Journal of Pathology
J Pathol 2006; **209**: 250–257
Published online 28 February 2006 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/path.1961

Original Paper

Immunoglobulin class-switch recombination occurs in mantle cell lymphomas

W Klapper,[1]* M Szczepanowski,[1] K Heidorn,[1] M Müschen,[2] S Liedtke,[2] A Sotnikova,[1] NS Andersen,[3] J Greeve[4] and R Parwaresch[1]

[1]*Department of Haematopathology and Lymph Node Registry Kiel, Schleswig-Holstein University Hospitals, Campus Kiel, Niemannsweg 11, 24105 Kiel, Germany*
[2]*Laboratory for Molecular Stem Cell Biology, Heinrich-Heine University, 40225 Düsseldorf, Germany*
[3]*Department of Haematology, Rigshospitalet, Copenhagen, Denmark*
[4]*Department of Internal Medicine, Inselspital-University Hospital Bern, 3010 Bern, Switzerland*

*Correspondence to:
W Klapper, Department of Hematopathology and Lymph Node Registry Kiel, Niemannsweg 11, 24105 Kiel, Germany.
E-mail:
wklapper@path.uni-kiel.de

Received: 29 June 2005
Revised: 6 December 2005
Accepted: 20 December 2005

Abstract

Mantle cell lymphoma (MCL) is an IgM-expressing B cell lymphoma that originates from naive B cells and responds poorly to chemotherapy. We show here that several MCLs harbour isotype-switched subclones. Similar to the situation in normal B cells, *in vitro* stimulation of MCL cell lines with CD40 ligand (CD40L) and interleukin-4 induced expression of activation-induced cytidine deaminase (AID) and germline transcription at the immunoglobulin heavy chain gene locus. Additionally, the occurrence of switch-circle transcripts and mature IgG transcripts after stimulation indicated ongoing class-switch recombination in mantle cell lymphoma cell lines. Furthermore, stimulation of primary MCL cells *in vitro* induced expression of class-switched IgG mRNA in the tumour cells. Our data indicate that mantle cell lymphomas have retained the ability to undergo class-switch recombination if appropriate stimuli, such as the CD40 ligand, are provided.

Keywords: class-switch; mantle cell lymphoma; immunoglobulin; activation-induced cytidine deaminase

Introduction

Mantle cell lymphoma (MCL) is a rare B cell non-Hodgkin's lymphoma that responds poorly to chemotherapy. MCL is characterized by the t(11;14) translocation, which juxtaposes the gene coding for cyclin D1 with the immunoglobulin heavy chain locus on one allele. The other intact immunoglobulin heavy chain allele is found to be unswitched and it produces IgM, which is expressed on the cell surface [1]. Recent studies have revealed that, contrary to naive B cells being the supposed cellular origin of MCL [2], a subset of mantle cell lymphomas harbour mutated variable regions of the immunoglobulin heavy chain gene (V_H) [3–5]. Since Babbage *et al* [6] reported the detection of switch-circle transcripts and class-switched subclones in samples of leukaemic MCL cells, it has become evident that a subset of MCL has initiated cellular processes corresponding to a germinal centre reaction.

Normal B cells and most B cell lymphomas express CD40, a surface receptor that regulates B cell proliferation and apoptosis. The physiological ligand of CD40 is CD154 (CD40L), which is expressed by T lymphocytes. *In vitro* stimulation of B cells by CD40L and appropriate cytokines, such as interleukin 4 (IL-4), induces proliferation and up-regulation of activation-induced cytidine deaminase (AID) followed by class-switch recombination (CSR) [7]. By analogy with normal B cells, CD40L and IL-4 are strong proliferative stimuli in primary MCL cells [8–10].

Previous reports failed to demonstrate class-switching in primary MCL cells after stimulation with CD40L and IL-4. However, these studies analysed protein expression by FACS analysis only [9,10]. We demonstrate here that MCL samples contain class-switched subclones. Furthermore, we show that class-switch recombination can be induced *in vitro* in MCL cell lines and primary MCL cells by stimulation with CD40L and IL-4. Since CD40L seems to stimulate class-switching in MCL, interaction with T cells might influence the biology of the lymphoma.

Material and methods

Tissue samples and primary MCL cells

The study was performed in accordance with the local ethical requirements for samples stored at the Lymph Node Registry Kiel. Fresh frozen tumour samples of MCL from the tissue collection of the Lymph Node Registry Kiel were cut with a cryostat and

tissue slides were transferred into RLT buffer for RNA extraction (RNeasy Mini Kit, Qiagen). Slides from the same level of the tissue block as that used for RNA extraction were stained with haematoxylin and eosin (H&E) and by immunohistochemistry for CD20, CD3, CD68, Ki-67 and CD23. Networks of follicular dendritic cells (FDC) were evaluated semi-quantitatively using staining for CD23. Residues of germinal centres (GC) were identified by staining for CD23 and Ki-67. Only lymph node samples revealing more than 80% tumour infiltration were used for this study. The diagnosis of MCL was confirmed by a panel of pathologists on paraffin-embedded tissue from the same cases stained for CD20, CD5, CD23 and cyclin D1. Primary MCL cells were recovered from the peripheral blood or lymph node cell suspensions from MCL patients, cultured and stimulated as described previously [8].

Cell lines

The MCL cell lines Granta 519, SP-49, HBL-2, JeKo-1 and NCEB-1 [11] were maintained in RPMI medium containing 10% FCS and stimulated with 33 ng/ml CD40L (Alexis or Strathmann) and 10 ng/ml IL-4 (Serva or Strathmann) for the indicated periods of time. All experiments were performed in triplicate. Total RNA was extracted using an RNeasy Mini Kit (Qiagen).

