

Short Communication

p52 Activation in Monomorphic B-Cell Posttransplant Lymphoproliferative Disorder/Diffuse Large B-Cell Lymphoma without BAFF-R Expression

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Posttransplantation lymphoproliferative disorders (PTLD) are associated with Epstein-Barr virus (EBV) and activate the NF- κ B pathway. B-cell activating factor (BAFF) modulates cell growth and survival in non-Hodgkin's lymphomas. However, there are few studies of EBV, BAFF/BAFF-R signaling, and NF- κ B1 and NF- κ B2 pathway activation in PTLD. Diffuse large B-cell lymphomas (DLBCL) in two different clinical contexts, immunocompetent patients (DLBCL/IC; $n = 30$) or posttransplantation solid-organ recipients (DLBCL/PTLD; $n = 21$), were characterized histogenically as germinal center (GC) or non-germinal center (NGC). Expression of BAFF, BAFF-R, and NF- κ B proteins p50 and p52 and the presence or absence of EBV were compared in these clinical contexts. Regardless of the GC or NGC pattern of DLBCL, BAFF-R was expressed in 37% of DLBCL/IC but in only 4.8% of DLBCL/PTLD. p52 was expressed in DLBCL/PTLD/NGC (12 of 19 cases) as compared with DLBCL/IC/NGC (0 of 18 cases). This pattern might be related to the presence of EBV and latent membrane protein 1 because p52 expression was observed primarily in EBV-positive DLBCL/PTLD cases expressing latent membrane protein 1. Thus, the activation profile or NGC pattern of DLBCL/PTLD was not associated with BAFF/BAFF-R expression, whereas nuclear p52 related to NF- κ B2 pathway activation might be linked to EBV. (*Am J Pathol* 2011, 179:1630–1637; DOI: 10.1016/j.ajpath.2011.07.003)

Posttransplantation lymphoproliferative disorders (PTLD) are among the worst prognostic complications after solid-organ transplantation.¹ These lymphoid proliferations are characterized by their heterogeneity. Several categories of PTLD have been identified by the World Health Organization (World Health Organization classification). Among these, the B-cell monomorphic subcategory fulfills the criteria for diffuse large B-cell lymphomas (DLBCL) described in immunocompetent individuals.² Studies of the histogenesis of PTLD also reflect this heterogeneity.³ Several studies of histogenesis origin using immunohistochemistry expression of CD10, BCL6, MUM1/IRF4, and CD138 demonstrated two patterns, germinal center (GC) and non-germinal center (NGC), including late GC/early post-GC and post-GC.^{4–7} Epstein-Barr virus (EBV)-positive cases were primarily associated with the NGC phenotype.⁶ EBV-negative PTLD are primarily described as late complications and are more aggressive than EBV-positive PTLD.^{8,9}

EBV is a member of the herpesvirus family and is usually associated with mononucleosis and a wide variety of malignant lesions.^{10,11} Latent infection leads to expression of nine proteins including latent membrane proteins LMP1, LMP2A, and LMP2B and nuclear proteins EBNA1, EBNA2, EBNA3A, EBNA3B, and EBNA3C, as well as the small nonencoded RNAs EBER1 and EBER2.⁸ Therefore, the transcriptional activator protein EBNA2 has been described as essential for B-cell transformation and LMP1 expression. The cytoplasmic C-terminal-located CTAR1 and CTAR2 regions of LMP1 activate the NF- κ B2 and NF- κ B1 pathways, respectively (canonical and non-canonical pathways).¹²

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NF- κ B is a dimeric transcription factor that is regulated by the I κ B protein family. Two pathways have been identified: the canonical pathway (NF- κ B1), which generates a p50/p65 dimer through I κ b α degradation by IKK β , and the non-canonical pathway (NF- κ B2) leading to p52/RelB dimer release via involvement of IKK α . These two pathways lead to translocation into the nucleus of these various dimers to modulate several genes.¹³ To date, several publications have reported constitutive expression of p65 in DLBCL subtypes.^{14,15}

