src* family tyrosine kinase *lck* is expressed highly in germinal centre B cells. Since *lck* is critical for T-cell receptor signalling, it is possible that germinal centre B cells use *lck* during B-cell receptor signalling.

Two proto-oncogenes, *LMO2* and *BCL-7A*, were found to be expressed highly at the germinal centre stage of differentiation. *LMO2* is translocated in acute T-cell leukaemias and causes a block in T-cell differentiation and subsequent leukaemia formation when overexpressed in transgenic mice (Rabbitts 1998). The high expression of *LMO2* in germinal centre B cells raises the possibility that it blocks terminal differentiation in the B lineage as well. *BCL-7A* is recurrently translocated in human non-Hodgkin's lymphomas, albeit infrequently (Zani *et al.* 1996). The germinal-centre-restricted expression of *BCL-7A* and its translocation in non-Hodgkin's lymphomas is strikingly reminiscent of *BCL-6* (Staudt *et al.* 1999). Thus, *BCL-7A*, like *BCL-6*, may control normal germinal centre B-cell physiology, and this normal function may be co-opted and deregulated in some germinal-centre-derived lymphomas.

3. THE GERMINAL CENTRE PHENOTYPE DEFINES A STABLE B-CELL DIFFERENTIATION STAGE

Lymphochip microarrays have also been used to profile gene expression human B-cell lymphomas, revealing

Figure 2. DNA microarray analysis of gene expression in purified normal human B-cell subpopulations and haematopoietic cell lines. Relative gene expression among the samples is depicted over a 16-fold range according to the grey scale shown at the bottom. Each row represents a different element on the Lymphochip microarray. Rows representing named human genes are indicated; other rows represent novel genes derived from a germinal centre B-cell library. The samples tested were: 1, total CD19 + blood B cells; 2, naive CD27 – blood B cells; 3, blood B cells; anti-IgM 6 h; 4, blood B cells; anti-IgM + CD40 ligand 6 h; 5, blood B cells; anti-IgM + IL-4 6 h; 6, blood B cells; anti-IgM + CD40 ligand + IL-4 6 h; 7, blood B cells; anti-IgM 24 h; 8, blood B cells; anti-IgM + CD40 ligand 24 h; 9, blood B cells; anti-IgM + IL-4 24 h; 10, blood B cells; anti-IgM + CD40 ligand + IL-4 24 h; 11 and 12, blood B cells; anti-IgM + CD40 ligand 48 h; 13, tonsil germinal centre B cells; 14, tonsil germinal centre centroblasts; 15, SUDHL6 (germinal centre B-like DLBCL cell line); 16, OCI-Ly3 (activated B-like DLBCL cell line); 17, Jurkat (T-cell line); 18, U937 (myeloid cell line). Data for this figure were obtained from <http://lmpp.nih.gov/lymphoma>.

unexpected heterogeneity within certain diagnostic categories (Alizadeh *et al.* 2000). Most non-Hodgkin's B-cell lymphomas appear to be derived either from germinal centre B cells or from a later stage of B-cell differentiation since they bear rearranged immunoglobulin genes with extensive somatic mutations (Kuppers *et al.* 1999). Gene expression profiling of the two most common forms of non-Hodgkin's lymphoma—diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma—defined the relationship between these malignancies and particular stages of B-cell differentiation. Follicular lymphomas expressed most of the genes within the germinal centre B-cell signature suggesting that this malignancy arises from a germinal centre B cell *per se*. In keeping with this notion, follicular lymphomas are capable of ongoing somatic hypermutation of their immunoglobulin genes (Bahler & Levy 1992), demonstrating that certain normal functions of germinal centre B cells are maintained even after oncogenic transformation. Indeed, the sustained expression of germinal centre genes in follicular lymphomas suggests that these malignant cells retain much of the biology of normal germinal centre B cells.

Diffuse large B-cell lymphomas were more heterogeneous in gene expression and could be divided into two subgroups, one that resembled germinal centre B cells in gene expression (germinal centre B-like DLBCL) and another that more closely resembled activated peripheral blood B cells (activated B-like DLBCL) (Alizadeh *et al.* 2000). This gene expression heterogeneity was accompanied by a striking difference in clinical response to chemotherapy: whereas three-quarters of the germinal centre B-like DLBCL patients were alive five years following initial therapy, less than one-quarter of the activated B-like DLBCL patients survived five years. Since these two subtypes of DLBCL appear to be derived from different stages of normal B-cell differentiation and also have distinct clinical courses, it is reasonable to consider these subgroups as two distinct diseases.