V_H sequencing

Immunoglobulin heavy chain constant regions (V_H) of cell lines, leukaemic MCL cells or tissue samples were amplified using cDNA and the primers described by Kuppers *et al* [12]. Sequencing of the resulting PCR products was carried out with Big Dye reagents on an ABI 310 sequencer (Applied Biosystems). Sequence alignments with germline sequences were determined from the IMGT database (http://imgt.cines.fr/). The number of somatic hypermutations was assessed in the region starting from the end of the forward primer until codon 94 of V_H and expressed as percentage of bases differing from the germline sequence. Clone-specific forward primers for MCL tissue samples and leukaemic MCL cells were designed based on the V_H gene sequence with the 3′-end of the primers located in the P- or N-nucleotides between the V- and D- or the D- and J-segment of V_H. The specificity of the clone-specific primers was evaluated by RT-PCR, using the clone-specific forward primer in combination with a reverse primer located in the IgM constant region [13] and cDNA from peripheral blood B cells as a template. If no PCR products were observed in peripheral B cells but there was strong amplification of IgM in the lymphoma sample, the forward primer was considered to be clone-specific (data not shown).

RT-PCR

cDNA synthesis was carried out using a Super-Script First-Strand Synthesis System and oligo-dT primers (Invitrogen). Quantitative PCR for *AID* was performed using a TaqMan 7000 and Quantitech reagents (Qiagen). Primer and probe sequences were as follows: *AID* forward, GAGGCAAGAAGACACT-CTGGACACCACTATGGACAG; *AID* reverse, CTT-CTGTGAGGACCGCAAGG; *AID* probe, CTTTCT-GCGAGGGAACCCCAACCTC. β-Glucuronidase (*b-GUS*) served as an internal control [14]. Quantitative PCR for germline transcripts was performed using Quantitech Sybr Green reagents (Qiagen) and the primers described by Cerutti *et al* [13]. Quantification was performed by the CT method as described in the user manual [15]. In detail, the threshold cycle for *b-GUS* was subtracted from the threshold cycle of the product of interest, e.g. *AID* (dCT). The dCT value of the control samples was subtracted from the dCT of the experimental samples (ddCT). The relative expression was calculated as $2^{-\mathrm{ddCT}}$. By this method the value of expression of the control sample is defined as 1 and the values for the experimental samples represent multiples of the control sample. The control sample for *AID* expression in MCL tissue samples was the MCL cell line NCEB-1, for *AID* expression in MCL cell lines the Burkitt lymphoma cell line Ramos and for germline expression the unstimulated cells from the corresponding cell line. For the detection of mature IgM or class-switched mature IgG transcripts ($V_H DJ_H$-C_H transcripts), V_H-specific primers [12] (for cell lines) or clone-specific primers (for tissue and blood samples) were combined with the IgM or IgG reverse primers also used for germline transcripts [13]. All PCR products were analysed on ethidium bromide-stained gels and selectively sequenced.

Switch-circle transcripts (CT) were amplified with the primers described by Cerutti *et al* [16]. Gel-eluted PCR fragments were reamplified and cloned into the pDrive vector (Qiagen) for sequencing.

Results

Class-switched subclones can be detected in MCL

In a study using a PCR assay with limited sensitivity we recently reported the absence of *AID* expression in MCL [17]. Using a highly sensitive real-time RT-PCR, we now observed low baseline expression of *AID* mRNA in all MCL tissue samples analysed, although at varying levels (Figure 1A). The levels of *AID* expression were low compared to the MCL cell line NCEB-1 and in some cases close to the detection limit using 40 PCR cycles (Figure 1A). *AID* mRNA expression has been reported to correlate with *AID* protein expression [18]. Since the level of *AID* mRNA expression is very low, we failed to detect *AID* protein by immunohistochemistry, using a tissue microarray containing 36 MCLs and the antibody described (data not shown) [19]. In agreement with previous reports, somatic hypermutations were detected in a subset of

