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Stages of Germinal Center Transit Are Defined by B Cell Transcription Factor Coexpression and Relative Abundance

Giorgio Cattoretti,1*† Rita Shaknovich,‡ Paula M. Smith,† Hans-Martin Jäck,§ Vundavalli V. Murty,*† and Bachir Alobaid*‡

The transit of T cell-activated B cells through the germinal center (GC) is controlled by sequential activation and repression of key transcription factors, executing the pre- and post-GC B cell program. B cell lymphoma (BCL) 6 and IFN regulatory factor (IRF) 8 are necessary for GC formation and for its molecular activity in Pax5+Pax1+ B cells. IRF4, which is highly expressed in BCL6− GC B cells, is necessary for class switch recombination and the plasma cell differentiation at exit from the GC. In this study, we show at the single-cell level broad coexpression of IRF4 with BCL6, Pax5, IRF8, and PU.1 in pre- and post-GC B cells in human and mouse. IRF4 is down-regulated in BCL6+ human GC founder cells (IgD+CD38+), is absent in GC centroblasts, and is re-expressed in positive regulatory domain 1-positive centrocytes, which are negative for all the B cell transcription factors. Activated (CD30+) and activation-induced cytidine deaminase-positive extrafollicular blasts coexpress Pax5 and IRF4. PU.1-negative plasma cells and CD30+ blasts uniquely display the conformational epitope of IRF4 recognized by the MUM1 Ab, an epitope that is absent from any other IRF4+PU.1+ lymphoid and hemopoietic subsets. Low grade B cell lymphomas, representing the malignant counterpart of pre- and post-GC B cells, accordingly express IRF4. However, a fraction of BCL6+ diffuse large B cell lymphomas express IRF4 bearing the MUM1 epitope, indicative of a posttranscriptional modification of IRF4 not seen in the normal counterpart. The Journal of Immunology, 2006, 177: 6930–6939.

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†Abbreviations used in this paper: TF, transcription factor; IRF, IFN regulatory factor; GC, germinal center; AID, activation-induced cytidine deaminase; BCL, B cell lymphoma; DLBCL, diffuse large BCL; HIC, immunohistochemistry; DAPI, 4′,6′-diamidino-2-phenylindole; TMA, tissue microarray; FCM, flow cytometry; CLL, chronic lymphocytic leukemia; PRDM1, positive regulatory domain 1.
mature B cell types, as detected by other Abs not limited to a conformational epitope recognized by the MUM1 Ab.

Materials and Methods

Cells and tissues

IRF4 knockout (17), BCL6 knockout mice (19), and their wild-type littermates (C57BL6 and F1 from C57BL6 × 129Sv) were gifts from U. Klein and R. Dalla-Favera (Columbia University, New York, NY). Anonymous

Table I. Abs used

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* C-term, C-terminal; N-term, N-terminal; Cytoplasm., Cytoplasmic.

Antibodies

Abs used in this study are listed in Table I. Multiple different anti-BCL6 Abs, which all reacted identically in various assays and combinations, will be referred to collectively as “BCL6.” The term “MUM1” will be restricted as above, washed in PBS, fixed in absolute cold methanol for 10 min, biotin. Therefore, cells were first stained with biotin-conjugated Abs, fixed which destroys prebound fluorochromes and some surface Ags, but not to anti-IRF4 Abs directed against aa 128–267 or aa 144–451 of the human protein.

Immunohistochemistry

Sections were essentially stained as previously published (36, 38), including double staining on 1 mM EDTA (pH 8) Ag-retrieved slides. Species-specific, alkaline phosphatase-conjugated secondary Abs, prescreened for specificity and absence of cross-reactivity, were developed with nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (Roche). The percentage and intensity of staining of CD20+ neoplastic B cells were independently scored in the DLBCL tissue microarray (TMA), each using a 10-tiered scale (0–9). The product of both was used as a case score and a value of 10 or greater was considered positive for a 10-tiered scale (0 –9). The product of both was used as a case score and independently scored in the DLBCL tissue microarray (TMA), each using

Immunofluorescence

Double immunofluorescence was performed with primary Abs raised in different species or of different isotypes, counterstained with FITC- or Cy3-conjugated, species- or isotype-specific secondary Abs (Jackson ImmunoResearch Laboratories) or biotin-conjugated secondary Abs, followed by conjugated avidin (Jackson ImmunoResearch Laboratories). Tyramide sig-
washed in PBS, transferred to PBS-BSA-Na$_2$HPO$_4$, and processed for intra-
cellular staining. Counterstaining of the nuclear Ag was followed by flu-
ochrome-avidin staining of the biotin-labeled Ab and staining for Ags
which survive the double fixation (e.g., IgD, CD38). IgD was stained with a
PE-conjugated rat anti-mouse IgD (Southern Biotechnology Associates)
or with a PE-conjugated mouse anti-human IgD (BD Biosciences), pre-
exced by excessive cold mouse Ig blocking of residual anti-mouse IgG
moieties. BCL6 staining is not affected by the methanol postfixation.