Interestingly, lymphoma cell lines were identified that mimicked, in part, the gene expression profile of each of the DLBCL subtypes (Alizadeh *et al.* 2000). Figure 2 shows one cell line (DLCL-GCB/SUDHL6) that has high expression of many, but not all, of the genes in the germinal centre B-cell signature. Indeed, other cell lines derived from DLBCL and Burkitt's lymphoma also express many germinal-centre-restricted genes (C. Ma and L. M. Staudt, unpublished data). By contrast, another cell line (DLCL-ABC/OCI-Ly3) lacks expression of most of the germinal centre markers (figure 2) and instead expresses genes characteristic of activated B-like DLBCL (Alizadeh *et al.* 2000). The fact that cultured cell lines can maintain the germinal centre B-cell gene expression profile demonstrates that at least part of this phenotype is stable in the absence of signals derived from other germinal centre cell types. This argues that the germinal centre B cell represents a distinct stage of differentiation rather than a specialized and reversible form of lymphocyte activation. However, lymphoma cell lines do not maintain high expression of all of the germinal centre B-cell genes (figure 2). Those germinal centre B-cell genes that are not expressed in the lymphoma cell lines may include genes that are upregulated by signals derived

from germinal centre T cells and/or follicular dendritic cells.

4. A GENOMIC PERSPECTIVE ON B-CELL DIFFERENTIATION

The analysis of gene expression at different B-cell differentiation stages using the Lymphochip has revealed how much we do not know about this differentiation system. Most of the differentially expressed genes discovered in this analysis are novel genes derived from a germinal centre B-cell library. Since these genes were identified using only a short, single-pass cDNA sequence, their coding regions are not currently known. To define further the differentiation stages in which these novel genes are expressed, we created representative cDNA libraries from resting and mitogenically activated peripheral blood B cells, and from tonsillar centroblasts, centrocytes and memory B cells (figure 1). To obtain sufficient mRNA to create high-quality cDNA libraries, at least 1×10^8 cells of each B-cell type were purified by a combination of magnetic and fluorescent cell sorting. cDNA prepared from these sorted cells was cloned directionally into phage vectors, yielding a minimum of 1×10^6 recombinants in each library. These libraries are valuable tools for the study of B-cell differentiation since they are, in essence, renewable repositories of the expressed genes in these relatively rare B-cell subpopulations.

These B-cell stage-specific cDNA libraries were used to confirm and extend the results from the Lymphochip gene expression analysis. Plasmid DNA was prepared from each library by *in vivo* excision in bacteria and this DNA was used as a template for the amplification of genes by PCR. PCR primers were prepared for 61 novel genes that were found by Lymphochip analysis to be differentially expressed among normal germinal centre B-cell samples and lymphoma samples (figure 3). Each of the libraries yielded comparable PCR signals for the control gene *GAPDH*. PCR products for a known germinal-centre-restricted gene, *A-myb*, were obtained from the centroblast, centrocyte and tonsillar memory libraries, but not from the resting or activated peripheral blood B-cell libraries or from an erythroleukaemia cell line (K562) library. By immunohistochemistry, A-myb protein has been reported to be restricted in expression to germinal centre B cells (Golay *et al.* 1998), despite the fact that A-myb mRNA was detected in the tonsillar memory B-cell library. This apparent discrepancy may be explained by the fact that tonsillar memory B cells are diffusely localized in the intraepithelial areas of the tonsil (Liu *et al.* 1995) and might be easily overlooked using immunohistochemistry.

The results from seven novel germinal centre genes are shown in figure 3. Each of these genes, as expected from the Lymphochip DNA microarray analysis, yielded signals from the total germinal centre library and not from the erythroleukaemia library. Roughly 20% of the novel genes, exemplified by *GC 4* in figure 3, showed the same expression pattern as *A-myb* by this PCR analysis. Another common expression pattern (*ca.* 20% of genes tested) was characterized by induced expression during mitogenic activation of peripheral blood B cells along

Figure 3. Analysis of gene expression using cDNA libraries derived from sorted B-cell subpopulations. PCR primers for seven novel germinal centre genes (*GC 1–7*), *A-myb*, and *GAPDH* were used to amplify these genes from the eight cDNA libraries shown. Amplification products were electrophoresed through agarose gels and were visualized by ethidium bromide staining. The nature of the B-cell populations used to make the cDNA libraries is illustrated in figure 1.

with expression in tonsillar germinal centre and memory subsets (e.g. *GC 5* in figure 3). A wide variety of different expression patterns were additionally detected, some of which are illustrated in figure 3. Two novel genes were found to be expressed only in centroblasts and centrocytes (e.g. *GC 1* in figure 3) and might conceivably play a role in germinal-centre-specific functions such as somatic hypermutation of immunoglobulin heavy chain class switching.