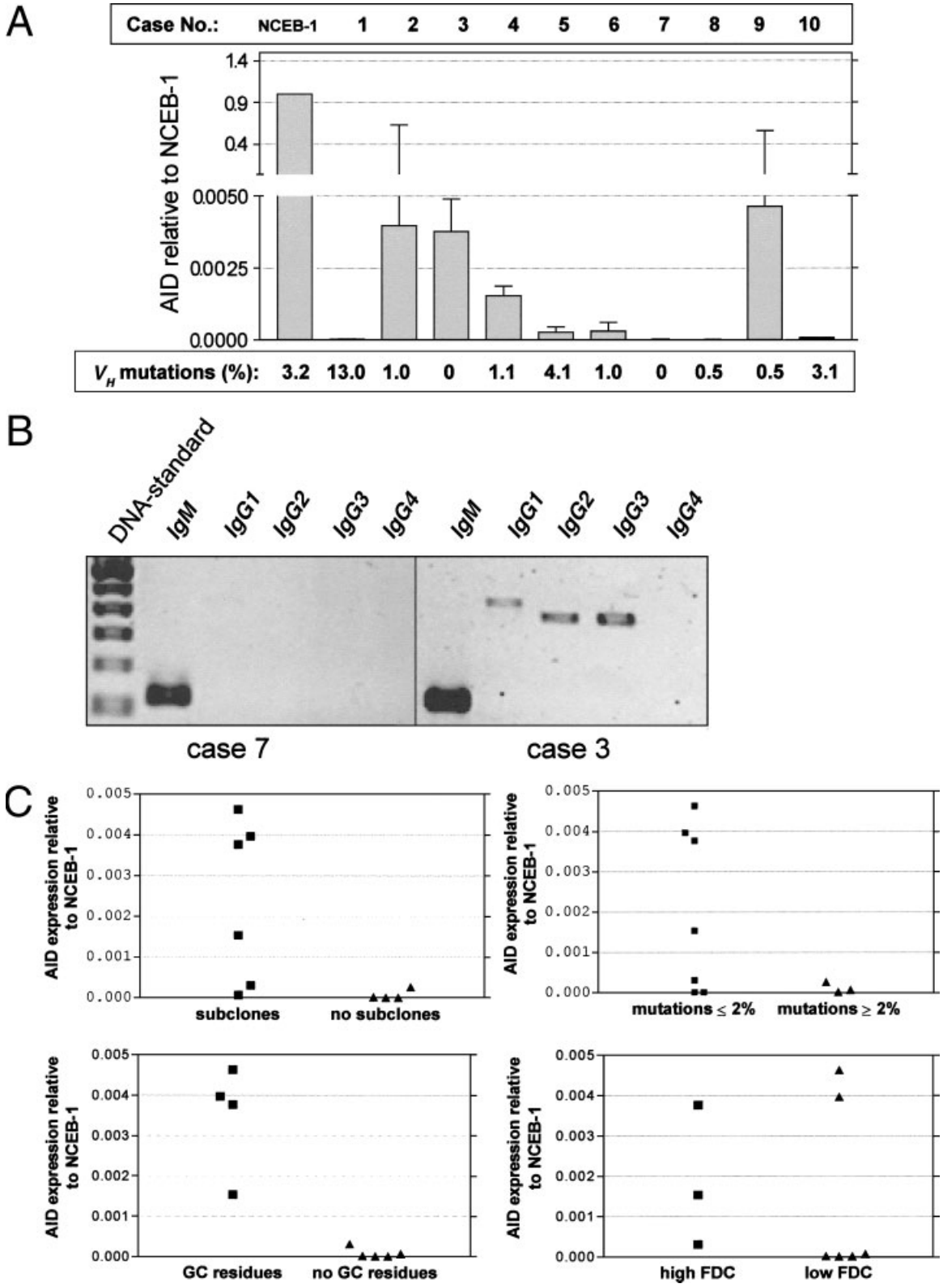

Figure 1. (A) Expression of activation-induced cytidine deaminase (*AID*) mRNA in 10 cases of mantle cell lymphoma (MCL), measured by quantitative RT-PCR. Expression in the MCL cell line NCEB-1 was set as 1 and the tissue samples were expressed as multiples of NCEB-1, as described above. All MCL cases expressed *AID*, although some only at minimal levels (cases 1, 7, 8 and 10). Somatic hypermutations are indicated as percentages of bases differing from the germline sequence. (B) Detection of class-switched subclones in MCL by RT-PCR, using clone-specific forward primers located in V_H and reverse primers located in the constant region of the immunoglobulin heavy chain. Examples from a primary lymphoma tissue sample without detectable subclones (case 7) and one with detectable subclones (case 3) are shown. As expected, *IgM* expression is strong in both cases, indicating that the majority of cells express *IgM*. (C) *AID* mRNA expression in the 10 MCL samples shown in (A) related to the presence or absence of detectable class-switched subclones, the presence and absence of somatic hypermutations (based on a threshold of 2% base exchanges compared to the germline sequence), the presence or absence of germinal centre (GC) residues and the amount of follicular dendritic cells (FDC)

MCL, in the range 0–13% of nucleotide changes compared to the germline sequence (Figure 1A). Based on the V_H sequence, clone-specific forward primers were designed and tested for lack of cross-reactivity on other B cells, as described in Material and methods (data not shown). RT-PCR analysis combining these forward primers with reverse primers located in the constant region revealed that all MCLs expressed predominantly IgM, but that a number of MCLs contained class-switched subclones (Figure 1B). Because these subclones carry identical V_H genes and differ only in the constant region, they cannot be detected by conventional clonality assays, which use primers located within V_H [20].

As shown in Figure 1C, MCLs with class-switched subclones tended to express higher levels of *AID* than MCL samples without detectable subclones. There was an obvious difference in *AID* expression between the group of MCLs with class-switched subclones and MCLs without subclones, but it did not reach statistical significance ($p = 0.0536$; t-test, Figure 1C). MCL cases with nucleotide exchanges of more than 2% compared to the germline sequence have been considered 'mutated' in previous studies [5]. In our study, MCLs with mutated V_H displayed lower *AID* expression than unmutated cases, although again the difference was not statistically significant ($p = 0.1548$; t-test, Figure 1C). MCLs often contain germinal centre (GC) residues and tumour-induced networks of follicular dendritic cells (FDC). We examined the presence of GC residues and the extent of FDC networks by immunohistochemistry in the areas directly adjacent to the areas that were used to study *AID* expression. All MCL cases examined showed a tumour cell content of more than 80%. The additional cells in the tumour are mainly macrophages and T cells that we identified by staining for CD68 and CD3. Using antibodies for Ki-67 and CD23 we were able to detect residues of germinal centres in a subset of cases examined. These residues comprised by far less than 1% of the whole sample. Nevertheless, stratifying the cases according to the presence or absence of GC residues revealed that the MCL samples containing GC residues tended to express higher *AID* levels than cases without GC residues ($p = 0.007$; t-test, Figure 1C). The extent of tumour-induced FDC networks, on the other hand, was independent of *AID* expression ($p = 0.7841$; t-test, Figure 1C). These data reveal that the level of *AID* expression might be influenced by the presence of GC residues in the tumour. Nevertheless, the detection of class-switched subclones using tumour-specific primers and analysis of the mutational status of the tumour was independent of contaminating cells. The mutational status, *AID* expression, the presence or absence of class-switched subclones and germinal centre residues and the quantitation of networks of follicular dendritic reticulum cells for all cases are shown in Table 1.