BAFF (B cell-activating factor), a member of the tumor necrosis factor family, increases the amount of peripheral B cells and is implicated in development of autoimmune diseases in a transgenic mice model overexpressing BAFF.^{16,17} Moreover, BAFF modulates cell growth and survival in multiple myeloma and B-cell chronic lymphocytic leukemia.^{18,19} BAFF is expressed in non-Hodgkin's lymphoma-like mantle and marginal zones, follicular lymphoma, and DLBCL.²⁰

BAFF-R, also known as BR3, TNFRSF13C, or CD268, is a type III transmembrane protein that is considered the principal receptor of BAFF leading to the survival and maturation of primary B cells.²¹ BAFF-R is the only specific receptor of BAFF.²² Expression of BAFF-R was first detected in tonsils, and demonstrated strong staining on B cells of the mantle zone and weak staining of GC B lymphocytes.²³ Similar staining has been also described in other studies. Furthermore, several non-Hodgkin's lymphomas have been investigated through BAFF-R expression, which was recently exhibited in most lymphoproliferative disorders.^{24–26}

BAFF-R signaling activates NEMO-independent processing of the NF- κ B2 pathway.²⁷ Moreover, LMP1 activates the BAFF gene promoter via NF- κ B. The ability of BAFF to increase cell survival and of BAFF-R and LMP1 to activate the NF- κ B pathway, and the strong association of EBV in PTLD constitute at least three reliable reasons to study the role of EBV, NF- κ B2, and BAFF-R in PTLD.

To analyze some differences in BAFF-R expression according to the activation level of B cells, we tested the expression of BAFF and BAFF-R in 51 DLBCL cases from the general population (30 patients) and immunodeficient patients (21 with PTLD). All cases were characterized according to morphologic features, immunophenotype, and GC or NGC histogenesis pattern. In EBV-positive cases, LMP1, EBNA2, and ZEBRA, an immediate replicative protein, were tested. The detection of nuclear or cytoplasmic expression of p50 and p52 proteins to analyze the activation of NF- κ B pathways demonstrated a correlation between p52 expression and the presence of EBV, as well as latency II or III, ie, expression of LMP1 in PTLD.

Materials and Methods

Patients

Fifty-one DLBCL cases were collected. Patients enrolled in the present study were followed up in two solid-organ transplantation centers, the Bicêtre and Paul Brousse University hospitals. All lymphomas in this series were

monomorphic B-cell lymphomas according to the criteria of the World Health Organization classification. Written informed consent was obtained from all included patients. The DLBCL cases comprised two subgroups: 30 immunocompetent patients with DLBCL (DLBCL/IC) and 21 solid-organ recipients with DLBCL (DLBCL/PTLD). Patient clinical data are given in Table 1. Most DLBCL cases, either DLBCL/IC or DLBCL/PTLD, were localized in lymph nodes (34 of 51). Mean age of patients with DLBCL/PTLD at diagnosis of lymphoma was 39 years (range, 1 to 74 years), and of patients with DLBCL/IC was 61.5 years (range, 18 to 92 years).

Conventional Morphologic Analysis and Immunostaining

Formalin-fixed, paraffin-embedded tissue sections from DLBCL biopsy specimens were stained using H&E for conventional morphologic analysis. The slides were reviewed by two hematopathologists (M.R. and T.L.) to reclassify cases according to the World Health Organization criteria. Immunostaining was performed on paraffin sections using an immunoperoxidase technique (ChemMate EnVision Detection Kit, DakoCytomation A/S, Glostrup, Denmark, or the Vectastain Elite ABC kit, Vector Laboratories, Inc., Burlingame, CA) according to the manufacturer's recommendations. To identify GC or NGC pattern, the following B-cell markers were tested: CD20 (clone L26; dilution 1:1950; DakoCytomation A/S), CD10 (clone 56C6; dilution 1:40; Novocastra, Newcastle-upon-Tyne, England), BCL6 (clone PG-B6p; dilution 1:40; DakoCytomation A/S), IRF4/MUM1 (clone M-17; dilution 1:400; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and CD138 (syndecan-1; clone B-A38; dilution 1:100; Diaclone, Stamford, CT).

