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MedB-1, A HUMAN TUMOR CELL LINE DERIVED FROM A PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

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Primary mediastinal B-cell lymphoma is a locally highly aggressive but poorly disseminating tumor composed of medium sized or large cells most probably of thymic medullary origin. It has a mature B-cell phenotype, typically lacks immunoglobulin expression and has variable defects in expression of HLA-molecules. We present here a cell line, MedB-1, derived from such a tumor. As is frequently found in mediastinal B-cell lymphomas *in situ*, MedB-1 is CD10$^-$, CD19$^+$, CD21$^-$, CD22$^+$, CD23$^+$, CD25$^-$, CD37$^+$, CD38$^-$, CD39$^+$, CD40$^+$, CD54$^+$, CD95$^+$. Like the parental tumor, MedB-1 lacks HLA-A,B,C α-chains and $\beta_2$microglobulin and expresses HLA-D molecules at decreased levels. Both parental tumor and MedB-1 cells are clonally related as shown by immunoglobulin heavy chain gene rearrangement analysis. Unlike the parental tumor tissue, the MedB-1 cell line cytoplasmically expresses IgG/κ in a very small subset of cells under standard culture conditions. MedB-1 does not contain any Epstein-Barr virus DNA. In a tissue adhesion assay MedB-1 cells showed an extensive binding to the medullary region of normal thymus. Altogether, MedB-1 is a suitable tool for functional and molecular analysis of this distinct lymphoma entity.

Key words: *human B-cell lymphoma; primary mediastinal B-cell lymphoma; human B-cell lymphoma cell line; immunophenotype; thymic medulla*

Primary mediastinal B-cell lymphoma is a tumor with clinical, histological, immunological, and cytogenetic features that, taken together, are almost unique. It occurs in all age groups but most frequently in young adults.[1–7] The typical localization is the anterior mediastinum, and thymic involvement has been repeatedly demonstrated. Therefore, it is assumed that this B-cell lymphoma is a primary thymic tumor.[2,8,9] As a normal counterpart to this neoplasm, a peculiar population of thymic medullary B-cells has been proposed.[10,11] Histologically, mediastinal B-cell lymphoma varies in cell size from medium to large and is, although inconsistently, associated with a sclerosing reaction.[12] The forthcoming World Health Organization Classification of Neoplastic Diseases of the Hematopoietic and Lymphoid Tissues currently lists *mediastinal (thymic) B-cell lymphoma* as a variant of diffuse large cell lymphoma[13] as it was formerly designated in the REAL classification.

Immunologically, this lymphoma is defined as CD10$^-$, CD19$^+$, CD20$^+$, CD21$^-$, CD22$^+$, CD37$^+$, CD40$^+$.[14–17] The expression of activation antigens is more variable but mostly CD23$^+$, CD25$^-$, CD30$^-$, CD95$^+$.[14,15,17] Recently, expression of the MAL gene, currently regarded as a T-lymphocyte restricted gene, was found in mediastinal but not in other diffuse large B-cell lymphomas.[18]

Although primary mediastinal B-cell lymphoma has its immunoglobulin genes regularly rearranged,[8,19,20,41] it does not, as a rule, contain any detectable immunoglobulin (Ig) constituents and has variable and often severe defects in expression of HLA class I and II molecules.[1,5,14,20–22] Clinically, mediastinal (thymic) B-cell lymphoma is characterized by aggressive local growth frequently involving the lung and, eventually it , "metastasizes" to other organs such as liver and kidney, and brain.[23] Although locoregional (*e.g.*, supraclavicular) lymph node involvement may occur, generalized lymphomatous spread or bone marrow involvement or leukemia has not been reported so far. We now present MedB-1, a novel B-cell line, and, by IgH gene rearrangement and immunology provide evidence for its clonal derivation from a typical primary mediastinal B-cell lymphoma.