Table 1. A summary of results for all MCL tissues examined. The expression of *AID* is described as multiples of expression in the MCL cell line NCEB-1, which was set as 1. The percentage of V_H mutations is indicated. The presence of germinal centre residues (GC residues) and the extent of networks of follicular dendritic cells (FDCs) within the tumour were evaluated by immunohistochemistry

Case number	AID	V_H mutations (%)	Subclones	GC residues	FDC
1	0.00002	13	No	No	Low
2	0.00397	1	Yes	Yes	Medium
3	0.00377	0	Yes	Yes	High
4	0.00154	1.1	Yes	Yes	High
5	0.00027	4.1	No	n.d.	n.d.
6	0.00031	1	Yes	No	High
7	0.00001	0	No	No	Medium
8	0.00001	0.5	No	No	Low
9	0.00463	0.5	Yes	Yes	Low
10	0.00007	3.1	Yes	No	Medium

n.d., not determined.

Class switching can be induced in MCL cell lines by CD40L and IL-4

Class-switched subclones in MCL might have evolved during malignant transformation and might coexist with the majority of IgM-expressing cells. To evaluate whether *de novo* class-switch recombination can occur in MCL, we stimulated MCL cell lines with CD40L and IL-4. Both reagents have been shown to induce proliferation in primary MCL cells [8–10]. Because the FACS analysis used in earlier studies failed to detect class-switch recombination, we analysed class-switch recombination by RT-PCR. The methods used in our study are very sensitive and additionally allow the analysis of intermediate stages of class switching, e.g. increased germline transcription and switch-circle transcripts (CT).

Expression of *AID* and germline transcripts are early steps towards class-switch recombination and can be observed within hours after the appropriate stimuli have been provided [21]. All MCL cell lines examined revealed increased *AID* expression after 24 h of stimulation (Figure 2A). Immunoglobulin germline transcription of γ-constant genes was detectable at baseline in all cell lines examined and increased hours after stimulation with CD40L, whereas IgM expression remained unchanged (Figure 2B). Switch-circle transcripts (CT) are short-lived transcripts derived from the excised circular DNA during class-switching and thus represent intermediate products of class-switch recombination [22]. Since CTs are degraded rapidly, their detection demonstrates active ongoing class-switch recombination [22]. As shown in Figure 2C, CTs were detectable in NCEB-1 cells after 3 days of stimulation with CD40L and IL-4 but were not detected in unstimulated NCEB-1 and unstimulated or stimulated Granta 519 cells. EBV infection of B cells has been shown to induce *AID* expression and class switching, independent of any stimuli such as CD40L [23]. Since NCEB-1 cells are EBV-positive we also