Using the same technique, expression of EBV latency proteins LMP1 (clone CS.1–4; dilution 1:100), EBNA2 (clone PE2; dilution 1:100), and the replicative protein ZEBRA (clone BZ.1; dilution 1:25) (all three from DakoCytomation A/S) were studied, as was expression of BAFF (clone buffy-2; dilution 1:100) and BAFF-R (clone 11C1; dilution 1:600) (both from Alexis Biochemicals Corp., Lausen, Switzerland). For BAFF and BAFF-R, cases exhibiting more than 30% of immunostained tumor cells were considered positive.⁵

Protein expression of p50 (dilution 1:40; Santa Cruz Biotechnology, Inc.) and p52 (dilution 1:400; Millipore Corp., Billerica, MA) was also assessed using immunoperoxidase to explore NF- κ B pathways. Localization of staining was specified as nuclear (activated NF- κ B pathway) or cytoplasmic.

Detection and Characterization of EBV

EBV status of all DLBCL was performed via *in situ* hybridization using EBER1 and EBER2 probes with the PNA In Situ Hybridization Detection Kit (DakoCytomation A/S) according to the manufacturer's recommendations. In EBV, EBER-positive cases, latency was defined as latency I (EBER-positive, LMP1-negative, or EBNA2-negative), latency II

Table 1. Clinical Data in Patients with DLBCL

Patients	Age at diagnosis (year)	Sex	Graft type	Delay of lymphoma occurrence (mo)	Localization	Histologic type
1	35	M	NR	NR	Lymph nodes	DLBCL/IC
2	55	F	NR	NR	Cavum	DLBCL/IC
3	32	M	NR	NR	Tonsils	DLBCL/IC
4	63	F	NR	NR	Lymph nodes	DLBCL/IC
5	81	F	NR	NR	Lymph nodes	DLBCL/IC
6	81	F	NR	NR	Lymph nodes	DLBCL/IC
7	65	M	NR	NR	Lymph nodes	DLBCL/IC
8	60	M	NR	NR	Lymph nodes	DLBCL/IC
9	75	M	NR	NR	Lymph nodes	DLBCL/IC
10	92	F	NR	NR	Lymph nodes	DLBCL/IC
11	18	M	NR	NR	Lymph nodes	DLBCL/IC
12	73	M	NR	NR	Lymph nodes	DLBCL/IC
13	52	M	NR	NR	Lymph nodes	DLBCL/IC
14	51	F	NR	NR	Lymph nodes	DLBCL/IC
15	85	M	NR	NR	Lymph nodes	DLBCL/IC
16	75	M	NR	NR	Lymph nodes	DLBCL/IC
17	82	F	NR	NR	ORL	DLBCL/IC
18	77	F	NR	NR	Lymph nodes	DLBCL/IC
19	79	F	NR	NR	Lymph nodes	DLBCL/IC
20	58	F	NR	NR	ORL	DLBCL/IC
21	80	F	NR	NR	Lymph nodes	DLBCL/IC
22	34	F	NR	NR	Kidney	DLBCL/IC
23	46	F	NR	NR	Cavum	DLBCL/IC
24	58	F	NR	NR	Lymph nodes	DLBCL/IC
25	71	M	NR	NR	Lymph nodes	DLBCL/IC
26	53	M	NR	NR	Lymph nodes	DLBCL/IC
27	58	M	NR	NR	Gut	DLBCL/IC
28	56	M	NR	NR	Gallbladder	DLBCL/IC
29	60	M	NR	NR	Lymph nodes	DLBCL/IC
30	89	M	NR	NR	Lymph nodes	DLBCL/IC
31	51	M	Liver	109	Liver	DLBCL/PTLD
32	16	M	Liver	5	Cavum	DLBCL/PTLD
33	22	M	Liver	1	Lymph nodes	DLBCL/PTLD
34	51	M	Lung	6	Lung	DLBCL/PTLD
35	64	F	Kidney	114	Lymph nodes	DLBCL/PTLD
36	3	F	Liver	27	ORL	DLBCL/PTLD
37	2	F	Liver	6	Liver	DLBCL/PTLD
38	5	F	Liver	32	Tonsil	DLBCL/PTLD
39	56	M	Liver	109	Lymph nodes	DLBCL/PTLD
40	3	F	Liver	2	Cavum	DLBCL/PTLD
41	66	F	Kidney	259	Small intestine	DLBCL/PTLD
42	39	M	Kidney	84	Lymph nodes	DLBCL/PTLD
43	74	M	Liver	49	Lymph nodes	DLBCL/PTLD
44	2	F	Liver	7	Tonsils	DLBCL/PTLD
45	54	M	Kidney + pancreas	5	Brain	DLBCL/PTLD
46	3	M	Liver	5	Lymph nodes	DLBCL/PTLD
47	49	M	Kidney + pancreas	162	Lymph nodes	DLBCL/PTLD
48	1	M	Liver	10	Tonsil	DLBCL/PTLD
49	36	F	Kidney	152	Peritoneum	DLBCL/PTLD
50	53	F	Kidney	192	Lymph nodes	DLBCL/PTLD
51	59	M	Kidney	81	Brain	DLBCL/PTLD