MATERIAL AND METHODS

Patient and tumor

In March 1990 a mediastinal mass was diagnosed in a 27-year-old male. Diagnostic thoracotomy was performed and a biopsy was taken from a bulky tumor situated in the anterior mediastinum. Histological and immunohistological examination (Fig.1*a*,*b*) revealed a diffuse large B-cell lymphoma compatible with primary mediastinal B-cell lymphoma. The patient was staged IIb and combined radiochemotherapy was applied. After initial tumor regression, progression under chemotherapy was noted. In July 1990, the left lung was partially invaded by tumor. A second thoracotomy was performed, debulking of the mediastinal tumor mass was carried out, and the left lung was resected. From this surgical specimen fresh and sterile tumor tissue was obtained immediately after removal. The patient died in January 1991 due to extensive local tumor progression.

Establishment of the cell line

The cell line was established from a 3 cm^3 tumor specimen. After the sample was washed several times with minimal essential medium including 10 μg/ml amphotericin and penicillin/streptomycin (100 U/100 μg/ml), it was minced into pieces of about 1 mm^3 and centrifuged. The erythrocytes were lysed with ammonium chloride buffer (0.15 M NH_4Cl; 0.01 M $KHCO_3$) and the cell suspension was placed in Iscove's medium (Seromed/Biochrom, Berlin, Germany), supplemented with 10% fetal calf serum and antibiotics as described above. The cultures were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide and the medium was replaced once a week. Outgrowing fibroblasts were not removed, they gradually decreased and finally disappeared. Since 1990, the tumor cells have been growing as a single cell suspension with a doubling time of about 3 days. Repeated controls for mycoplasma infection carried out regularly over the years were always negative. Karyotyping using classical Giemsa-band-

Abbreviations: β-2 m, beta-2 microglobulin; EBV, Epstein-Barr virus; LCL, lymphoblastoid cell line; Ig, immunoglobulin; H, Ig heavy chain; MALT, mucosa-associated lymphoid tissue; PCR, polymerase chain reaction.

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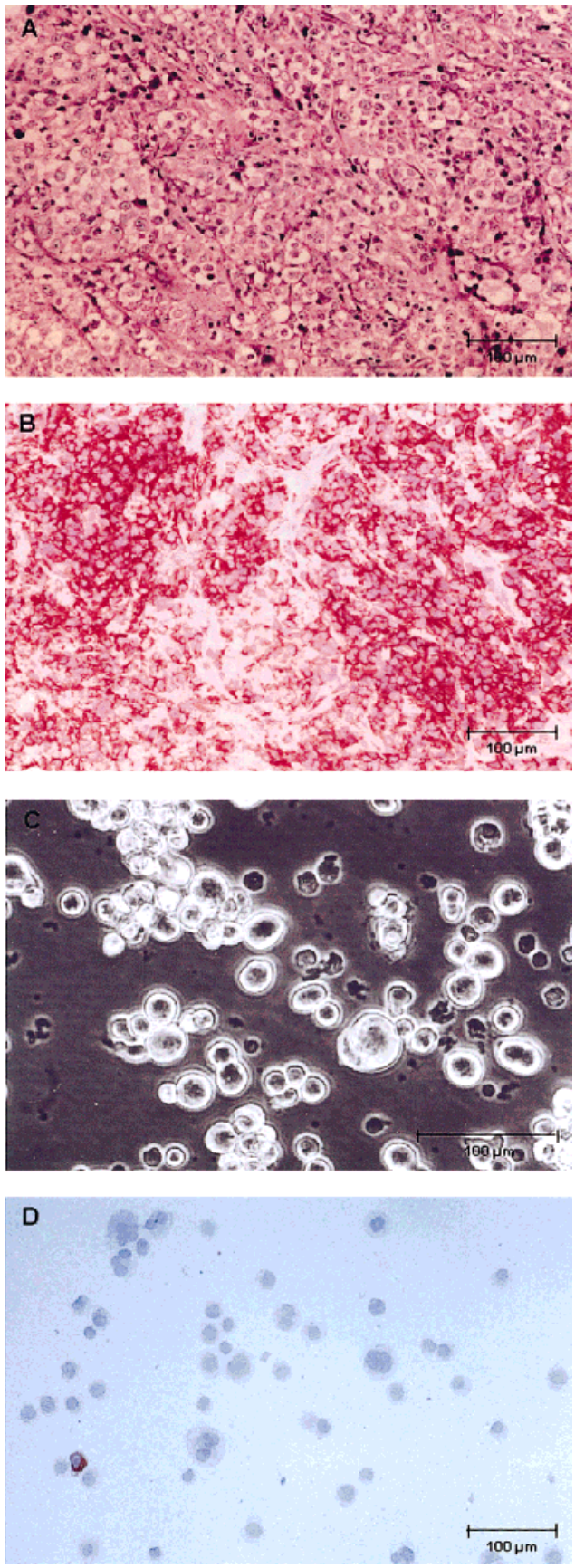