Goat and rabbit primary Abs (IRF4, PU.1, negative control) were used at
1 µg/ml, incubated overnight, and counterstained with either donkey
anti-rabbit Cy5 (Jackson ImmunoResearchLaboratories) or donkey
anti-goat-FITC (1/500; Jackson ImmunoResearch Laboratories). For
double IRF4, PU.1 and BCL6 stainings, human cells were surface-
stained first with mouse anti CD3-biotin (BD PharMingen), followed by
nuclear staining, exclusion by gating of the CD3$^+$ cells, and analysis of
the non-T cells (>95% B cells).

More than 30,000 events were acquired on a FACScalibur (BD Bio-
sciences) and CellQuest acquisition software and analyzed with FlowJo 6.4
(Tree Star).

cDNA microarray analysis of human samples

Data were obtained from published databases (1), obtained by Affymetrix
U95A chip analysis of normal tonsil subsets.

Fluorescence in situ hybridization

LSI BCL6 dual-color, break-apart rearrangement, and Spectrum Green-
labeled CEP 8 probes were obtained from Abbott Molecular. Four 5-µM
thick tissue sections of two TMAs were cut onto adhesive-coated slides.

Paraffin-sections were baked overnight at 60°C before hybridization. TMA
slides were subjected to protease treatment using paraffin pretreatment kit
(Abbot Molecular). Fluorescence in situ hybridization was performed by
standard methods and hybridization signals were scored on at least 200
interphase nuclei on DAPI-stained slides (26). The sensitivity of hybrid-
ization on paraffin-embedded tissues was determined by performing the
same analysis on analogous sections from normal tonsils and similarly
processed cell lines of known genomic asset. Cases were diagnosed as
rearranged if the fraction of cells showing the rearrangement in >5% cells.

Results

Distribution of B cell TF by tissue IHC

The distribution of the transcription factors Pax-5, Oct-2, PU.1, and
IRF8 (Fig. 1) in mouse and human lymphoid tissues is broad and
overlaps among different B cell subsets (morphologically and
phenotypically defined follicular mantle, marginal, and GC cells) as
shown by in situ immunostaining with Abs validated for speci-

fically (Fig. 2). BCL6 and IRF4 instead show a mutually exclusive
distribution; BCL6 is highly expressed in GC, IRF4 instead is
positive in mantle and marginal zone B cells, and negative in most GC
B cells. The expression of IRF4 in IRF8$^+$, PU.1$^+$, Oct-2$^+$, and
Pax5$^+$ follicular mantle B cells could be seen only with Abs dis-

rected against the C-terminal and Asp$^{175}$, and not with two other
Abs directed against aa 128–267 of the IRF4 protein (hence named
MUM1 epitope). Abs against this region of IRF4 detect only
expression profiling in purified B cell subsets (1). As shown in Fig.
I, the RNA levels of each TF in GC, mantle, and marginal zone B
cells correspond to the distribution of the respective protein Ags,
including IRF4 by C-terminal and Asp$^{175}$ staining.

Single-color staining and tissue RNA or protein extraction do not allow a
detailed analysis of the coexpression of two given Ags on a cell-by-cell basis. Therefore, we analyzed by double immu-

nophenotype and well-defined zones, comprised predominantly of B cells,
which correspond to different functional and/or maturational sub-
sets. Extrafollicular B cells, however, are more dispersed and
therefore better defined by double staining (36, 40) rather than by
topographic location. We analyzed the coexpression of IRF4 and
other B cell TF in four B cell subsets: the CD30$^+$ activated cells,
the extrafollicular AID$^+$ blasts (36), the centrocytes in the GC
light zone, and the extrafollicular memory/marginal zone IRTA1$^+$
cells (41).

CD30$^+$ extrafollicular blasts are largely B cells showing evid-
cence of BCR plus cofactor-mediated acute activation (36), and
IRF4 has been shown to be part of this signature (42). Accord-
ingly, CD30$^+$ cells were largely IRF4$^+$ and Pax5$^+$. The intensity of
Pax5 expression was inversely related to intensity of IRF4 ex-
pression (Fig. 3H).

