American Journal of Pathology, Vol. 156, No. 2, February 2000

Up-Regulation of BOB.1/OBF.1 Expression in Normal Germinal Center B Cells and Germinal Center-Derived Lymphomas

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The BOB.1/OBF.1/OCAB.1 protein is a lymphocyte-specific transcriptional coactivator. It interacts with the Oct1 and Oct2 transcription factors and contributes to the transcriptional activity of octamer motifs. The analysis of established B cell lines had suggested that BOB.1/OBF.1 is constitutively expressed at all stages of B cell development. Here we show that expression of BOB.1/OBF.1 is regulated within the B cell lineage. Specifically, germinal center B cells show highly increased BOB.1/OBF.1 levels. We can induce the up-regulation by stimulating primary splenic B cells, eg, by triggering CD40 signaling in the presence of interleukin-4. Expression of BOB.1/OBF.1 is detectable but reduced in spleens from mice unable to undergo the germinal center reaction due to mutations in the TNF receptor p55 or lymphotoxin β (LTβ) receptor genes. Furthermore, we demonstrate that BOB.1/OBF.1 expression is highly regulated in human B cell lymphomas. Whereas lymphomas representing pre- and postfollicular B cell developmental stages are negative for BOB.1/OBF.1, high-level expression of BOB.1/OBF.1 is characteristic of germinal center-derived tumors. In these tumors BOB.1/OBF.1 is typically coexpressed with high levels of Bcl6. These results imply that overexpression of BOB.1/OBF.1, like overexpression of Bcl6, might play a role in the pathogenesis of germinal center-derived B cell lymphomas. Furthermore, overexpression of BOB.1/OBF.1 represents a characteristic feature of these tumors that is useful in their identification. *(Am J Pathol 2000, 156:501–507)*

The octamer motif is an important regulatory element for B-cell-specific transcription.[1] It is conserved in virtually all immunoglobulin heavy and light chain gene promoters as well as in several immunoglobulin enhancer elements. It is essential for the B-cell-specific promoter function and contributes to immunoglobulin enhancer activity in B cells.[2] B-cell-specific transcription of octamer-dependent promoters has been shown to require additional coactivators, which functionally interact with the Oct1 and/or Oct2 transcription factors.[3–6] The BOB.1/OBF.1 coactivator was identified a few years ago and was shown to be a critical determinant of octamer-dependent gene transcription in B lymphocytes.[7–10] This coactivator does not recognize the octamer motif with high affinity,[11] but rather is recruited into the transcription complexes via protein-protein interactions with the POU domains of the Oct proteins.[12]

Expression of BOB.1/OBF.1 is restricted largely to B lymphocytes. Analyses of BOB.1/OBF.1 expression in a variety of established B cell lines representing different stages of B cell development had suggested a constitutive, B-cell-specific expression pattern.[13] Furthermore, cell fusion experiments between B cells and fibroblasts, where the resulting somatic cell hybrids show a dominance of the fibroblast phenotype, revealed that BOB.1/OBF.1 expression is extinguished in these hybrids.[14] Interestingly, expression of BOB.1/OBF.1 can be induced in T lymphocytes by costimulation with phorbol ester (PMA) and ionomycin.[15] Furthermore, it was demonstrated that BOB.1/OBF.1 transactivation function in T cells is regulated by costimulation-induced phosphorylation of the transactivation domain.[15] Recently, a specific up-regulation of BOB.1/OBF.1 expression in murine germinal center B cells has been demonstrated.[16]

Mice deficient for the BOB.1/OBF.1 coactivator showed specific defects, which were largely restricted to late stages of B cell development.[17–19] The most prominent phenotype was the complete absence of germinal centers in the secondary lymphoid organs of these mice. No gross alterations of antigen-independent B cell development was observed, although the actual number of mature B cells in the spleen was reduced two- to fourfold in these mice. Neither rearrangement of immunoglobulin

Supported by Deutsche Forschungsgemeinschaft grants to the Forschergruppe "Defekte transkriptionelle Aktivierung lymphoider Tumore" to A. G. and T. W. and a grant from DFG to T. W. (SFB 465, B7).

Accepted for publication October 2, 1999.

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genes nor expression of the μ-heavy chain were measurably affected in these mice. However, consistent with the failure of germinal center development, BOB.1/OBF.1-deficient mice showed a strong reduction in humoral responses to both T-cell-dependent and T-cell-independent antigens. The levels of secondary immunoglobulin isotypes other than IgM were dramatically reduced.