ing technique was carried out and revealed 47, XY, inv(X) (p22; q13), +der(1)t(1;14) (q10,q10), +9, −14, −21, i(21q).[24]

Detection of Epstein Barr virus (EBV)-DNA by Southern blot analysis

Genomic DNA was prepared from MedB-1 cells. The Burkitt's lymphoma cell line Namalwa[25] that contains 2 integrated copies of EBV-DNA[26] together with a lymphoblastoid cell line (LCL) from a healthy donor were used as positive controls. Cells were lyophilized and treated with proteinase K (1 mg/ml) overnight at 37°C followed by phenol/chloroform extraction and ethanol precipitation. Ten µg of cellular DNA were digested with *Bam*HI, electrophoresed on a 1% agarose gel and blotted onto a nylon membrane. The EBV *Bgl*II-U fragment[27] was radiolabeled with ^{32}P by random primed labeling and hybridized under stringent conditions. Radioactive bands were visualized by exposure to X-ray films using intensifying screens.

Monoclonal antibodies

The monoclonal antibodies used are listed in Table I. CD95(anti-APO-1) was kindly supplied by P.H. Krammer, Heidelberg, Germany; anti-MHC-class I α-chain (clone LA-45)[28] was a generous gift from W. Knapp, Vienna, Austria; CD27(CLB-27) was kindly supplied by R.A.W. Van Lier, Amsterdam, Netherlands, and CD40(G28-5) by J.A. Ledbetter, Seattle, WA; CD19 (HD237), CD23(HD50), CD37(HD28), and anti-IgD (clone HD9) were raised and characterized in the laboratory of one of the authors (G.M.). CD21(OKB7), CD24(OKB2), and CD38 (OKT10) were obtained from Ortho (Raritan, NJ). Anti-κ (clone TB28-2), anti-λ (clone 1-155-2), anti-HLA-DP (clone B7/21), and CD25(2A3) were supplied by Becton Dickinson (San Jose, CA); CD39(AC2) was purchased from The Binding Site (Birmingham, UK), and CD20 (B1) from Coulter Cytometry (Hialeah, FL). Anti-IgM (clone R/169) and CD30(Ber-H2) were obtained from Dakopatts (Copenhagen, Denmark). Anti-IgG (clone 8a4), anti-IgA (clone NIF2) were supplied by Dianova (Hamburg, Germany).

Immunohistological/-cytological staining procedures

The method is described in detail elsewhere.[29] Monoclonal antibodies in culture supernatants were used undiluted; ascites preparations were used as 1:200 dilutions, purified reagents were used at a protein concentration of 10 µg/ml. A polyclonal biotinylated sheep antibody to mouse Ig (reactive with all mouse isotypes) and a streptavidin-biotinylated peroxidase complex, all obtained from Amersham, High Wycombe, UK, served as a detection system for the primary antibodies. 3-Amino-9-ethylcarbazole (Sigma, St. Louis, MO) was used as substrate for the enzyme; the peroxidase reaction resulted in an intense red precipitate. The sections or cytospin preparations were faintly counterstained with Harris' hematoxylin.

Flow cytometry

Immunofluorescent staining was performed in polystyrene round-bottom tubes (Falcon, San Jose, CA). Throughout, dilution and washing were carried out in RPMI 1640 medium (Gibco) containing 2% heat-inactivated fetal calf serum (FCS), 0.1% so-

FIGURE 1 – *a*: Histology of the parental tumor reveals a medium sized and large cell lymphoma with diffuse growth pattern. The cytoplasm of the lymphoma cells is pale; nuclei are large and contain small nucleoli. There is some degree of sclerosis with fine collagen bands surrounding larger groups of tumor cells (Giemsa). *b*: Immunolabeling of the B-cell specific CD19 antigen reveals strong cytoplasmic CD19 expression (frozen section and indirect immunoperoxidase technique). *c*: MedB-1 cells in culture. The cell line grows in suspension, does not adhere to plastic and spontaneously displays poor homotypic adhesion (phase contrast microscopy). *d*: Cytoplasmic immunoglobulin expression of IgG/κ type is observed in less than one per cent of cells (immunolabeling of IgG heavy chain by indirect immunoperoxidase technique).