B cells express AID upon in vitro activation (43) and IRF4 is
necessary for AID expression (17). Approximately half of the
AID$^+$ extrafollicular blasts were IRF4$^+$, MUM1$^-$ (Fig. 3, A and
B), with the intensity of IRF4 often noticeably dim, compared with
that of CD30$^+$ extrafollicular blasts. This was in sharp contrast to
the GC where AID and IRF4 were rigorously mutually exclusive
both in the dark and outer zone (Fig. 3A). Thus, appreciable levels
of IRF4 are detectable in extrafollicular AID$^+$ cells, possibly be-
fore they enter the GC reaction, when IRF4 is shut off.

Commitment to the plasma cell lineage within the GC is marked
by PRDM1 expression, at first in Pax5$^+$ centrocytes, then in
CD20$^+$, Pax5$^+$, and CD138$^+$ preplasma cells (38). The bulk of
bright IRF4$^+$ centrocytes coexpress PRDM1 and are Pax5 nega-
tive (Fig. 3, I, L, and M). In addition, a minority of Pax5-positive
or Pax5 weakly positive centrocytes express IRF4 and/or PRDM1
(Fig. 3I). The MUM1 epitope is not detected on these latter cells
(Fig. 3L).

The fourth B cell subset we focused on is defined by the FCRL4/
IRTA1 Ag (41, 44). IRTA1$^+$ cells are monocytoid B cells with
evidence of Ag selection and variable Ig gene mutation (44), thus
memory B cells, located within the tonsil festooned epithelium and
scattered in the interfollicular areas. Although intraepithelial
IRTA1$^+$ cells were largely IRF4$^-$, interfollicular and intrafollicu-
lar IRTA1$^+$ cells contained IRF4$^+$ (Fig. 3C). This is consistent
with the reported expression of IRF4 by gene expression profiling
in purified memory B cells (1) (Fig. 1). IRTA1$^+$ B cells were
previously reported negative for IRF4 with the MUM1 Ab.

In summary, IRF4 is coexpressed with the other B cell TF in
pre- and post-GC B cells, but not inside the GC, where it is spe-
cifically down-regulated during transit.

Distribution of IRF4 and BCL6 in lymphoid tissues by flow cyto-
metry

BCL6 staining in the follicle mantle is usually negative, however,
occurantly we are able to detect it by IHC only in selected mouse
samples (Fig. 2). BCL6 is among the most variably expressed
genes in the mouse (45) and our inconsistent results may be due to
either sample-to-sample variation and/or insufficient sensitivity.

To assess in a quantitative fashion the association of IRF4 by gene expression profiling in purified memory B cells (1) (Fig. 1). IRTA1$^+$ B cells were
previously reported negative for IRF4 with the MUM1 Ab.

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In summary, IRF4 is coexpressed with the other B cell TF in
pre- and post-GC B cells, but not inside the GC, where it is spe-
cifically down-regulated during transit.
FIGURE 1. B cell TF staining in mouse spleen and human tonsil. A, Serial sections of immunized mouse spleen are stained for B cell TF and proliferation-associated Ag (MCM7). Low-power (×10) images are on top, high power (×40) on bottom. oPALS, outer periarteriolar lymphoid sheet; MC, mantle cells; MZ, marginal zone. Note the reciprocal staining pattern of IRF4 vs the other TF in the GC, and the coexpression in the MZ and MC. B, Serial sections of human tonsil are stained for B cell TF and proliferation-associated Ag (MCM7). Low-power (×4) images are on top, high power (×40) in the middle. Lower panel, The U95 Affymetrix cDNA chip raw values, depicted as bars, indicating cDNA expression for the corresponding transcription factors in memory (Mem), naive, and GC-purified cells. IE, intraepithelial; SE, subepithelial; MC, mantle cell; GC, germinal center. Note the reciprocal staining pattern of IRF4 vs the other TF in the GC, and the coexpression in the MC and IE.
tained a BCL6⁺, IRF4⁺ population, corresponding to mantle zone B cells and a BCL6⁺, IRF4⁻ negative or weak population, corresponding to GC founder cells. MUM1 Ab, shown to be reactive with IRF4 by FCM on lymphoma cell lines (data not shown), was negative on all these B cell fractions.

We conclude that each B cell subset coexpressing BCL6, IRF4, and the other B cell TF is characterized by discrete protein levels of such TF, which are typical of each cell type.