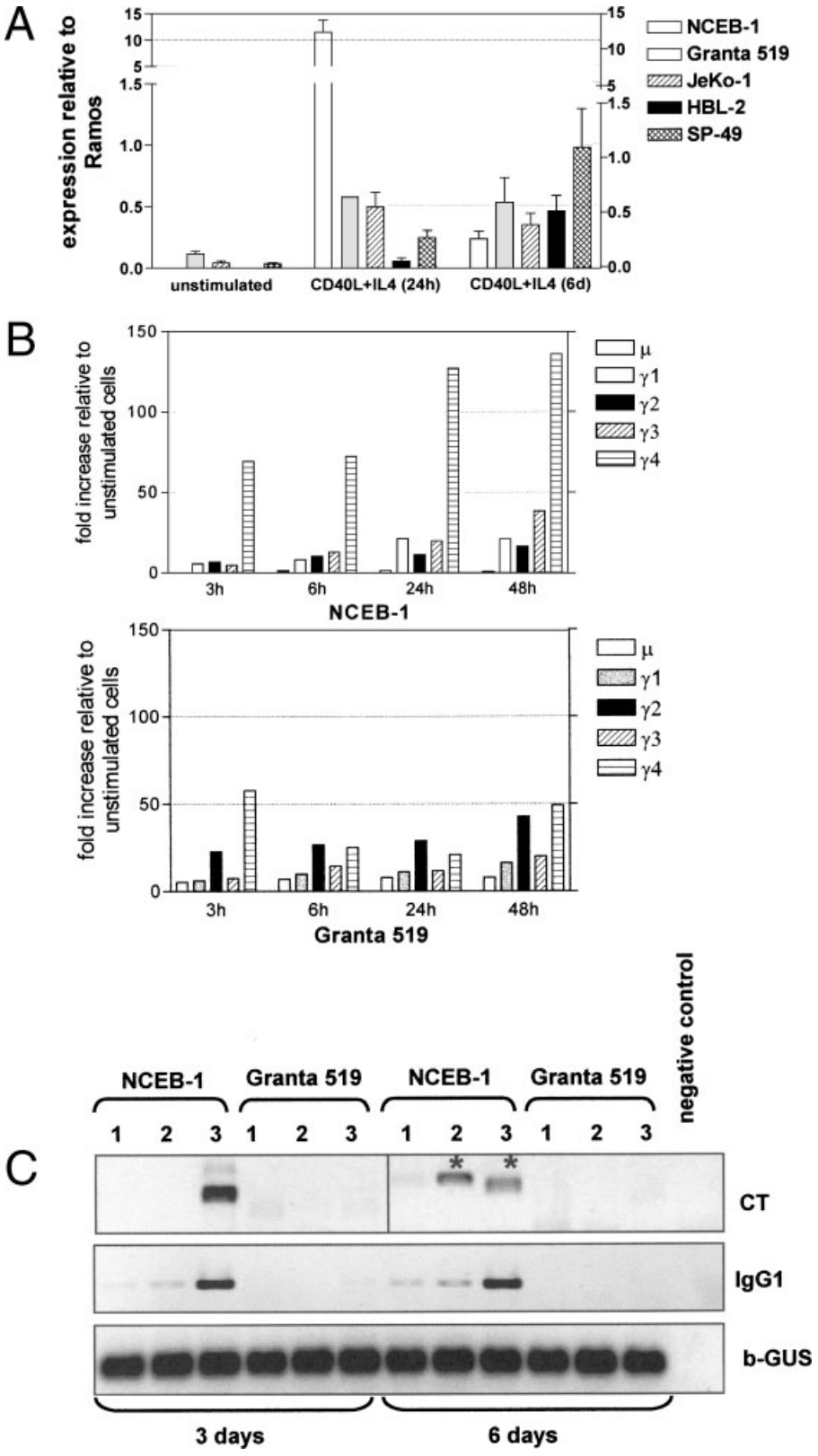

Figure 2. (A) *AID* mRNA expression in MCL cell lines at baseline and after 24 h and 6 days of stimulation with CD40L and IL-4. The level of expression is defined relative to Ramos cells, a Burkitt lymphoma cell line known to express high levels of *AID* [33]. (B) Immunoglobulin germline transcription in two MCL cell lines at baseline and after 3, 6, 24 and 48 h of stimulation with CD40L and IL-4. The level of transcription is expressed as multiples of the expression detected in unstimulated cells. (C) Expression of circle transcripts (CT) and mature class-switched *IgG1* transcripts in two MCL cell lines at baseline (1) and stimulated with CD40L (2) or CD40L and IL-4 (3) for 3 and 6 days. In NCEB-1 cells, CTs occur after 6 days of stimulation by CD40L alone and already after 3 days of stimulation with CD40L and IL-4. Asterisks indicate bands that were sequenced to confirm the correct sequence. Mature *IgG1* transcripts were detectable in NCEB-1 cells at low levels at baseline (1) and strongly increased after stimulation with CD40L (2) or CD40L and IL-4 (3). Expression of b-GUS served as an internal control [14]. In Granta 519 cells no CTs or mature *IgG1* transcripts were detectable

examined EBV-negative MCL cell lines for CTs after stimulation with CD40L and IL-4 and discovered that SP-49 cells, a EBV-negative cell line, expressed CTs after stimulation (data not shown). The sequences of the CT products were amplified in several independent experiments, cloned and sequenced (GeneBank Accession Nos AY748 447 and AY748 448). The final product of class-switch recombination, mature class-switched *IgG* ($V_H DJ_H$-*CH*) transcripts, were detected in several MCL cell lines. An example is shown in Figure 2C. Similar to primary MCL samples, pre-existing class-switched subclones can be detected, e.g. in NCEB-1 cells, by their low background expression of *IgG1*. Since CTs were additionally detectable in these cells, the increase in mature *IgG* $V_H DJ_H$-C_H transcripts in NCEB-1 cells after stimulation does not simply reflect a transcriptional up-regulation of *IgG* in a class-switched subclone, but at least partially reflects *de novo* class-switch recombination.

Class switching in primary MCL cells after stimulation with CD40L and IL-4

To evaluate whether CD40L and IL-4 can induce class switching in primary MCL cells, we stimulated two cases of MCL *in vitro* with CD40L and IL-4. The examined samples contained more than 90% MCL cells. In order to recover as many viable cells as possible, the samples were not sorted to remove minimal residual normal B cells. Thus, the *AID*, germline transcripts and CTs detected after stimulation with CD40L and IL-4 cannot be definitely ascribed to the tumour cell population. Nevertheless, both samples examined expressed *AID*. The expression level of *AID* decreased after stimulation (data not shown). This might be explained by the high rate of cell death observed in primary MCL cultures that counteracts the expected increase in *AID* expression. Since the surviving cells might successfully undergo class-switch, we sequenced the V_H and designed clone-specific forward primers that do not anneal to peripheral B cells (data not shown). Case T displayed an unmutated V_H and case 46 showed 5.1% mutations in V_H. Using these primers in combination with reverse primers located in the constant region of the immunoglobulin heavy chain, we were able to detect *IgM* mRNA derived from the tumour cells in both cases examined (Figure 3A). One of the two cases (case T) did not express *IgG* mRNA before stimulation but clearly expressed *IgG1* and *IgG2* after 72 h of stimulation with CD40L and IL-4 (Figure 3B). The second case (case 46) displayed a subclone expressing *IgG3* at baseline without stimulation, but did not show an increase in *IgG3* or the occurrence of other *IgG* mRNAs after stimulation (Figure 3B). CTs, although not definitely ascribable to the tumour cells, were detected only in case T, supporting the finding that this MCL case did undergo *de novo* class switching after stimulation with CD40L and IL-4 (Figure 3C).