Here we have analyzed the expression of BOB.1/OBF.1 in mouse and human primary B cells representing distinct stages of B cell development. Given the presumed role of BOB.1/OBF.1 in germinal center development, we were very interested in determining its expression levels in germinal center B cells compared to different B cell differentiation stages. In addition, we have investigated BOB.1/OBF.1 expression in a variety of human B cell lymphomas, which represented both germinal center-derived and non-germinal center-derived tumors.

Materials and Methods

Immunohistochemical stainings were performed on 4-μm cryostat sections of fresh frozen surgical specimens. Biopsy tissues were kept at −70°C as snap-frozen blocks until sections were prepared at the time of the experiments. Tumor samples were selected randomly from the German lymph node registry of the Institute of Pathology in Würzburg and classified according to REAL classification.[20] The immunoperoxidase method was applied using a three-step incubation procedure with diluted affinity-purified rabbit anti-BOB.1/OBF.1 antibodies and the preimmune serum as control, as described in detail elsewhere.[21] For double stainings on cryostat sections the immunoperoxidase-alkaline phosphatase double staining technique was used according to Krenn et al.[22] In brief, BOB.1/OBF.1 was first detected using indirect immunoperoxidase staining. Thereafter, the alkaline anti-alkaline phosphatase (APAAP) method was applied for Bcl6 detection (mAb D-8; Santa Cruz Biotechnology, Santa Cruz, CA).

The BOB.1/OBF.1-specific antibody used in these studies has been described earlier.[10] In brief, the antibody was raised in rabbits against the amino terminal domain of murine BOB.1/OBF.1, which is highly homologous to the human protein. The rabbit serum was affinity-purified using the recombinant BOB.1/OBF.1 protein. Control experiments showed that the antibody reacts equally well with murine and human BOB.1/OBF.1 in all experimental procedures.

Viable single-cell suspensions of lymphocytes were isolated from reactive human tonsils by density-gradient centrifugation and depleted of T cells and macrophages using magnetic beads coupled either with anti-CD2 or anti-CD14 (Dynal, Hamburg, Germany). Thereafter, lymphocytes were >95% CD19+ as determined by fluorescence-activated cell sorting (FACS). They were further purified by positive selection with anti-CD38 (clone ACT 13.5, Serotec, Heidelberg, Germany)-coupled magnetic beads to isolate human germinal center B cells and anti-IgD (clone HJ9, Sigma, Heidelberg)-coupled beads to isolate mantle zone B cells. The remaining memory B lymphocytes stained CD19+/IgD−/CD38−/sIg+ as described elsewhere.[23,24] If purity of cell isolates was 95% or better, cell suspensions were subjected to further analyses. Each fraction was checked by morphology using Pappenheim-stained cytosmears. Furthermore, three color flow cytometric analyses were performed with a FACScan (Becton Dickinson, Heidelberg, Germany) using an Argon ion laser tuned at 488 nm, with LYSIS II (FACS analysis software, Becton Dickinson) for data acquisition and analysis using directly conjugated mAb (CD19 HD 37, Sigma; CD3 UCHT-1, Sigma; IgD, HJ9, Sigma; CD14, Leu-M3, Becton Dickinson; CD38, ACT-13.5, Serotec; κ, KP-53, Sigma; λ, HP6054, Sigma).

For Percoll gradient separation, single-cell suspensions of mouse spleens were depleted for T cells using a cocktail of Thy1-, CD4-, and CD8-specific antibodies, followed by treatment with low toxicity guinea pig complement prepared as described. Preparation of discontinuous Percoll gradients has been described previously.[25] Preparation of whole cell and whole organ protein extracts, nuclear and cytoplasmic cell extracts, and isolation of total RNA have been described.[15,26,27]

Protein immunoblots and Northern blotting of RNA were performed as described previously.[10,15] Stimulation of fractionated B cells with antibodies to CD40 (10 μg/ml) and recombinant interleukin-4 (IL-4; 100 U/ml) was performed as described.[28] Lipopolysaccharide (Sigma) was used in a final concentration of 50 μg/ml. The Oct1-specific antibody has been described previously,[10] and the RelA and IκBα-specific antibodies were bought from Santa Cruz.

Wild-type control mice, TNFRp55−/−,[29] or LTβR−/− mice[30] were immunized by i.p. injection of 200 μg (4-hydroxy-3-nitrophenyl-acetyl) chicken γ globulin (molar ratio 19:1) adsorbed to alum per mouse. Spleens were removed and snap-frozen 10 days after immunization.