**Distribution of IRF4 and BCL6 in B cell non-Hodgkin lymphoma**

We then evaluated by IHC the mutual distribution of IRF4 and BCL6 in a series of 40 DLBCL. We found 45% (18 of 40) coexpressed BCL6 and IRF4, 45% were BCL6⁺ only, 7.5% (3 of 40) were IRF4⁺ only, and 2 were negative for both (Fig. 6). A semiquantitative evaluation of the staining showed two distinctly distributed groups of predominantly BCL6- or IRF4-positive cases and a heterogeneous cluster of coexpressing cases (Fig. 6A). A similar inversely related distribution has been obtained for the RNA expression levels by gene expression profiling (Ref. 47 and data not shown). Cases with known BCL6 translocation were low in BCL6 and (3 of 4) coexpressed IRF4.

IRF4 and MUM1 staining were highly correlated (Fig. 6B), with only two IRF4⁺ cases not expressing the MUM1 epitope (Fig. 6B).

To understand the mutual relationship of BCL6 and IRF4 at the single-cell level, we costained for both selected double-positive DLBCL cases. A range of coexpression patterns was obtained, from mutual exclusion to substantial costaining (Fig. 7).

A small group of low-grade lymphomas representative of the neoplastic counterpart of pre- and post-GC cells was also evaluated. Six cases of chronic lymphocytic leukemia (CLL), two mantle cell lymphomas, and two marginal zone lymphomas, showed more extensive but not consistent IRF4 staining, compared with MUM1 (Fig. 8). One CLL case was BCL6⁺ by IHC (data not shown). BCL6 and IRF4 expression was confirmed by FCM in two representative CLL cases, one of which showed coexpression (data not shown).

Thus, B cell lymphomas, as their normal B cell counterparts, show coexpression of BCL6 and IRF4. However, in tumors of putative GC origin, such as a group of DLBCL, IRF4 bears the MUM1 conformational epitope in BCL6⁺ cells.

**Discussion**

BCL6 is a potent transcriptional repressor expressed at high levels in GC cells, and presumably prevents terminal B cell differentiation by repressing key genes, such as PRDM1/Blimp-1 (48). BCL6 may also have other important functions, such as antiapoptotic activity (49). Recently, Pax5 has been shown to have an essential role in maintaining the B cell phenotype in GC cells (5). IRF8 and IRF4 are also needed to complete essential activities within the GC, such as somatic mutation and class switch recombination of Ig genes through AID induction (13, 17). The emerging picture requires an understanding of the topographic distribution of all these genes through AID induction (13, 17). The emerging picture requires an understanding of the topographic distribution of all these genes through AID induction (13, 17). The emerging picture requires an understanding of the topographic distribution of all these genes through AID induction (13, 17). The emerging picture requires an understanding of the topographic distribution of all these genes through AID induction (13, 17). The emerging picture requires an understanding of the topographic distribution of all these genes through AID induction (13, 17). The emerging picture requires an understanding of the topographic distribution of all these genes through AID induction (13, 17). The emerging picture requires an understanding of the topographic distribution of all these genes through AID induction (13, 17).
expected according to previously published molecular and immunohistochemical studies (1, 31, 51). The mutual relationship of these TF outside the GC is fine tuned by the amount of each TF in a cell type-specific fashion, as demonstrated by quantitative flow cytometry.

The role of BCL6 in pre- and post-GC B cells is unknown and probably redundant for B cell maturation and survival, because mantle zone B cells in BCL6 knockout mice are unaffected (19). In addition, the protein levels of BCL6 in pre-GC cells are maintained low by posttranscriptional down-regulation (31) and transcriptional-negative autoregulation (52). However, unimmunized BCL6-transgenic mice with only moderately raised baseline BCL6 levels in pre-GC cells experience GC formation to a level comparable to that of a potent polyclonal immunization (53), suggesting a “tonic” role for such low protein levels. What is probably crucial is the ability to rapidly increase BCL6 levels upon activation. We suggest this increase to happen in GC founder cells.

Low levels of BCL6 are also physiologically relevant in myeloid-derived cells, in which BCL6, undetectable by routine IHC, but detected by FCM, is necessary to repress an otherwise lethal immune dysregulation (33) and to control IL-6 signaling (32). Thus, BCL6 is continuously expressed at various levels before and during the GC reaction, and is eventually down-regulated in terminally differentiated plasma cells.