TABLE I – IMMUNOSTAINING OF THE NEOPLASTIC POPULATION OF THE PARENTAL TUMOR TISSUE *IN SITU*, OF CYTOPLASMIC ANTIGEN IN CYTOSPIN PREPARATIONS, AND SURFACE ANTIGEN EXPRESSION OF MEDB-1 CELLS AS MEASURED BY FLOW CYTOMETRY.

Antigen	Clone	Tumor	MedB-1		
		in situ	Cytoplasm	Cell surface	
				mF[1]	%pos.
HLA-A,B,C	W6/32	−	−	−	−
β-2 m	BBM.1	−	−	−	−
Class I α	LA-45	−	−	−	−
HLA-DR	ISCR3	+>−	+/−	291	80.7
HLA-DP	B7/21	−/+	+/−	110	90.0
HLA-DQ	Tü22	−/+	−>+	86	51.1
IgM	R/169	−	−	−	−
IgD	HD9	−	−	−	−
IgG	8a4	−	−>+	7	9.4
IgA	G20-359	−	−	−	−
κ	TB28-2	−	+>−	−	−
λ	1-155-2	−	−	−	−
CD10	J5	−	−	−	−
CD19	HD237	+	+	176	99.1
CD20	B1	+	−>+	14	22.1
CD21	OKB7	−	−	−	−
CD22	HD39	+	+	18	53.9
CD23	HD50	+	+	640	96.9
CD24	OKB2	−>+	−>+	13	18.8
CD25	2A3	−	−	−	−
CD27	CLB-27	−	−	−	−
CD30	Ber-H2	−>+	+	151	96.9
CD37	HD28	+	+	47	95.7
CD38	OKT10	−	−	8	7.8
CD39	AC2	+	+	47	95.6
CD40	G28-5	+	+	315	99.5
CD54	MEM-112	+	+	153	99.2
CD74	BU-45	+/−	+>−	−	−
CD95	anti-APO-1	+	+	200	99.2

[1]mF, mean fluoresence; %pos., percent positive cells above background; −, no detectable antigen; +, antigen detected, irrespective of staining intensity; +/−, positive and negative cells in about equal parts; +>−, more positive than negative cells; −>+, *vice versa*.