Results

The lack of germinal centers in BOB.1/OBF.1-deficient mice prompted us to investigate BOB.1/OBF.1 protein expression in germinal centers of normal human individuals. We therefore characterized BOB.1/OBF.1 expression *in vivo* in normal reactive human tonsils. This was done by immunohistochemistry using an affinity-purified antibody and cryostat sections of normal tonsils. Strong nuclear and weak cytoplasmic BOB.1/OBF.1 expression was found in the majority of normal tonsillar germinal center B cells. Nuclear expression was highest in the dark zone and decreased in the apical light zone of the germinal center (Figure 1a). In contrast to this, only scattered mantle zone and marginal zone lymphocytes were stained, but no plasma cells (Figure 1b and data not shown). Double staining experiments demonstrated that BOB.1/OBF.1 was confined to CD19-positive B cells, almost all of which coexpressed Bcl6 in normal tonsillar tissues (Figure 1c). Neither dendritic cells nor macrophages expressed BOB.1/OBF.1 or Bcl6.

To strengthen the immunohistochemistry results we purified different B cell populations from human tonsils

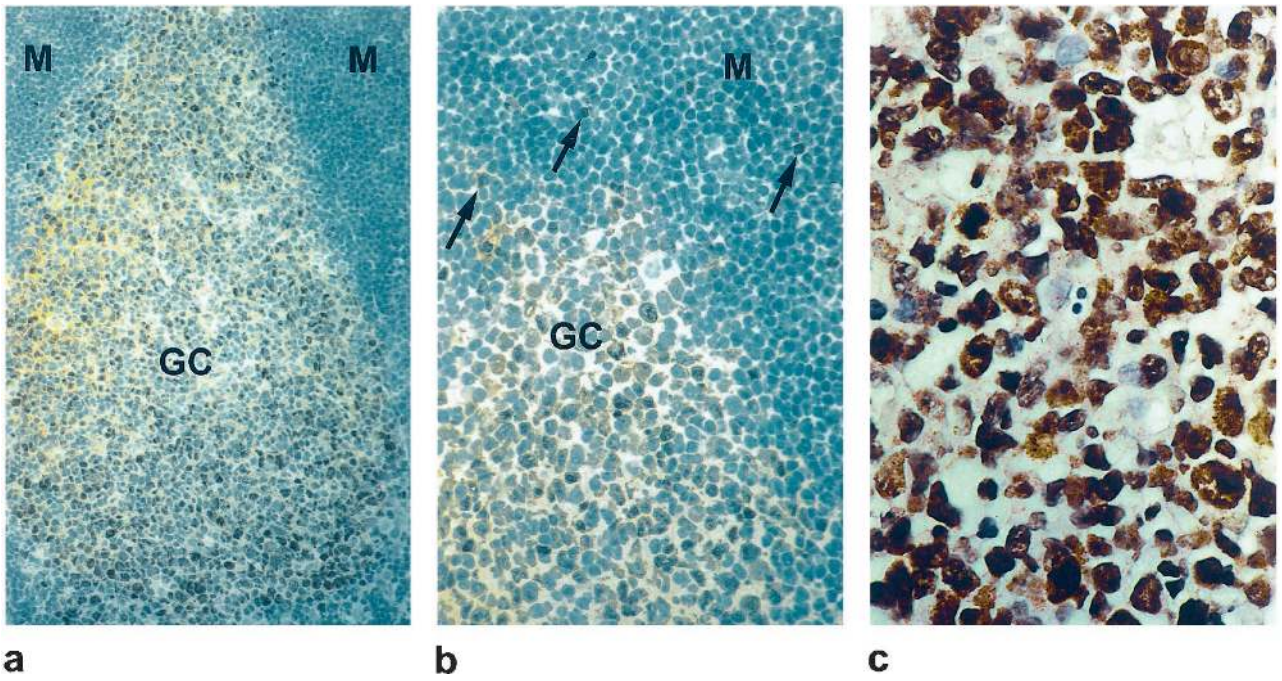

Figure 1. Expression of BOB.1/OBF.1 in sections from normal human tonsils. **a:** Cryostat section of normal tonsil showing intense brown staining for BOB.1/OBF.1 in germinal center lymphocytes (GC). Immunoperoxidase; original magnification, ×100. **b:** A detail of the same tonsil shows the border between GC and mantle zone (m) lymphocytes, where only few cells show positive nuclear staining (**arrow**). Immunoperoxidase; original magnification, ×250. **c:** BOB.1/OBF.1 and Bcl-6 are colocalized in nuclei of most dark zone germinal center lymphocytes of normal human tonsils. Original magnification, ×400. Double staining with BOB.1/OBF.1 (immunoperoxidase, brown) and Bcl6 (APAAP, red). Note the weak cytoplasmic staining in B cells and lack of staining for BOB.1/OBF.1 and Bcl6 in macrophages (**arrow**).