Discussion

In line with one previous report, we describe here the detection of class-switched subclones in MCL [6]. In our study we used RT-PCR methods to detect class-switch recombination, but some primary MCL cells [6] and cell lines additionally express IgG protein (M. Müschen, unpublished observations). Nevertheless, RT-PCR analysis, like that in our study, can also detect intermediate products of class-switch recombination, such as CTs and mature class-switched mRNA. Since RT-PCR detection of CTs and mature class-switched mRNA is independent of protein translation, these methods are more specific and sensitive indicators of the genetic event of class-switch recombination. The information in the literature on the role of *AID* expression in MCL is contradictory [4,6,24]. Generally *AID* expression is low in MCL, but it can be detected by quantitative RT-PCR, as in our study. The level of *AID* mRNA has been shown to correlate with *AID* protein expression [18]. Our data indicate that *AID* expression varies in MCL and tends to be higher in lymphomas with detectable class-switched subclones but low levels of somatic hypermutation. MCL and B-CLL with low or no *AID* expression display somatic hypermutation and class-switched subclones more frequently and thus might arise from GC-experienced B cells [25]. The dissociation between class-switch recombination and somatic hypermutation in *AID*-expressing B-CLL and MCL, on the other hand, favours the view that AID may act differentially on CSR and SHM. *AID* expression might be necessary but not sufficient for the initiation of somatic hypermutation. Additional levels of regulation could be required for the efficient function of this protein.

In our study the level of *AID* expression is higher in samples containing GC residues, which are a rich source of *AID* expression. Thus, from our experimental data it cannot at present be concluded that the low levels of *AID* detected in MCL tumour biopsies reflects expression in MCL cells. Nevertheless, the detection of class-switched subclones by using clone specific primers, as in our study, and the measurement of mutational status, are not influenced by contaminating GC cells. The data also suggest that MCL cells can interact with GC residues and perform class-switch due to this interaction. These data have to be confirmed in future studies using larger series of MCL.

Our results indicate that class-switched subclones occur in nodal MCLs *in vivo* and that *de novo* class-switch recombination can be induced in MCL cell lines and primary MCL cells by CD40L and IL-4 *in vitro*. MCL cells have thus retained the ability to respond to environmental class switch-inducing stimuli, such as CD40L and IL-4. Under normal conditions, expression of *AID* and class-switch recombination are initiated in centroblasts under the control of the microenvironment of the germinal centre, e.g. by follicular dendritic cells. *AID* has been discussed as playing a role