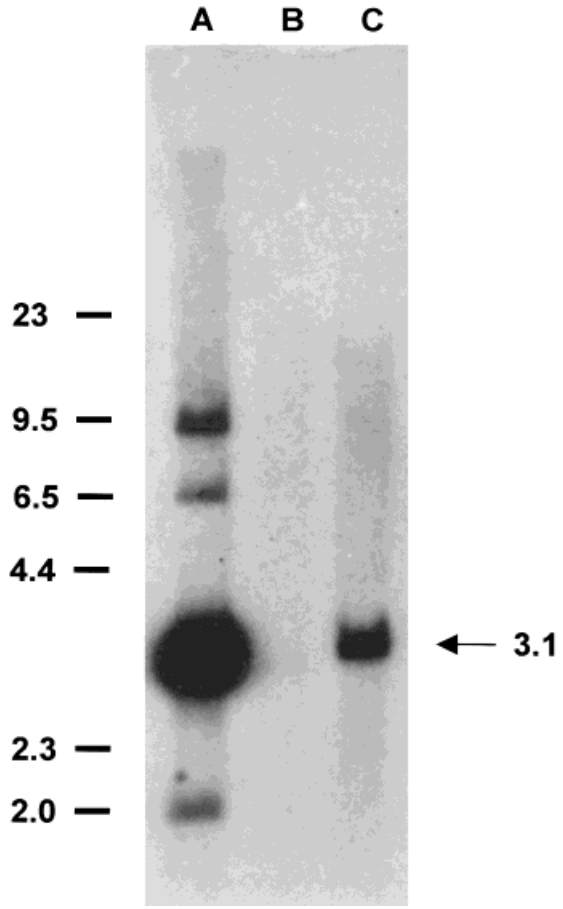

FIGURE 2 – Southern blot hybridization using the EBV *Bgl*II-U probe of *Bam*HI digested DNAs obtained from LCL cells (lane *a*), from MedB-1 (lane *b*), and Burkitt's lymphoma cell lines Namalwa (lane *c*). The position of the EBV 3.1 kb *Bam*HI-W repeat fragment is indicated by an arrowhead. This blot reveals that both, BJAB and MedB-1 are devoid of EBV-DNA.

dium azide and 10 mM HEPES. Approximately 10^6 cells/sample, suspended in 50 μl of medium, were incubated at 4°C with an equal volume of the appropriate dilution of each MAb. After 45 min, cells were washed twice in 500 μl of cold medium, and 2 μg of F(ab′)2 goat anti-mouse IgG and IgM FITC conjugate (Jackson ImmunoResearch, West Grove, PA) was added for 45 min at 4°C. Cells were washed again twice and resuspended in 300 μl of medium containing 1 μg/ml propidium iodide (Sigma). From each sample the green fluorescence of 10^4 cells was analyzed. Dead cells were excluded by selectively gating on propidiumiodine fluorescence, forward and side scatter parameters. Flow cytometry was performed on a FACScan cytometer with the LYSIS II software (Becton Dickinson).

IgH-gene rearrangement analysis

DNA was extracted from frozen lymphoma tissue and from pelleted MedB-1 cells using the salting out method as described by Miller et al.[30] Five hundred ng of DNA were submitted to IgH-gene rearrangement analysis by polymerase chain reaction (PCR). Consensus primers against the FRII region of the V-segments (FR2, FR2A)[31] and against the conserved sequences of the J-segments (LJH, VLJH)[41] of the immunoglobulin heavy chain gene served to amplify the IgH-gene rearrangement in a semi-nested fashion. PCR was carried out in a reaction mix of 50 μl, containing 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 50 pmol of each primer and 2.5 U Taq polymerase (Pharmacia, Uppsala, Sweden) in the presence of a reaction buffer supplied by the manufacturer. Amplification was performed with 35 cycles at an annealing temperature of 55°C for the first round, using primers FR2 and LJH, followed by a second amplification step with primers FR2A and VLJH for 20 cycles at 60°C. Aliquots of the PCR products were size fractionated on a 7.5% non-denaturing polyacrylamide-gel and stained with ethidium bromide.

Tissue adhesion assay

To investigate adhesive properties of MedB-1 cells on tissue sections, a modified Stamper-Woodruff assay was applied.[32] Briefly, frozen tissue sections of about 5μm from genetically unrelated normal human tonsil, lymph node, colon mucosa, and thymus were thawed at room temperature and pre-incubated in a humid atmosphere at 37°C for 15 min. Sections were then covered with IMDM medium containing 10^6/ml MedB-1 cells and incubated at 37°C for 1 hr in a humid atmosphere. Finally, after carefully washing away non-adherent cells with PBS, sections were fixed in 3% paraformaldehyde for 10 min at room temperature and stained according to Pappenheim.

RESULTS

Growth properties

The MedB-1 cell line grows as single cell suspension and does not show plastic adherence (Fig. 1*c*).

Southern blot analysis for Epstein-Barr virus (EBV)DNA

To exclude the possibility that a lymphoblastoid B-cell line had been generated, Southern blot analysis of *Bam*HI digests of MedB-1 DNA was carried out using EBV *Bgl*/II-U as probe. As