and analyzed BOB.1/OBF.1 expression by protein immunoblotting. In a first step, CD19-positive cells (B cells) were purified. These cells were then further subdivided into the CD38-positive fraction (germinal center B cells) and CD38-negative fraction. The CD38-negative cells were further sorted for expression of IgD on the surface (naive B cells). When protein extracts from these different populations were characterized for BOB.1/OBF.1 expression, the strongest signal was evident in the CD38-positive germinal center B cell fraction (Figure 2A). The other fractions also showed BOB.1/OBF.1 expression, albeit at a clearly reduced level. We also analyzed these fractions for Bcl6 expression as an additional marker for germinal center B cells. Consistent with the immunohistochemistry results, Bcl6 was also strongly up-regulated in the germinal center B cell fractions. The observed differences were, however, specific for these two proteins, in that RelA expression was rather uniform in the various samples (Figure 2A).

The immunostainings shown above had revealed some cytoplasmic staining for BOB.1/OBF.1. Given the known function of BOB.1/OBF.1 as a nuclear regulator, this staining pattern came as a surprise. To investigate whether BOB.1/OBF.1 could be found in the cytoplasm of established B cell lines, cell fractionation followed by protein immunoblotting experiments were performed. This analysis revealed that BOB.1/OBF.1 can be found in the nuclear as well as the cytoplasmic fraction. In contrast, proteins known to be in the cytoplasm of PD31 cells like RelA and IκBα were found exclusively in the cyto-

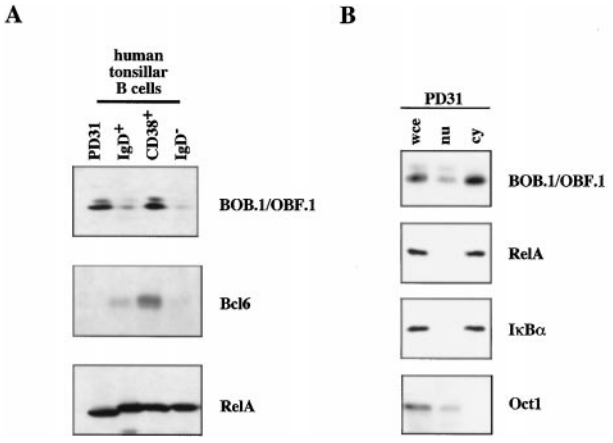

Figure 2. Analysis of BOB.1/OBF.1 expression in sorted B cells and after subcellular fractionation. **A:** Human tonsillar B cells (CD19 positive cells) were sorted into CD38 positive and CD38 negative fractions, the CD38 negative fraction was further divided into an IgD+ (naive B cells) and an IgD− (memory B cells) fraction. Whole cell protein extracts (40 μg) of these fractions were analyzed with antibodies specific for BOB.1/OBF.1 (**top panel**), for Bcl6 (**middle panel**) and for RelA (**bottom panel**) by protein immunoblotting. Extracts from murine PD31 pre-B cells were used as an additional control. Note that murine RelA is somewhat smaller than human RelA. **B:** Whole cell extracts (wce), nuclear extracts (nu), and cytoplasmic extracts (cy) were prepared from the murine PD31 cell line. 50 μg of proteins were analyzed by protein immunoblotting with antibodies specific for the proteins indicated. As expected, RelA and IκB[α] proteins are exclusively found in the cytoplasm of PD31 cells and Oct1 is only found in the nuclear fraction. In contrast, BOB.1/OBF.1 is found in both fractions. The lack of the slower migrating (weaker) BOB.1/OBF.1 band in the cytoplasmic extracts was not seen consistently.