The presence of IRF4 in pre-GC B cells is expected because it is required for IgD+IgM− B cell phenotypic maturation (16). In addition, IRF4 in human and murine mantle and marginal zone B cells are quantitatively lower than the levels found in plasma cells and, notably, qualitatively different. The large majority of IRF4+ cells in mouse and human tissue cannot be detected by MUM1-like epitopes (85), suggesting that the molecule is in a peculiar conformational configuration which masks the MUM1-like epitope in most B cells and myelomonocytic cells.
IRF4 is structurally modified by an immunophilin (FKBP52 or FKBP4) with peptidyl-prolyl isomerase and chaperone-like activity (54), broadly expressed in all B cell subsets at the mRNA level (data obtained from Ref. 1) which binds to IRF4 in a segment centered on aa 150–237. FKBP4 binding induces a structural conformation which reduces the interaction of IRF4 with PU.1 and the transcriptional activity on PU.1-binding target genes (54). FKBP4 binding is inhibited when the C-terminal autoinhibitory domain folds back on the N-terminal DNA-binding domain (54).

Interestingly enough, the aa 128–267/MUM1 epitope is found predominantly on PU.1-negative cells (T cells, plasma cells, and melanocytes) (15, 55) and never present in myelomonocytic cells (G. Cattoretti, manuscript in preparation) or mantle zone B cells, where IRF4 has been shown to be functionally active and required, in cooperation with PU.1 (16, 56). This suggests that the acquisition of the MUM1 conformation by IRF4 is associated with a change in interacting partners or with reduced interaction with PU.1. Future studies with biochemical assays are needed to identify the interacting partners and the target DNA sequences.

At the entry of the GC, IRF4 is quickly down-regulated, while BCL6 is up-regulated, as demonstrated by the absence of double IRF4−BCL6−positive cells by FCM and IHC inside the GC. The situation is reversed in the light zone centrocytes committed to the plasma cell lineage. There, down-regulation of BCL6 is followed by PRDM1 expression in cells which still express Pax5. Then, Pax5, IRF8, and PU.1 are gradually down-regulated and IRF4 is substantially up-regulated in cells which have already lost the B cell program (38, 48) (Fig. 9). BCL6 is eventually totally lost on these cells. The factors initiating the exit process are unknown at the present time. We detected nuclear relocation of cREL, an NF-κB member, in BCL6− and negative centrocytes in the light zone (Ref. 57 and data not shown), largely in IRF4-negative but also in some IRF4+ and PRDM1+ cells (38). Unfortunately, we could not investigate nuclear relocation of other NF-κB members, therefore, the role of this group of TF in down-regulating BCL6 remains elusive. Recent studies have suggested that Pax5, not BCL6, may be the key regulator to be switched off before plasma cell differentiation (5), and PRDM1 would be the TF that seals the plasma cell fate (58).

The light zone contains Pax5− centrocytes which lack both high levels of BCL6, as well as PRDM1 and IRF4. Some of these may be memory B cell precursors, which did not acquire yet memory B cell Ags, such as IRTA1 (41), usually not detectable in the GC. However, the lack of IRF4 would be in contrast with the gene expression and flow cytometry data that we have generated, indicating that memory B cells are IRF4+. One possibility is that
Pax5+ memory B cell precursors are rapidly exported from the GC and they acquire memory B cell Ags and IRF4 outside the GC boundaries. The detection of IRF4 in IRTA1 cells at the edge of the GC may be evidence of such maturation. Another nonexclusive possibility is that centrocytes may be quite promiscuous in terms of lineage commitment, once they reach the BCL6-negative stage. IRF4+ and/or PRDM1+ cells may still be able to enter the memory B cell lineage and would then quickly exit the GC. Promiscuity in lineage commitment is not a new concept in B and myeloid TF literature (59). The hypothesis that PRDM1 is expressed in a common memory and plasma cell precursor has been published in the past (60). Both hypotheses would require a “common centrocyte” (Fig. 9) which would then give rise to either memory or plasma cells or both.

This putative “common centrocyte” is an AID-negative cell. We have previously shown that AID+ cells in the dark and outer zone of the GC are MUM1 negative and now we confirm these data with a broader anti-IRF4 reagent. Because IRF4 is needed for class switch recombination (17), its absence in AID+ cells through the GC is in contrast with the common knowledge that the light zone is the site where such activity occurs. It is possible that IRF4 function in the GC is replaced by another TF of the same class; IRF8 and IRF4 have been previously shown to be partially redundant (56), thus IRF8 (which promotes AID expression (13)) may be a candidate. Yet, the absence of AID in centrocytes suggests that the molecular lesions initiating class switch recombination occur in a cell upstream, which would be AID+ and may express IRF4 at a certain point. We have identified putative cells fulfilling such criteria. IRF4 is expressed in rare AID+ blasts at the edge of the GC.