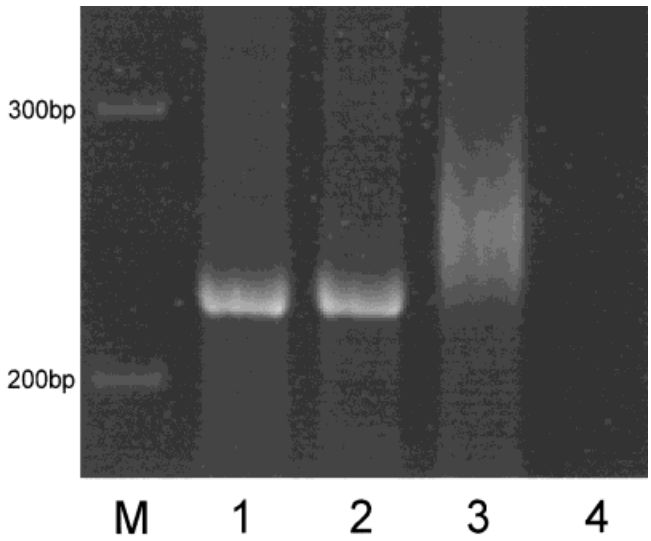

FIGURE 3 – PCR analysis of the IgH gene rearrangement. MedB-1 cell line DNA (lane 1) and DNA obtained from parental lymphoma tissue (lane 2) generated distinct clonal bands of identical size, whereas buffy coat DNA (lane 3) yielded a polyclonal smear. Lane 4: water; M, molecular size marker.

shown in Figure 2, no EBV signal was detected in MedB-1 DNA (lane *b*). The sensitivity of the assay is shown by blotting Namalwa DNA that is known to carry 2 integrated copies of the EBV genome (lane *c*). This excludes the possibility that the MedB-1 cell line is a lymphoblastoid line. Furthermore, it is extremely unlikely that MedB-1 contains any EBV DNA.

IgH-gene rearrangement

PCR analysis of the IgH-gene rearrangement with both DNAs obtained from lymphoma tissue and MedB-1 cells, respectively, generated a single distinct band of identical size after electrophoresis on a high resolution polyacrylamide gel (Fig. 3, lanes 1 and 2). Thus, the MedB-1 cell line is clonally derived from the solid lung tumor tissue.

Immunophenotype

The immunoprofiles of the neoplastic population of the parental tumor and of the cell line MedB-1 are listed in Table I. Both the original lymphoma and the MedB-1 cell line expressed a series of B-cell restricted (CD19, CD20, CD22) and B-cell associated (CD37, CD39) surface molecules. Ig expression was not detectable by immunohistology on either frozen or paraffin sections. Immunocytology, however, revealed IgG/κ in the cytoplasm of a very small subset of MedB-1 cells. Correspondingly, we measured very small amounts of IgG on about 10 % of cells by FACS in the absence of other Ig isotypes. The early B-cell and follicular center stage-associated antigen CD10 and a marker for mature but resting follicular mantle B-cells, CD21, were lacking. Instead, a number of activation antigens (CD23, CD30, CD40, and CD95) were surface exposed. The cell line proved inhomogeneous in expression of CD20, CD22, CD24 and comprised a small subfraction of CD38-positive cells and subpopulations of HLA-DR-, -DP-, and -DQ-negative cells. The HLA-D associated invariant chain, CD74, was cytoplasmically expressed in a subset of tumor cells *in situ* and in the cytoplasm of a major subset of MedB-1 cells but was not surface exposed. As is commonly found in primary mediastinal B-cell lymphomas,[21,22] the original tumor and the MedB-1 cell line completely lacked HLA-A,B,C products and β_2m which, in a B cell context, is highly abnormal. The immunophenotype of MedB-1 cells proved stable during the last nine years and, in essence, still corresponds to that of the parental lymphoma.