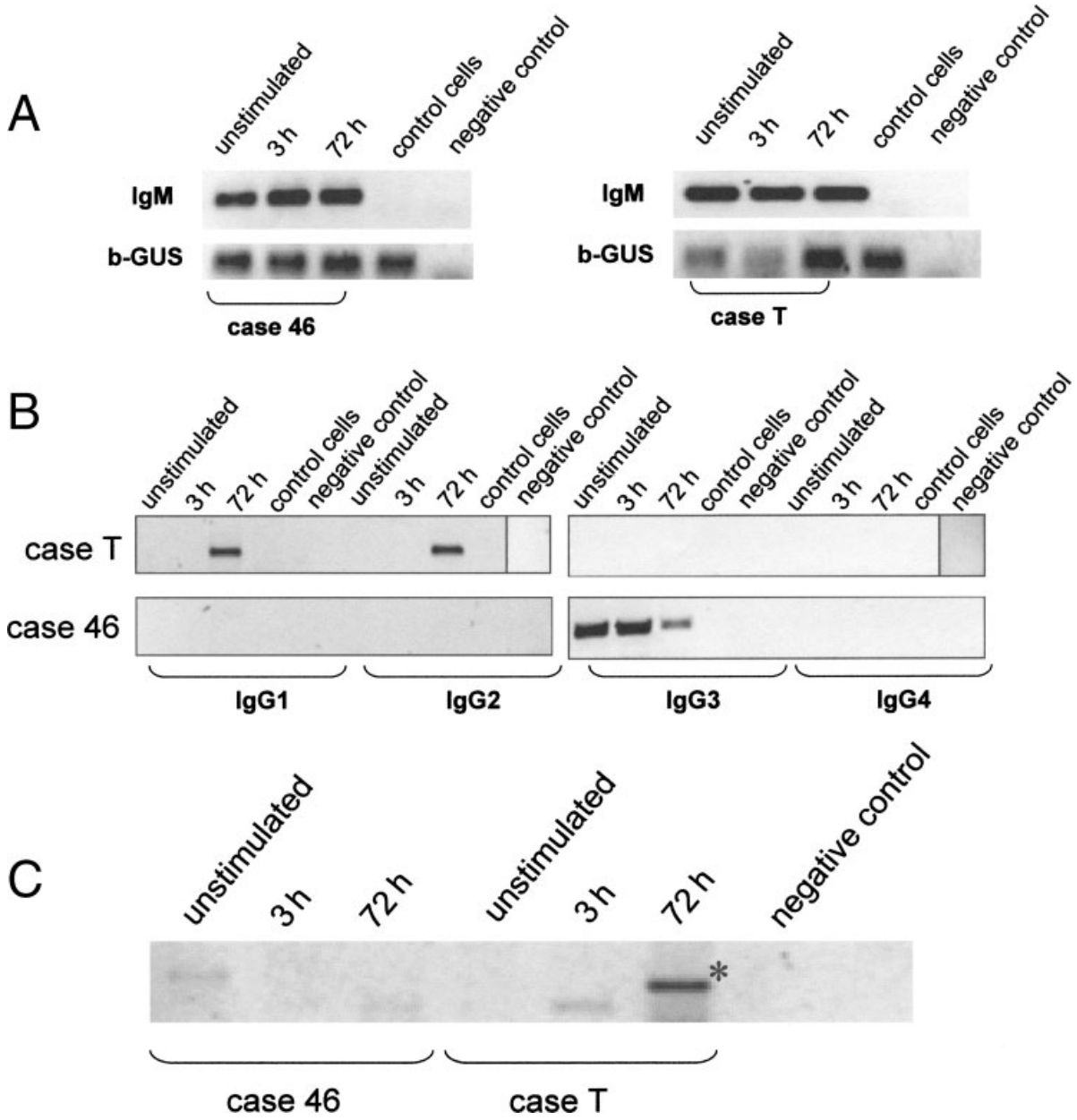

Figure 3. (A) Expression of *IgM* mRNA in cells from two patients with leukaemic MCL stimulated for 3 and 72 h with CD40L and IL-4 *in vitro* using clone specific primers. An unrelated cDNA sample (control) or no template (negative control) did not result in any PCR product, demonstrating the specificity of the primers used. b-GUS expression served as an internal control [14]. (B) Expression of class-switched mature *IgG* mRNA in two leukaemic MCL cases stimulated *in vitro* with CD40L and IL-4. Case 'T' displayed class-switch recombination after stimulation, as indicated by the occurrence of mature *IgG1* and *IgG2* transcripts. The second case ('46') showed an IgG3 expressing subclone at baseline but no class switching to other *IgG* subtypes after stimulation. (C) Detection of CTs in the two MCL cases stimulated *in vitro*. The asterisk indicates a band that was sequenced

in the development of *c-myc/IgH* translocations in IL-6 transgenic mice [26], and over-expression of *AID* in transgenic mice induced mutations in other genes besides the immunoglobulin genes [27,28]. It is tempting to speculate that the induction of *AID* and the initiation of class-switch recombination in malignant MCL cells that have escaped the control mechanisms of the germinal centre might lead to the accumulation of secondary genetic damage. High levels of proliferation [29,30] or secondary genetic abnormalities [31,32] are predictive of a poor clinical outcome in MCL. Because CD40L and IL-4 induce proliferation and class-switch recombination in MCL, these stimuli might influence the clinical course of the disease. T cells are the primary source of CD40L *in vivo*. Future studies should evaluate the impact of T cell content on proliferation, the occurrence of class-switched subclones and the clinical outcome of MCL.

Acknowledgements

The authors would like to thank K Dege, C Schulte, M Krams, C Napp and G Xu for their help. HBL-2 cells were a gift from R Siebert. This work was supported by the European Mantle Cell Lymphoma Network (No. LSHC-CT-2004-503351) and the Kinderkrebsinitiative Buchholz, Holm-Seppensen.