FIGURE 7. Coexpression of BCL6 and IRF4 in DLBCL. Four representative cases of DLBCL (A–D), double stained for BCL6 and IRF4 (MUM1), are shown, the two stains are split and shown in black and white. Arrowheads indicate nuclei with mutually exclusive staining, arrows coexpression. Note the variety of ratios of the two Ags.

FIGURE 8. Comparison of MUM1 and IRF4 polyclonal Ab staining on a sample of low grade non-Hodgkin lymphoma. Selected cases of low grade B cell lymphomas (CLL, MC, mantle cell lymphoma, MZ, marginal zone lymphoma) and tonsil are stained respectively with the MUM1 and the polyclonal IRF4 Ab. Identical fields on serial sections are shown. A low-power field (×4) is shown at the sides. The polygon marks the tonsil GC, the star the mantle. Note the more frequent positivity of the polyclonal IRF4 Ab.

FIGURE 9. Scheme of B cell TF distribution across the human GC transit. A, B cell Ags and TF distribution is shown from naive mantle cell (MC) B cells, through the outer and dark zone (OZ, DZ) of the GC, the GC light zone (LZ), and post GC. Only the plasma cell exit is shown. T, T cell; FDC, follicular dendritic cell; NcREL, nuclear cRel. B, B cell Ags and TF distribution in the GC light zone (LZ) and in the memory cell (left) and plasma cell (right) arms of post-GC differentiation. Extrafollicular CD30+ cells are shown on the left as precursors of both the GC and the extrafollicular memory B cell pathway. On the right, a CD30+ intermediate is hypothesized (dashed) although no evidence of plasma cell commitment has been identified so far. Vertical gray bars are drawn to identify boundaries of Ag expression per cell type or stage of differentiation. Extent of coexpression is approximate and not quantitative.
and, more conspicuously, in extrafollicular AID+ cells. We have shown that these cells are losing the phenotype of the acute BCR stimulation typical of CD30+ blasts (Myc, JunB, CCL22) and acquire characteristics that are similar to GC cells (36). Once these cells enter the GC reaction, if they do, then they may start the molecular processes, completed later in the light zone. The remarkable finding is that IRF4 is broadly coexpressed with other B cell TF outside the GC but it is repressed through most of the GC transit except for the plasma cell exit, where it acquires posttranscriptional modifications which may change its function. A fraction of diffuse large B cell lymphomas, some of which are identified as “activated B cell type” (22, 61), coexpress BCL6 and IRF4 is almost exclusively bearing the MUM1 epitope. In addition they are often AID+ and IgM+ (Ref. 62 and G. Cattoretti, unpublished data), suggestive of a centroblastic or outer zone origin rather than centrocytic (36).

The conformation of IRF4 resulting in the exposure of the MUM1 epitope may reduce the transcriptional activity of PU.1, as suggested by in vitro studies (54). In addition, binding of Krüppel-type zinc finger proteins such as BCL6 and PRDM1/Blimp-1 to IRF4 is mediated by partially overlapping portions of the molecule, whose posttranscriptional modification may change both the interacting partners and the effect of IRF4 transactivating ability (63).

The biological consequences of a modified IRF4 conformation are unknown. Pre-GC cells need IRF4 for terminal differentiation and never express the MUM1-like form of it (except for CD30+ blasts and some AID+ cells); IRF4 is also needed for plasma cell differentiation (17), but is unclear whether the required form in this case is the modified type found in PU.1+ cells. To add to the complexity, DLBCL containing a modified IRF4 may at the same time contain deregulated BCL6 or lack PRDM1 expression, the other necessary factor to drive plasma cell differentiation (26, 63, 64). Finally, the mutual interactions between all these TF is only partially elucidated and posttranscriptional regulation will add yet another layer of complexity to this multiplexer system.

Low-grade, pre-, or post-GC human B cell lymphomas (in contrast to DLBCL) resemble their normal counterpart by expressing low levels of IRF4 and BCL6. However, there is significant variability among different cases, possibly reflecting the state of activation or the fluctuation of baseline BCL6 expression (45). From a practical point of view, use of MUM1 Ab for prognostication in low-grade GC B cell lymphomas (in contrast to DLBCL) is not advisable because of a high percentage of false-negative cases (21).

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