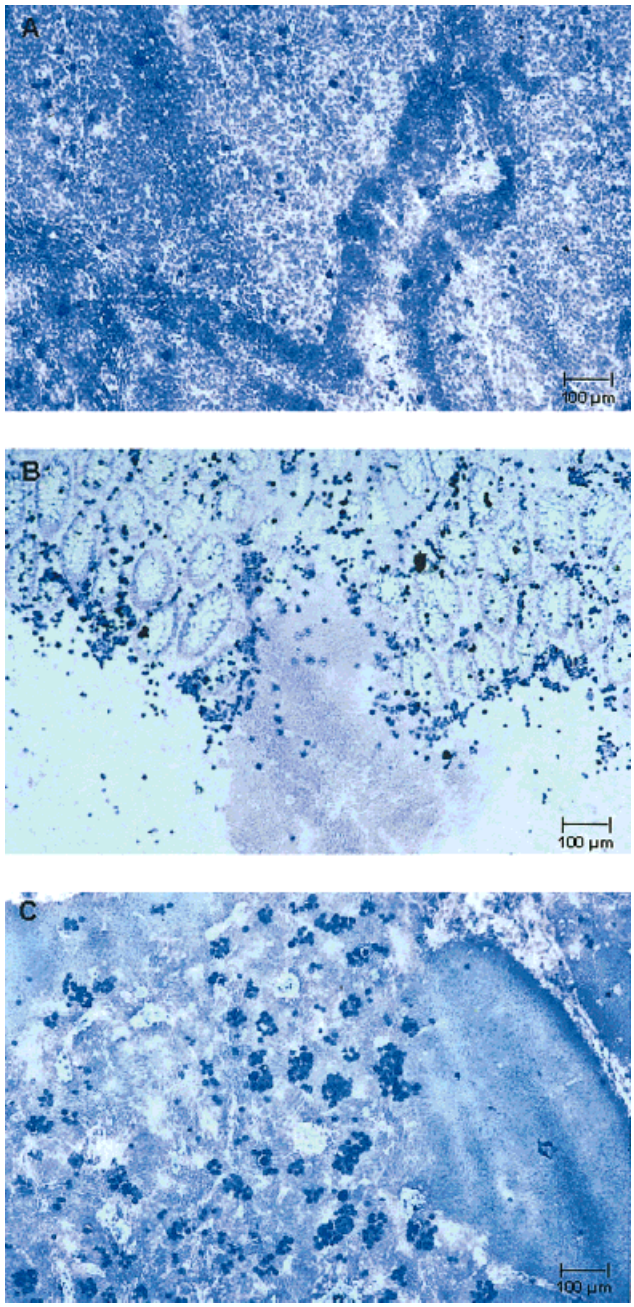

FIGURE 4 – Adhesion assay probing the adhesive potential of MedB-1 cells on frozen tissue sections of normal, genetically non-related human lymphoid tissues. (*a*) On tonsil, cells rarely adhere in a seemingly random fashion. (*b*) On colonic tissue, cells preferentially adhere in the *Lamina propria mucosae* whereas enterocytes, mucosa-associated lymph follicles, and outer layers of the gut are nearly devoid of adherent cells. (*c*) In thymus, cells form clusters of adherent cells in thymic medullary areas.

Immunoglobulin expression and secretion

In extension of the flow cytometric immunoprofile, immunocytology was performed on cytospin preparations of MedB-1 cells to further analyze the expression of Ig constituents. Heavy chains of IgD, IgM and IgA were undetectable as was the λ light chain. IgG/κ was detected cytoplasmically in a small minority of MedB-1 cells, at well below 1% under standard culture conditions (Fig. 1*d*). As determined by quantitative enzyme-linked immunosorbent assay, the amounts of immunoglobulin released in the supernatant

under standard culture conditions were likewise very low (around 15 ng/ml; data not shown).

Tissue adherence

To test whether MedB-1 cells show preferential binding to defined tissue structures, cells were seeded on frozen sections of tonsil, lymph node, thymus and mucosa-associated lymphoid tissue (MALT). Adherence of MedB-1 cells to tonsil and lymph node was very low (Fig. 4*a*). In gut-associated lymphoid tissue there was no binding to lymph follicles. A propensity for selectively adhering to the lamina propria was observed (Fig. 4*b*). Surprisingly, MedB-1 cells showed an extensive binding to thymus that was clearly restricted to the medullary region (Fig. 4*c*). Thus, MedB-1 cells, although growing in suspension *in vitro*, have conserved adhesive properties recognizing the extrafollicular compartment of MALT and, especially, thymic medulla.

In summary, the MedB-1 line is a novel, permanent B-cell line derived from a typical case of primary mediastinal B-cell lymphoma featuring a characteristic immunophenotype shared by the tumor *in situ*.