DLBCL, diffuse large B-cell lymphoma; DLBCL/IC, DLBCL in immunocompetent patients; F, female; M, male; NR, not relevant; ORL, otorhinolaryngology; PTLD, post-transplantation lymphoproliferative disorder.

(EBER-positive, LMP1-positive, or EBNA2-negative), and latency III (EBER-positive, LMP1-positive, or EBNA2-positive).

Controls

External controls tested included reactive lymph nodes or tonsils and lymphomatous tissues known to express BAFF and BAFF-R: follicular lymphoma, 6 cases; mantle cell lymphoma, 5 cases; marginal zone B-cell lymphoma, 4 cases; MALT (mucosa-associated lymphoid tissue) lymphoma, 6 cases; and Burkitt's lymphoma, 6 cases.

Results

Characterization and Histogenesis of DLBCL/IC and DLBCL/PTLD

The definition of GC origin means a GC B-cell pattern having the expression of CD10 and/or BCL6 or activated GC B-cell pattern according to expression of at least one GC B-cell marker, either BCL6 or CD10, and at least one activation marker, either IRF4/MUM1 or CD138. The activated NGC B-cell pattern means expression of activation markers IRF4/

Table 2. Immunohistochemical Staining of DLBCL Samples

DLBCL	CD10	BCL6	IRF4	CD138	BAFF	BAFF-R	EBER	LMP1	EBNA2	ZEBRA	P52	P50	Origin
1	-	+	-	-	+++	+++	-	NR	NR	NR	-	-	GC
2	-	+	-	-	+++	+	-	NR	NR	NR	Nu	Nu	GC
3	+	+	-	-	+++	+	-	NR	NR	NR	-	-	GC
4	+	-	-	-	+++	-	-	-	NR	NR	-	-	GC
5	-	+	-	-	+++	-	-	-	NR	NR	Nu	-	GC
6	+	+	-	-	+++	-	-	NR	NR	NR	-	-	GC
7	+	+	-	-	++	-	-	NR	NR	NR	-	-	GC
8	+	+	-	-	+++	-	-	NR	NR	NR	Nu	Nu	GC
9	+	+	-	-	+	+	-	NR	NR	NR	-	-	GC
10	+	+	-	-	+++	-	-	NR	NR	NR	Nu	-	GC
11	+	+	-	-	+++	+++	-	NR	NR	NR	Nu	-	GC
12	+	+	-	-	-	+++	-	NR	NR	NR	-	Nu	GC
13	-	+	+	-	+++	++	-	NR	NR	NR	-	-	NGC
14	-	+	+	-	+++	+	-	NR	NR	NR	-	-	NGC
15	-	+	+	-	+++	+	-	NR	NR	NR	-	-	NGC
16	-	-	+	-	+	+	-	NR	NR	NR	-	-	NGC
17	-	-	+	-	+++	-	-	NR	NR	NR	-	-	NGC
18	-	-	+	-	+++	-	-	NR	NR	NR	-	-	NGC
19	-	-	+	-	+++	-	-	NR	NR	NR	-	-	NGC
20	-	-	+	-	+++	-	-	NR	NR	NR	-	Nu	NGC
21	-	-	+	-	+++	-	-	NR	NR	NR	-	-	NGC
22	-	-	+	+	+++	-	+	+	+	-	-	Nu	NGC
23	-	+	+	-	+++	-	-	NR	NR	NR	-	-	NGC
24	-	-	+	+	-	-	+	-	-	+	-	Nu	NGC
25	-	-	+	-	++	-	-	NR	NR	NR	-	-	NGC