5. SUMMARY

In a sense, these early genomic experiments are allowing us to peer over the canyon's edge into a rich valley of B-cell biology yet to be explored. Already these technologies have yielded surprises that have altered our definitions of lymphoid malignancies. In the future, the clues provided by the high-throughput technologies described in this report will guide functional genomics experiments in an effort to understand the machinery and logic of B-cell differentiation. The wide variety of gene expression fluxes evident in figure 3 highlights the complex orchestration of responses within the germinal centre microenvironment. Some of these gene expression changes may relate to the state of cellular proliferation while others may reflect signalling from germinal centre T cells and follicular dendritic cells. One approach to dissect these issues will be to employ *in vitro* cultures of germinal centre B cells in which cell signals can be manipulated. It will clearly also be important to extend these efforts into the mouse to make use of genetic tools to dissect the functions of the many novel germinal centre genes defined in this work.

We wish to thank the many individuals in Louis Staudt's laboratory and in the laboratories of Patrick Brown and David Botstein at Stanford University who contributed to the Lymphochip project. We are especially grateful to Bob Strausberg, Rick Klausner and the Cancer Genome Anatomy Project at the National Cancer Institute for their support in creating the Lymphochip microarray. We also thank Fatima Abbasi, Howard Mostowski and Gerald Marti, Center for Biologics Evaluation and Research, Food and Drug Administration, for their help in sorting B-cell subpopulations and Holy Cross Hospital, Silver Spring, MD, USA, for providing tonsil specimens.

REFERENCES

- Alizadeh, A. (and 19 others) 1999 The Lymphochip: a specialized cDNA microarray for the genomic-scale analysis of gene expression in normal and malignant lymphocytes. *Cold Spring Harbor Symp. Quant. Biol.* **64**, 71–78.
- Alizadeh, A. A. (and 30 others) 2000 Identification of molecularly and clinically distinct types of diffuse large B-cell lymphoma by gene expression profiling. *Nature* **403**, 503–511.
- Allman, D., Jain, A., Dent, A., Maile, R. R., Selvaggi, T., Kehry, M. R. & Staudt, L. M. 1996 *BCL-6* expression during B-cell activation. *Blood* **87**, 5257–5268.
- Bahler, D. W. & Levy, R. 1992 Clonal evolution of a follicular lymphoma: evidence for antigen selection. *Proc. Natl Acad. Sci. USA* **89**, 6770–6774.
- Bruner, S. D., Norman, D. P. & Verdine, G. L. 2000 Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature* **403**, 859–866.
- Castigli, E., Alt, F. W., Davidson, L., Bottaro, A., Mizoguchi, E., Bhan, A. K. & Geha, R. S. 1994 CD40-deficient mice generated by recombination-activating gene-2-deficient blastocyst complementation. *Proc. Natl Acad. Sci. USA* **91**, 12 135–12 139.
- Dent, A. L., Shaffer, A. L., Yu, X., Allman, D. & Staudt, L. M. 1997 Control of inflammation, cytokine expression, and germinal center formation by *BCL-6*. *Science* **276**, 589–592.
- DeRocco, S. E., Iozzo, R., Ma, X. P., Schwarting, R., Peterson, D. & Calabretta, B. 1997 Ectopic expression of *A-myb* in transgenic mice causes follicular hyperplasia and enhanced B lymphocyte proliferation. *Proc. Natl Acad. Sci. USA* **94**, 3240–3244.
- Eisen, M. B., Spellman, P. T., Brown, P. O. & Botstein, D. 1998 Cluster analysis and display of genome-wide expression patterns. *Proc. Natl Acad. Sci. USA* **95**, 14 863–14 868.
- Fearon, D. T. & Carroll, M. C. 2000 Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *A. Rev. Immunol.* **18**, 393–422.
- Forman, M. S. & Pure, E. 1991 T-independent and T-dependent B lymphoblasts: helper T cells prime for interleukin 2-induced growth and secretion of immunoglobulins that utilize downstream heavy chains. *J. Exp. Med.* **173**, 687–697.
- Fukuda, T. (and 11 others) 1997 Disruption of the *Bcl6* gene results in an impaired germinal center formation. *J. Exp. Med.* **186**, 439–448.
- Galibert, L., Burdin, N., De Saint-Vis, B., Garrone, P., Van Kooten, C., Banchereau, J. & Rousset, F. 1996 CD40 and B cell antigen receptor dual triggering of resting B lymphocytes turns on a partial germinal center phenotype. *J. Exp. Med.* **183**, 77–85.
- Golay, J. (and 10 others) 1998 The A-Myb transcription factor is a marker of centroblasts *in vivo*. *J. Immunol.* **160**, 2786–2793.
- Kawabe, T., Naka, T., Yoshida, K., Tanaka, T., Fujiwara, H., Suematsu, S., Yoshida, N., Kishimoto, T. & Kikutani, H. 1994 The immune responses in CD40-deficient mice: impaired immunoglobulin class switching and germinal center formation. *Immunity* **1**, 167–178.
- Kelsoe, G. 1996 Life and death in germinal centers (redux). *Immunity* **4**, 107–111.