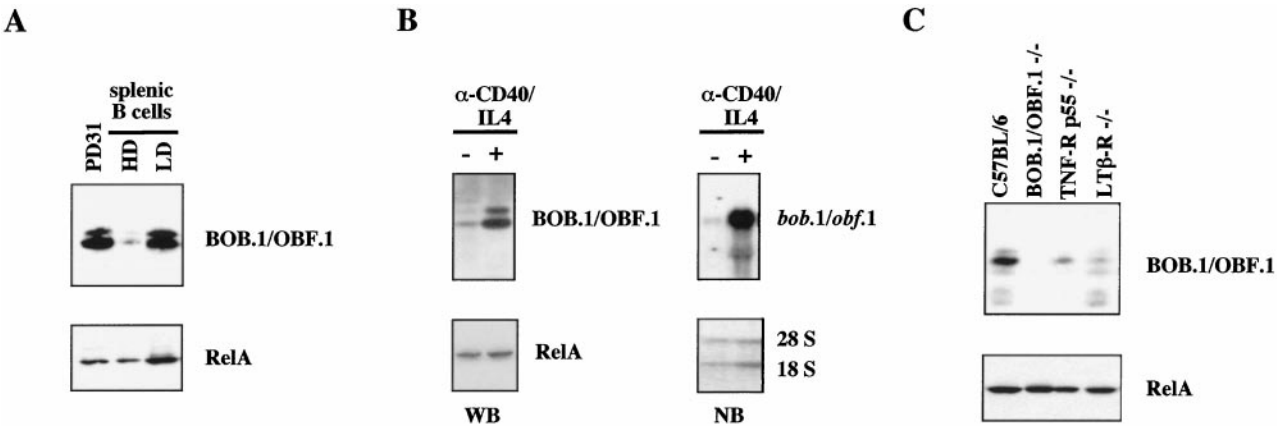

Figure 3. Regulation of BOB.1/OBF.1 expression in primary B cells following activation by LPS and α-CD40 plus IL-4. **A:** Protein immunoblot analysis of 40 μg whole cell extract from Percoll gradient-enriched B cell fractions as indicated. The blot was probed with antibodies specific for BOB.1/OBF.1 and RelA as indicated. HD, high density; LD, low density B cell fractions. See text for details. **B:** Costimulation with anti-CD40 antibodies in the presence of IL-4 induces BOB.1/OBF.1 expression. Protein immunoblots with 40 μg whole cell extract from either unstimulated HD B cell fractions, or cells stimulated for 72 hours are shown in the **left panel** (WB, Western blot). The blots were probed with antibodies specific for BOB.1/OBF.1 and RelA as above. The **right panel** shows a Northern blot (NB) with RNA from HD B cells untreated or stimulated with α-CD40/IL4 for 72 hours probed with a BOB.1/OBF.1-specific probe. The **lower panel** shows a the EtBr-stained RNA and the 28S and 18S ribosomal RNAs are marked. **C:** Expression of BOB.1/OBF.1 in spleens from immunized wild-type and mutant mouse strains. Protein extracts (100 μg) from spleens C57B1/6 wild type mice, p55-TNF-RI-deficient mice, and LTβ-R-deficient mice, all immunized for 10 days with DNPG, as well as unimmunized BOB.1/OBF.1-deficient mice were analyzed by protein immunoblot with antibodies specific for BOB.1/OBF.1 and RelA.