26	-	++	+	-	+++	-	-	NR	NR	NR	-	-	NGC
27	-	+	+	-	++	+++	-	NR	NR	NR	-	-	NGC
28	-	-	+	-	++	-	-	NR	NR	NR	-	-	NGC
29	-	+	+	-	++	-	-	NR	NR	NR	-	-	NGC
30	-	+	+	-	+	-	-	NR	NR	NR	-	-	NGC
31	+	-	-	+	+++	-	-	NR	NR	NR	-	-	NGC
32	-	+	+	-	+++	-	-	NR	NR	NR	Nu	Nu	NGC
33	-	+	-	+	-	-	+	+	ND	+	Nu	ND	NGC
34	-	-	+	+	+++	-	-	NR	NR	NR	-	Nu	NGC
35	-	-	+	-	+	-	-	NR	NR	NR	-	-	NGC
36	-	-	+	+	+++	-	+	+	-	+	-	Nu	NGC
37	-	-	-	+	+++	-	+	+	+	+	Nu	Nu	NGC
38	-	-	+	+	+	+	+	+	+	+	Nu	Nu	NGC
39	-	-	+	-	+	-	-	NR	NR	NR	-	ND	NGC
40	-	-	+	+	+	-	+	+	+	+	Nu	Nu	NGC
41	-	-	+	-	+	-	+	-	-	-	-	-	NGC
42	-	-	+	-	-	-	-	NR	NR	NR	-	-	NGC
43	-	-	+	-	+	-	-	NR	NR	NR	Nu	-	NGC
44	-	-	+	+	+	-	+	+	+	+	Nu	-	NGC
45	-	+	+	+	+	-	+	+	+	-	Nu	Nu	NGC
46	-	-	+	+	+++	-	+	+	+	-	Nu	-	NGC
47	-	-	+	-	++	-	-	NR	NR	NR	Nu	-	NGC
48	-	-	+	+	+++	-	+	+	-	+	Nu	-	NGC
49	-	-	+	+	+++	-	-	NR	NR	NR	Nu	-	NGC
50	-	-	-	-	+++	-	+	-	-	-	Nu	Nu	ND
51	-	-	-	-	+++	-	+	+	+	+	Nu	-	ND

Nos. 1 to 30, DLBCL/IC; 31 to 51, DLBCL/PTLD.

BAFF/BAFF-R staining, semiquantitative evaluation: +++, >60%; ++, 30% to 60%; +, <30%; -, negative. For other markers: +, >30%; -, negative. GC, germinal center; ND, not determined; NGC, non-germinal center; NR, not relevant; Nu, nuclear staining.

MUM1 and/or CD138 but not GC B-cell markers.⁷ DLBCL/PTLD specimens generally demonstrated an activated pattern (Tables 2 and 3): of 21 cases, 19 expressed the NGC phenotype, and 2 could not be characterized because of nondetection of the four markers.²⁸ Of 30 DLBCL/IC cases, 12 expressed the GC pattern, and 18 expressed the NGC pattern (Table 3).

Detection and Characterization of EBV

EBV was detected in 12 of 21 DLBCL/PTLD cases (57%) versus only 2 of 30 DLBCL/IC cases that were EBV-positive. The EBV latency status was characterized in 11

of 12 DLBCL/PTLD EBV-positive cases exhibiting different types of latency: latency I, 2 cases; latency II, 3 cases; and latency III, 6 cases (Table 2).

Nine cases expressed ZEBRA: one DLBCL/IC case with latency II, and eight DLBCL/PTLD cases, of which five cases demonstrated latency III, two exhibited latency II, and latency in one case was undetermined.