References

1. Vaandrager JW, Schuuring E, Kluin-Nelemans HC, Dyer MJ, Raap AK, Kluin PM. DNA fiber fluorescence *in situ* hybridization analysis of immunoglobulin class switching in B cell neoplasia: aberrant *CH* gene rearrangements in follicle center-cell lymphoma. *Blood* 1998;**92**(8):2871–2878.
2. Kuppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B cell lymphomas. *N Engl J Med* 1999;**341**(20):1520–1529.
3. Camacho FI, Algara P, Rodriguez A, Ruiz-Ballesteros E, Mollejo M, Martinez N, *et al*. Molecular heterogeneity in MCL defined by the use of specific V_H genes and the frequency of somatic mutations. *Blood* 2003;**101**(10):4042–4046.
4. Orchard J, Garand R, Davis Z, Babbage G, Sahota S, Matutes E, *et al*. A subset of T(11;14) lymphoma with mantle cell features displays mutated *IgVH* genes and includes patients with good prognosis, nonnodal disease. *Blood* 2003;**101**(12):4975–4981.
5. Kienle D, Krober A, Katzenberger T, Ott G, Leupolt E, Barth TF, *et al*. V_H mutation status and VDJ rearrangement structure in mantle cell lymphoma: correlation with genomic aberrations, clinical characteristics, and outcome. *Blood* 2003;**102**(8):3003–3009.
6. Babbage G, Garand R, Robillard N, Zojer N, Stevenson FK, Sahota SS. Mantle cell lymphoma with T(11;14) and unmutated or mutated V_H genes expresses AID and undergoes isotype switch events. *Blood* 2004;**103**(7):2795–2798.
7. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 2000;**102**(5):553–563.
8. Andersen NS, Larsen JK, Christiansen J, Pedersen LB, Christophersen NS, Geisler CH, Jurlander J. Soluble CD40 ligand induces selective proliferation of lymphoma cells in primary mantle cell lymphoma cell cultures. *Blood* 2000;**96**(6):2219–2225.
9. Visser HP, Tewis M, Willemze R, Kluin-Nelemans JC. Mantle cell lymphoma proliferates upon IL-10 in the CD40 system. *Leukemia* 2000;**14**(8):1483–1489.
10. Castillo R, Mascarenhas J, Telford W, Chadburn A, Friedman SM, Schattner EJ. Proliferative response of mantle cell lymphoma cells stimulated by CD40 ligation and IL-4. *Leukemia* 2000;**14**(2):292–298.
11. Drexler HG, MacLeod RA. Malignant hematopoietic cell lines: *in vitro* models for the study of mantle cell lymphoma. *Leuk Res* 2002;**26**(9):781–787.
12. Kuppers R, Zhao M, Rajewsky K, Hansmann ML. Detection of clonal B cell populations in paraffin-embedded tissues by polymerase chain reaction. *Am J Pathol* 1993;**143**(1):230–239.
13. Cerutti A, Zan H, Schaffer A, Bergsagel L, Harindranath N, Max EE, Casali P. CD40 ligand and appropriate cytokines induce switching to IgG, IgA, and IgE and coordinated germinal center and plasmacytoid phenotypic differentiation in a human monoclonal IgM + IgD + B cell line. *J Immunol* 1998;**160**(5):2145–2157.
14. Godfrey TE, Kim SH, Chavira M, Ruff DW, Warren RS, Gray JW, Jensen RH. Quantitative MRNA expression analysis from formalin-fixed, paraffin-embedded tissues using 5′ nuclease quantitative reverse transcription-polymerase chain reaction. *J Mol Diagn* 2000;**2**(2):84–91.
15. Applied Biosystems. ABI prism 7700 sequence detection system user bulletin 2. *User Bulletin*, 2nd edn. Applied Biosystems: Foster City, CA, USA, 1997.
16. Cerutti A, Zan H, Kim EC, Shah S, Schattner EJ, Schaffer A, Casali P. Ongoing *in vivo* immunoglobulin class switch DNA recombination in chronic lymphocytic leukemia B cells. *J Immunol* 2002;**169**(11):6594–6603.
17. Greeve J, Philipsen A, Krause K, Klapper W, Heidorn K, Castle BE, *et al*. Expression of activation-induced cytidine deaminase in human B cell non-Hodgkin lymphomas. *Blood* 2003; **101**(9:):3574–3580.
18. Pasqualucci L, Guglielmino R, Houldsworth J, Mohr J, Aoufouchi S, Polakiewicz R, *et al*. Expression of the AID protein in normal and neoplastic B cells. *Blood* 2004;**104**(10):3318–3325.
19. Greiner A, Tobollik S, Buettner M, Jungnickel B, Herrmann K, Kremmer E, Niedobitek G. Differential expression of activation-induced cytidine deaminase (AID) in nodular lymphocyte-predominant and classical Hodgkin lymphoma. *J Pathol* 2005; **205**(5):541–547.
20. van Dongen JJ, Langerak AW, Bruggemann M, Evans PA, Hummel M, Lavender FL, *et al*. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98–3936. *Leukemia* 2003;**17**(12):2257–2317.
21. Nagumo H, Agematsu K, Kobayashi N, Shinozaki K, Hokibara S, Nagase H, *et al*. The different process of class switching and somatic hypermutation; a novel analysis by CD27(−) naive B cells. *Blood* 2002;**99**(2):567–575.
22. Kinoshita K, Harigai M, Fagarasan S, Muramatsu M, Honjo T. A hallmark of active class switch recombination: transcripts directed by I promoters on looped-out circular DNAs. *Proc Natl Acad Sci USA* 2001;**98**(22):12620–12623.
23. He B, Raab-Traub N, Casali P, Cerutti A. EBV-encoded latent membrane protein 1 cooperates with BAFF/BLyS and APRIL to induce T cell-independent Ig heavy chain class switching. *J Immunol* 2003;**171**(10):5215–5224.
24. Guikema JE, Rosati S, Akkermans K, Bende RJ, van Noesel CJ, van Krieken JH, *et al*. Quantitative RT-PCR analysis of activation-induced cytidine deaminase expression in tissue samples from mantle cell lymphoma and B cell chronic lymphocytic leukemia patients. *Blood* 2005;**105**(7):2997–2999.
25. Heintel D, Kroemer E, Kienle D, Schwarzinger I, Gleiss A, Schwarzmeier J, *et al*. High expression of activation-induced cytidine deaminase (AID) MRNA is associated with unmutated IGV_H gene status and unfavourable cytogenetic aberrations in patients with chronic lymphocytic leukaemia. *Leukemia* 2004;**18**(4):756–762.
26. Ramiro AR, Jankovic M, Eisenreich T, Difilippantonio S, Chen-Kiang S, Muramatsu M, *et al*. AID is required for *C-Myc*/*IgH* chromosome translocations *in vivo*. *Cell* 2004;**118**(4):431–438.
27. Okazaki IM, Hiai H, Kakazu N, Yamada S, Muramatsu M, Kinoshita K, Honjo T. Constitutive expression of AID leads to tumorigenesis. *J Exp Med* 2003;**197**(9):1173–1181.
28. Kotani A, Okazaki IM, Muramatsu M, Kinoshita K, Begum NA, Nakajima T, *et al*. A target selection of somatic hypermutations is regulated similarly between T and B cells upon activation-induced cytidine deaminase expression. *Proc Natl Acad Sci USA* 2005;**102**(12):4506–4511.
29. Schrader C, Meusers P, Brittinger G, Teymoortash A, Siebmann JU, Janssen D, *et al*. Topoisomerase II alpha expression in mantle cell lymphoma: a marker of cell proliferation and a prognostic factor for clinical outcome. *Leukemia* 2004;**18**(7):1200–1206.
30. Argatoff LH, Connors JM, Klasa RJ, Horsman DE, Gascoyne RD. Mantle cell lymphoma: a clinicopathologic study of 80 cases. *Blood* 1997;**89**(6):2067–2078.
31. Rubio-Moscardo F, Climent J, Siebert R, Piris MA, Martin-Subero JI, Nielander I, *et al*. Mantle cell lymphoma genotypes identified with CGH to BAC microarrays define a leukemic subgroup of disease and predict patient outcome. *Blood* 2005;(in press).
32. Bea S, Ribas M, Hernandez JM, Bosch F, Pinyol M, Hernandez L, *et al*. Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants. *Blood* 1999;**93**(12):4365–4374.
33. Zhang W, Bardwell PD, Woo CJ, Poltoratsky V, Scharff MD, Martin A. Clonal instability of V region hypermutation in the Ramos Burkitt's lymphoma cell line. *Int Immunol* 2001;**13**(9): 1175–1184.