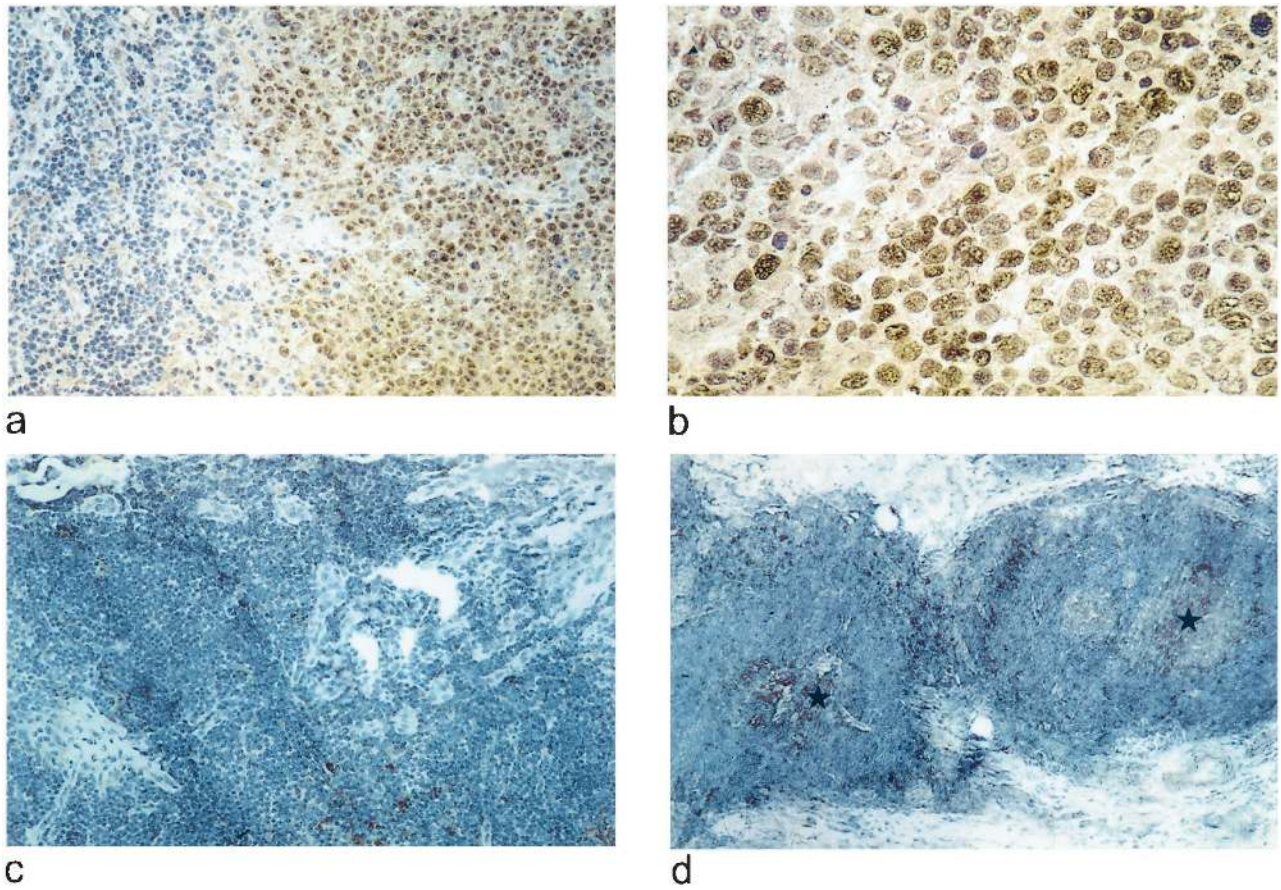

Figure 4. Immunohistochemical analysis of BOB.1/OBF.1 expression in B cell non-Hodgkin's lymphomas. **a:** Strong nuclear BOB.1/OBF.1 is present in high-grade DLBL (immunoperoxidase; original magnification, ×100), whereas the surrounding normal lymph node tissue stains negative. **b:** Same case as **a** in detail, stained with Bcl-6 as well (immunoperoxidase; original magnification, ×250). **c:** BOB.1/OBF.1 staining in low grade MALT. There is no staining detectable in the low grade lymphoma (immunoperoxidase; original magnification, ×100). **d:** A parallel section of the low grade lymphoma was stained for Bcl6 (immunoperoxidase; original magnification, ×40). The reactive GC is stained as well as some perifollicular cells, whereas the tumor cells surrounding the follicle are negative.

Table 1. Immunohistochemical Stainings of Normal and Malignant Lymphatic Tissues for BOB.1/OBF.1 and Bcl6

Normal tonsillar lymphoid tissue	Expression of BOB.1/OBF.1 I Bcl6	Malignant compartment (*n*)	BOB.1/OBF.1 pos./ Bcl6 pos.
Early naive B cell	n.d.	B-CLL (5)	(0/0)
Mantle cell (naive B cell)	± /−	Mantle cell lymphoma (16)	(0/0)
Germinal center B cell (dark zone)	+++ /+++	Burkitt (8)	(8/8)
Germinal center B cell	++ /++	Follicular lymphoma (18)	(17/16)
Germinal center B cell	++ /++	DLBL (31)	(29/26)
Marginal zone B cell (memory B cell)	± /−	MALT-type lymphoma (14)	(0/0)
Plasma cell	− /−	Plasmacytoma (7)	(0/0)

Sections of the indicated normal and malignant tissues were stained with antibodies specific for BOB.1/OBF.1 and Bcl6 as indicated.
−, no stainings; ±, <10% of positive cells; +, ≥10% <30% of positive cells; ++, ≥30% <70% of positive cells; +++, ≥70% of positive cells. DLBL, diffuse large cell B cell lymphoma.

plasmic fraction,[31] whereas the Oct1 transcription factor was found exclusively in the nucleus (Figure 2B).