Controls of BAFF and BAFF-R Immunostaining

BAFF and BAFF-R expression obtained in different lymphomatous tissues demonstrated the same positivity as has been previously described in the literature (Figure 1). In

Table 3. Expression of BAFF/BAFF-R, NF- κ B Proteins, and EBV in Patients with DLBCL

Lymphoma type	Origin	No. of patients	BAFF	BAFF-R	p52	p50	EBV	LMP1
IC	GC	12	11 of 12	6 of 12	5 of 12	3 of 12	0	ND
IC	NGC	18	17 of 18	5 of 18	0 of 18	3 of 18	2/18	2/2
PTLD	NGC	19	17 of 19	1 of 19*	12 of 19*	7 of 17	10 of 19	9 of 10
PTLD	ND	2	2 of 2	0 of 2	2 of 2	1 of 2	2 of 2	1 of 2

*Statistically significant: $P < 2.10^{-4}$ for p52, $P < 0.008$ for BAFF-R.

GC, germinal center; IC, immunocompetent; EBV, Epstein-Barr virus; LMP1, latent membrane protein 1; ND, not determined; NGC, non-germinal center; p50 and p52 positive staining, nuclear localization (>10%); PTLD, post-transplantation lymphoproliferative disorders.

brief, all lymphomas expressed BAFF, whereas BAFF-R was expressed in follicular, mantle, and marginal zone lymphomas but was negative in Burkitt's lymphoma.^{24,26}

Expression of BAFF and BAFF-R

BAFF expression was predominantly detected in almost all cases in the entire series of DLBCL [47 of 51

cases (92%)]. Only two DLBCL/PTLD cases exhibiting the NGC pattern and two DLBCL/IC cases exhibiting the NGC and GC patterns, respectively, did not express BAFF. Of note, regardless of the histogenesis pattern, BAFF-R was weakly expressed in only 1 of 21 (4.88%) DLBCL/PTLD cases, in contrast with DLBCL/IC cases, of which 11 of 30 (37%) were positive for BAFF-R. This distinct BAFF-R expression was statistically significant ($P < 0.008$).