- Klein, U., Rajewsky, K. & Kuppers, R. 1998 Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J. Exp. Med.* **188**, 1679–1689.
- Kuo, F. C. & Sklar, J. 1997 Augmented expression of a human gene for 8-oxoguanine DNA glycosylase (MutM) in B lymphocytes of the dark zone in lymph node germinal centers. *J. Exp. Med.* **186**, 1547–1556.
- Kuppers, R., Klein, U., Hansmann, M. L. & Rajewsky, K. 1999 Cellular origin of human B-cell lymphomas. *New Engl. J. Med.* **341**, 1520–1529.
- Kurosaki, T. & Tsukada, S. 2000 BLNK: connecting Syk and Btk to calcium signals. *Immunity* **12**, 1–5.
- Liu, Y. J., Barthelemy, C., De Bouteiller, O., Arpin, C., Durand, I. &anchereau, J. 1995 Memory B cells from human tonsils colonize mucosal epithelium and directly present antigen to T cells by rapid up-regulation of B7-1 and B7-2. *Immunity* **2**, 239–248.
- MacLennan, I. C. 1994 Germinal centers. *A. Rev. Immunol.* **12**, 117–139.
- Qin, X. F., Reichlin, A., Luo, Y., Roeder, R. G. & Nussenzweig, M. C. 1998 OCA-B integrates B cell antigen receptor-, CD40L- and IL 4-mediated signals for the germinal center pathway of B cell development. *EMBO J.* **17**, 5066–5075.
- Rabbitts, T. H. 1998 LMO T-cell translocation oncogenes typify genes activated by chromosomal translocations that alter transcription and developmental processes. *Genes Dev.* **12**, 2651–2657.
- Staudt, L. M. & Brown, P. O. 2000 Genomic views of the immune system. *A. Rev. Immunol.* **18**, 829–859.
- Staudt, L. M., Dent, A. L., Shaffer, A. L. & Yu, X. 1999 Regulation of lymphocyte cell fate decisions and lymphomagenesis by BCL-6. *Int. Rev. Immunol.* **18**, 381–403.
- Tangye, S. G., Liu, Y. J., Aversa, G., Phillips, J. H. & De Vries, J. E. 1998 Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J. Exp. Med.* **188**, 1691–1703.
- Wakimoto, B. T. 1998 Beyond the nucleosome: epigenetic aspects of position-effect variegation in *Drosophila*. *Cell* **93**, 321–324.
- Wheeler, K. & Gordon, J. 1996 Co-ligation of surface IgM and CD40 on naive B lymphocytes generates a blast population with an ambiguous extrafollicular/germinal centre cell phenotype. *Int. Immunol.* **8**, 815–828.
- Ye, B. H. (and 13 others) 1997 The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. *Nature Genet.* **16**, 161–170.
- Yokouchi, M., Suzuki, R., Masuhara, M., Komiya, S., Inoue, A. & Yoshimura, A. 1997 Cloning and characterization of APS, an adaptor molecule containing PH and SH2 domains that is tyrosine phosphorylated upon B-cell receptor stimulation. *Oncogene* **15**, 7–15.
- Zani, V. J., Asou, N., Jadayel, D., Heward, J. M., Shipley, J., Nacheva, E., Takasuki, K., Catovsky, D. & Dyer, M. J. 1996 Molecular cloning of complex chromosomal translocation t(8;14;12)(q24.1;q32.3;q24.1) in a Burkitt lymphoma cell line defines a new gene (*BCL7A*) with homology to caldesmon. *Blood* **87**, 3124–3134.