The germinal center reaction requires a complex interplay of antigen-specific B cells, antigen-specific T cells, and antigen-presenting follicular dendritic cells. As a consequence, specific B cells are activated, proliferate, perform isotype-switching and somatic hypermutation, and finally differentiate into either plasma cells or memory B cells. We were, therefore, interested to determine whether the activation status of B cells determines the level of BOB.1/OBF.1 expression. To this end, we separated murine splenic B cells on discontinuous Percoll density gradients into different fractions, which grossly represent distinct activation stages of B lymphocytes. The high density fraction (HD) largely represents naive resting B cells, whereas the low density fraction (LD) is comprised predominantly of large activated B cells. In the high-density B cell fraction there was only a low level of BOB.1/OBF.1 protein, whereas expression levels were significantly higher in the fraction representing large activated B cells (Figure 3A). Consistent with an earlier report,[16] these low levels of BOB.1/OBF.1 expression in the high density B cell fraction could be increased by costimulation with CD40 and IL-4 at both the RNA and protein levels (Figure 3B).

To address the question whether most or all B cells express at least baseline levels of BOB.1/OBF.1 protein and expression is up-regulated in germinal center B cells, or, alternatively, that the low expression levels detected in non-germinal center B cells might be due to contaminating germinal center B cells, we analyzed BOB.1/OBF.1 expression in spleens from mice that showed a defect in germinal center formation. Two such mouse mutants were investigated, one carrying a mutation in the TNFRI (p55 gene)[32,33] and the second carrying a mutation in the gene encoding the lymphotoxin (LT) β receptor.[30] Both mutant mice have previously been demonstrated to lack germinal centers; this defect is not intrinsic to the B lymphocytes, but rather affects accessory cells. When spleen extracts from these mutant mice were compared with wild-type mice for their levels of BOB.1/OBF.1 expression, a reduction of the BOB.1/OBF.1 signal was evident (about two- to fourfold). Nevertheless, these mice still express significant levels of BOB.1/OBF.1 (Figure 3C). This finding suggests that B cells not engaged in germinal center reactions constitutively express a lower level of BOB.1/OBF.1.

The Bcl6 protein plays an important role in regulating B cell differentiation within germinal centers.[34–36] In addition, its expression is often deregulated in germinal center-derived lymphomas.[37–41] The observation that BOB.1/OBF.1 and Bcl6 showed a high degree of coexpression in normal germinal center B cells prompted us to investigate BOB.1/OBF.1 expression in a variety of human B cell lymphomas. To do so, 99 different B cell lymphomas were selected randomly from the German lymph node repository. The tumors had been classified according to the REAL guidelines and represented different types of B cell lymphomas, which correlate well with distinct stages of normal B cell development.[20] In addition, 18 different T cell lymphomas such as T-lymphoblastic lymphomas (5 cases), large granular lymphocytic leukemia of T type (2 cases), angioimmunoblastic T cell lymphoma (AILD, 6 cases), and intestinal T cell lymphomas (5 cases) were also included in this study.

All tumors were analyzed for the presence of BOB.1/OBF.1 as well as Bcl6 by immunohistochemistry. A representative example is shown in Figure 4. A summary of the results of the immunohistochemistry is given in Table 1. Overall, 54% (54/99) of the B cell lymphomas analyzed were BOB.1/OBF.1-positive. In contrast, all T cell lymphomas investigated were negative for BOB.1/OBF.1. All Burkitt's lymphomas, most follicular lymphomas (17/18), and 94% of high-grade diffuse large cell B cell lymphomas (29/31) stained strongly, whereas all low-grade lymphomas (B-CLL, mantle cell lymphomas, MALT-type lymphomas, plasmacytomas; 42/42) were negative for BOB.1/OBF.1 and Bcl6. Within low-grade lymphomas, only a few scattered large cells as well as reactive nonneoplastic germinal centers were positive for BOB.1/OBF.1 and Bcl6 (Figure 4, c and d). Strikingly, BOB.1/OBF.1 staining was paralleled by Bcl-6 staining in most high-grade B cell lymphomas (Figure 4, a and b). However, two diffuse large B cell lymphomas (DLBL) stained for neither BOB.1/OBF.1 nor Bcl6, and three other cases were positive for BOB.1/OBF.1 but negative for Bcl6.

Discussion

The most obvious phenotype of the BOB.1/OBF.1 knockout mice was a complete lack of germinal centers in spleen and lymph nodes. Consistent with this defect, we see a significant up-regulation of BOB.1/OBF.1 expres-

sion in normal germinal center B cells. This up-regulation can be mimicked by stimulating the B cells in culture with the potent mitogen LPS or by triggering CD40 signaling in the presence of IL-4. Similar results have recently been obtained by Qin and colleagues.[16] In addition, we found that in contrast to established B lymphoma cell lines, which all expressed significant levels of BOB.1/OBF.1 regardless of their differentiation state, such tumors *in situ* show striking differences with respect to BOB.1/OBF.1 expression. Like their normal counterparts, germinal center B cell-derived B cell lymphomas stained strongly positive for BOB.1/OBF.1 in immunohistochemistry, whereas lymphomas representing other stages of B cell development were negative. Although these results do not prove a role for BOB.1/OBF.1 in the pathogenesis of germinal center-derived tumors, the high level expression can at least be regarded as a consistent marker for the classification of this type of lymphomas.