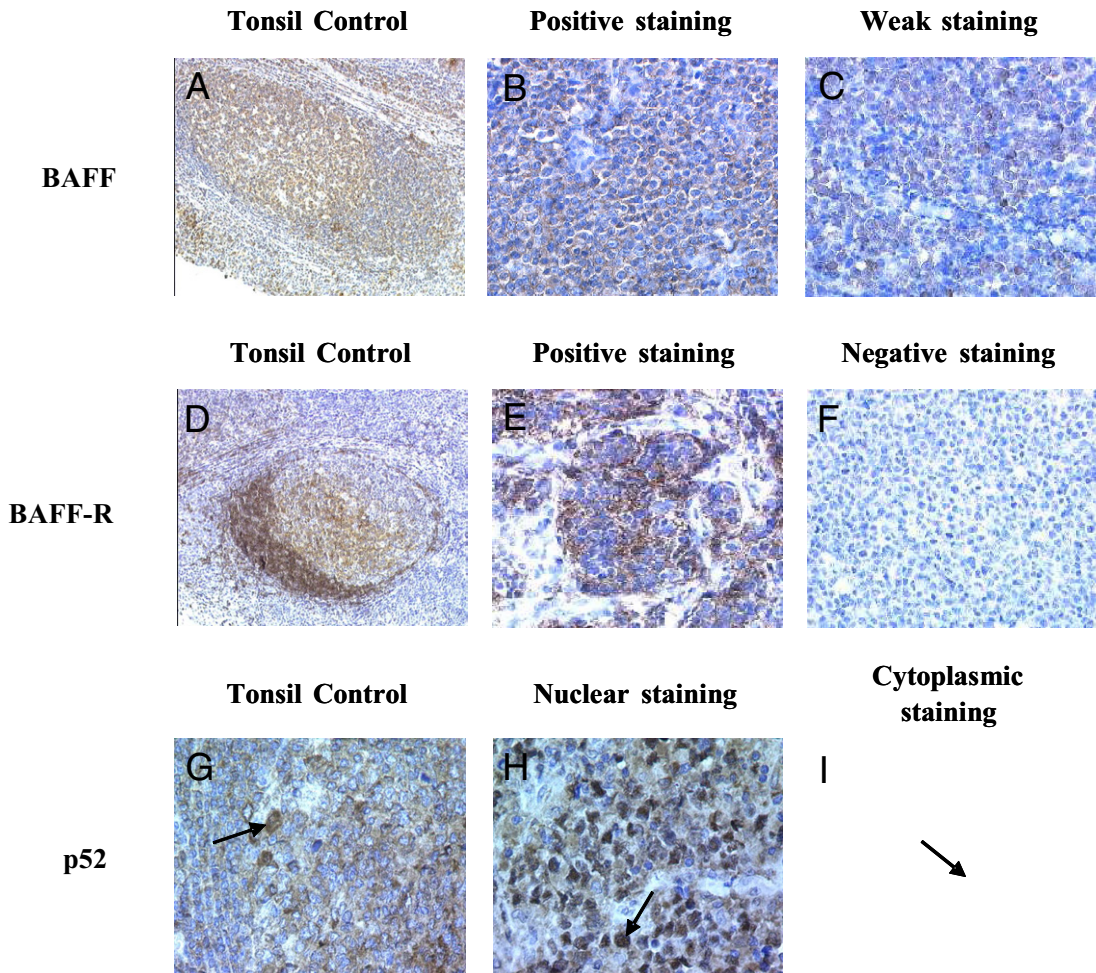


Figure 1. BAFF, BAFF-R, and p52 staining. **A–C:** BAFF staining. **A:** Tonsil control. High expression in GC cells, weak expression in mantle cells, and heterogeneous staining in the interfollicular zone. **B:** Positive staining. **C:** Weak staining. **D–F:** BAFF-R staining. **D:** Tonsil control. High expression in mantle cells and heterogeneous staining in GC cells. **E:** Positive staining. **F:** Negative staining. **G–I:** p52 staining. **G:** Tonsil control. Nuclear (arrow) and cytoplasmic staining in GC cells. **H:** Positive staining, nuclear localization (arrow). **I:** Negative staining, cytoplasmic localization (arrow).

Expression of NF- κ B Pathways

In the entire series of DLBCL (DLBCL/IC or DLBCL/PTLD) demonstrating the NGC pattern (37 cases), LMP1 expression observed in 11 cases was associated with NF- κ B activation, regardless of the pathway (p50 and/or p52). Among these 11 LMP1-positive cases, 8 (73%) expressed nuclear p52 protein. Nine of these 11 cases were from the subgroup DLBCL/PTLD, associated with p50 in 4 cases. In contrast, in the LMP1-negative subgroup (EBV-positive or EBV-negative), p52 nuclear expression was observed in only 4 of 26 (15%) NGC DLBCL cases (Figure 1). This difference was statistically significant ($P = 0.0014$). No correlation was observed between p52 and BAFF-R protein expression. p52 expression with the NGC pattern was positive in 12 of 19 cases (63%). Furthermore, none of 18 DLBCL/IC cases with the NGC pattern expressed p52 (Table 3). This distinct p52 expression was highly statistically significant ($P < 2.10^{-4}$). p52 expression between DLBCL/PTLD and DLBCL/IC with the GC profile could not be compared because of the absence of GC pattern in our series of DLBCL/PTLD cases. Moreover, p52 expression seems to be associated with ZEBRA expression in DLBCL/PTLD because almost all cases expressing ZEBRA [7 of 8 cases (87%)] demonstrated an NF- κ B2-activated profile at nuclear p52 staining.

Discussion

In the present study, 51 DLBCL cases, divided into two subcategories, DLBCL/PTLD and DLBCL/IC, characterized by the level of activation of lymphomatous B cells and their histogenesis profile, ie, GC or NGC pattern, were investigated for BAFF-R and p52 expression.⁷ In cases demonstrating the NGC pattern, a statistically significant difference of p52 expression was observed between DLBCL/PTLD [12 of 19 cases (63%)] and DLBCL/IC (0 of 18 cases). Despite BAFF-R expression, no association with p52 expression was observed. However, regardless of the histogenesis profile (GC versus NGC), BAFF-R expression, which was rarely observed in DLBCL/PTLD [1 of 21 cases (4.8%)], was statistically different from DLBCL/IC, in which 11 of 30 cases (37%) were positive.