DISCUSSION

MedB-1 is a novel B-lymphoma line derived from a mediastinal large B-cell lymphoma. The MedB-1 line has retained a considerable number of immunophenotypic features of the original tumor that, at the same time, are also characteristics of the lymphoma subtype itself. The absence of EBV genomic material in MedB-1 excludes a lymphoblastoid B- cell line and is at the same time a consistent feature of mediastinal B-cell lymphoma (Pawlita, unpublished).[33] This data together with the identical IgH rearrangement analysis are evidence of the clonal derivation of MedB-1 from a typical case of mediastinal B-cell lymphoma.

As recently published in a comprehensive molecular cytogenetic study on mediastinal B-cell lymphoma, MedB-1 cells have an abnormal, though stable 47, XY, inv(X) (p22;q13), +der(1)t(1;14) (q10,q10), +9, −14, i(21)(q10) karyotype.[24] Probing mediastinal B-cell lymphomas by molecular cytogenetics we and others reported frequent overrepresentation of genomic material on chromosomes X and 9,[34,35] the consensus region on chromosome 9 being 9p23–9p24.[36] The Janus kinase 2 gene, *Jak2*, was recently identified as one of the candidate genes in this genomic region.[37] The parental tumor of MedB-1 was Case 14 of our series and also showed an overall gain on chromosome 9 already pointing at trisomy.[34] Recently, it was shown by Southern blot analysis that *JAK2* signals in MedB-1 DNA were 4 times more intense than in normal DNA.[37] This again makes MedB-1 an interesting tool for research into the pathogenesis of mediastinal B-cell lymphoma. Karpas 1106, another B-cell line with a complex translocation involving 18q21.3 and genetic aberrations involving chromosomes 9 and X has been reported to be derived from a lymphoma with predominantly mediastinal manifestation.[38] The parental lymphoma of Karpas 1106, however, was diagnosed as a lymphoblastic lymphoma. In a later publication, the same cell line was regarded as a marginal zone lymphoma[39] that is in accordance with our view that histomorphology and immunophenotype of mediastinal B-cell lymphoma bear considerable resemblance to monocytoid B-cells.[9,12] Thus, it seems possible that Karpas 1106 might also have been derived from a primary mediastinal B-cell lymphoma.

The MedB-1 line has retained a considerable number of immunophenotypic features of the parental tumor that, at the same time, are also characteristic of the lymphoma type itself.[14–16] MedB-1 cells are negative for germinal center cell stage-associated surface antigen CD10 and show a marginal positivity for the germinal center cell- and plasma cell-associated CD38 antigen but strongly express CD23 together with other B-cell-associated activation antigens such as CD39, CD40, CD54, and CD95. This immunoprofile clearly argues against a germinal center stage of maturation/differentiation of this B-lymphoma cell line. Mediastinal B cell lymphoma typically is predominantly CD30-negative.[15] The parental tumor of MedB-1, however, contained a CD30-positive subpopulation. The MedB-1 cell line is strongly CD30-positive. Under reactive conditions, CD30-positivity is an optional characteristic of a subset of plasma cells. Thus, CD30-positivity might be due to further maturation of the neoplastic clone under *in vitro* conditions. This hypothesis is supported by the low expression of CD20, the minimal CD38 expression, the partial loss of HLA-D subloci products, and the cytoplasmic IgG/κ expression in a very small subset of cells. A presumed pre-plasma cell stage of maturation is in keeping with published data characterizing mediastinal B-cell lymphoma as a post germinal center stage tumor[40] with immunological features of terminal steps of B-cell development.[14]

Med-B1 cells lack HLA-A,B,C α heavy chains and β-2m, which is very often the case in mediastinal B-cell lymphoma.[1,21] This is also frequently encountered in mediastinal B-cell lymphoma.[1,22] Thus, the cell line features the very typical defect in major histocompatibility complex class I expression, the molecular mechanism of which remains elusive.

Mediastinal B-cell lymphoma is currently regarded as a primary thymic tumor.[2,9] As a normal counterpart, asteroid B-cells have been described in the normal thymic medulla.[10,11] It is intriguing that MedB-1 cells preferentially adhere to normal thymic medulla even after 10 years of growing in suspension.

To conclude, MedB-1 is a novel B-lymphoma line and a useful model for future functional and molecular investigation of mediastinal B-cell lymphoma.

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