The earlier studies on expression of BOB.1/OBF.1 in the B lymphoid lineage had relied largely on the analysis in established tumor cell lines. The most detailed study along this line had revealed a rather constant expression level throughout B lymphoid development.[13] Interestingly, expression levels in transformed pre-B cell lines was shown to be unaffected by LPS treatment.[13] Indeed, we also did not see an induction of BOB.1/OBF.1 expression on LPS treatment of the WEHI231 cell line representing the stage of an immature B cell (Boehm J, Wirth T, unpublished observations). These results indicate a significant difference between regulation of BOB.1/OBF.1 expression in primary B cells as compared to established B cell lines.

Recently, a critical role for BOB.1/OBF.1 up-regulation in B cell transformation has been suggested.[16] The in-depth analysis of BOB.1/OBF.1 expression in B lymphomas *in situ* performed in the present study indicates that deregulation of BOB.1/OBF.1 expression is not a general event in B cell tumorigenesis. Rather, the human lymphomas analyzed reflected the situation observed in their normal counterparts, namely low (undetectable by immunohistochemistry) expression of BOB.1/OBF.1 in tumors not derived from germinal center B cells and high expression in germinal center-derived tumors. Therefore, our results do not allow us to conclude whether high level expression of BOB.1/OBF.1 contributes to the transformation of these germinal center-derived lymphomas. It remains unclear why the initial difference in BOB.1/OBF.1 expression in different types of B lymphomas is lost when B cell lines are established. A potential explanation is that selection for continuous growth in tissue culture may result in the up-regulation of BOB.1/OBF.1 expression. This could be a consequence of permanent stimulation of BOB.1/OBF.1 expression by some component in the tissue culture medium or a stable genetic/epigenetic alteration acquired during the cell culture adaptation process.

Bcl6 (also called Laz3) is a member of the family of zinc-finger transcription factors which contains an amino-terminal POZ/ZIN domain.[37,42] It functions as a sequence-specific transcriptional repressor.[36,43] Chromosomal translocations involving the *bcl6* gene have been found in about 40% of diffuse large cell lymphomas and to a lesser extent (approximately 14%) in follicular lymphomas.[37–39,42] As a consequence of these translocations, expression of the *bcl6* gene is controlled by heterologous promoters.[41] Interestingly, in at least one case the translocation of *bcl6* resulted in the fusion to the BOB.1/OBF.1 gene promoter.[44,45] Although normal expression of Bcl6 is not confined to the lymphocyte lineage, within the B cell lineage Bcl6 is specifically expressed in germinal center B cells.[46,47] Interestingly, like the defect observed in the BOB.1/OBF.1-deficient mice, Bcl6-deficient B cells fail to generate germinal centers.[34–36] It was recently demonstrated that Bcl6 protein expression is unaffected by the BOB.1/OBF.1 mutation.[16] However, it is possible that expression of the two genes is coregulated by some so far unknown mechanism in the B cell lineage.

The analysis of BOB.1/OBF.1 expression in normal lymphoid tissues and primary tumors suggests that BOB.1/OBF.1 might be a good marker for germinal center-related B cell transformation as described for bcl6 expression recently.[48] In most tumor cases Bcl6 and BOB.1/OBF.1 were coexpressed at a high level, but we noted three cases of DLBL positive for BOB.1/OBF.1 but lacking detectable Bcl6 expression. However, we could not find a tumor positive for Bcl6 expression that lacked expression of BOB.1/OBF.1. Therefore, BOB.1/OBF.1 seems to be an appropriate marker for high grade malignant germinal center derived B cell lymphomas. Interestingly, we found two cases of DLBL negative for both Bcl6 and BOB.1/OBF.1. These two tumors were morphologically indistinguishable from the other DLBL cases and the reasons for their lacking BOB.1/OBF.1 expression are currently not known.

Acknowledgments

We thank B. Kistler for the anti-CD40 antibodies and IL-4 as well as for many helpful suggestions. We thank M. Reichert, H. Haber, and A. Zant for invaluable technical assistance and A. Schimpl for many helpful comments and critical reading of the manuscript.

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