Among the 21 DLBCL/PTLD cases, the NGC phenotype was expressed in 19, whereas 18 of 30 DLBCL/IC cases demonstrated the NGC profile. EBV was present in 14 cases, 2 DLBCL/IC and 12 DLBCL/PTLD. All but two EBV-associated cases demonstrated the NGC histogenesis pattern. The percentage of EBV-associated DLBCL/PTLD was 57%. In the present series, this low percentage of EBV-positive PTLD can be related to the high number of PTLD cases occurring later than 24 months after transplantation, as has previously been described.²⁹

BAFF expression was detected in 92% of the 51 DLBCL cases. This result could reflect ligand-receptor engagement as well as environmental- or cellular-secreted BAFF. Indeed, monoclonal antibodies against human BAFF recognized membrane-bound and soluble

BAFF.¹⁶ The autocrine pathway could explain this expression, as described in other models such as chronic lymphocytic lymphoma and multiple myeloma.^{18,30} Study of BAFF-R expression was performed to respond to this hypothesis of the autocrine signaling pathway.

Little is known about BAFF-R expression relative to the level of DLBCL activation. Only one study has explored this correlation in association with Lck protein.³¹ Our results were similar to those of Paterson et al.³¹; indeed, no differences were detected between the histogenesis profile and BAFF-R expression in the DLBCL/IC subgroup. We observed that BAFF-R was expressed in 11 of 30 DLBCL/IC cases (37%), which corresponds to the mean percentage of previously published results.^{24,26,31} However, regardless of the histogenesis profile, BAFF-R expression was higher in DLBCL/IC (37%) than in DLBCL/PTLD (4.8%), with a statistically significant correlation. Thus, the hypothesis of the autocrine pathway through BAFF-R could be excluded. Nevertheless, receptors TACI and BCMA could also be implicated in this signaling pathway; however, their expression was not tested in the present series. The high level of BAFF expression in NHL, based on transcription enhancement through BAFF promoter, could be related to the possible effect of the polymorphism -871C→T (or other genetic variation) or to the nuclear translocation of the CD40-cRel dimer.³¹⁻³⁴ In addition, BAFF-R weak expression could be explained by possible down-regulation of BAFF-R arising from BAFF binding.³⁵⁻³⁷

EBV activates the NF- κ B pathway through LMP1 protein, and the link between this activation pathway and lymphomas has also been described.^{12,15} As expected, in our series, all 11 NGC DLBCL cases positive for LMP1 were associated with NF- κ B activation. Among these cases, p52 was generally expressed (8 of 11 cases); however, it was positive in only 4 of 26 cases in the LMP1-negative subgroup whether EBV was or was not present. BAFF-R expression could not explain these results because there was no correlation with p52 nuclear localization, which suggests that the BAFF/BAFF-R signaling pathway could not be directly implicated in DLBCL lymphomagenesis mediated by p52 signaling. The dissociation between BAFF/BAFF-R and p52 signaling pathways could be interpreted via repression of NF- κ B activity mediated by BCL6 protein expression or the posttranslational modification of RelB.^{38,39}

The nuclear expression of p50 and/or p52 in six cases demonstrating the GC pattern in DLBCL/IC could be related to deregulation of the NF- κ B pathway caused by multiple genetic events including negative and positive regulators of NF- κ B, as previously reported by Compagno et al.⁴⁰ The higher expression of p52 related to the NF- κ B2 activation pathway in DLBCL/PTLD, which is statistically different from the NGC pattern in DLBCL/IC, could be related not only to genetic events but also to activation of NF- κ B2 via LMP1.

Moreover, it must be emphasized that in seven of eight cases expressing ZEBRA in EBV-positive DLBCL/PTLD cases, p52 demonstrated nuclear staining, which suggests a relationship between the replicative cycle of EBV

and p52 nuclear expression. However, this result must be confirmed in a larger series.

Thus, our results demonstrating rare expression of BAFF-R and the frequency of NF- κ B2 pathway activation in DLBCL/PTLD as compared with DLBCL/IC confirm the importance of EBV, especially LMP1, and suggest other mechanisms of NF- κ B2 pathway deregulation in these tumors.

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