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 β2-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 cytologically expresses IgGκ 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 mediastinal 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.8,11 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 lymphoma13 as it was formerly designated in the REAL classification.

Immunologically, this lymphoma is defined as CD10+, CD19+, CD20+, CD21+, CD22+, CD23, CD25, CD37+, CD38, CD39+, CD40+, CD54, CD95.14-17 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,18,19,20,41 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.1,5,14,20,23 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 loco-regional (e.g., supraclavicular) lymph node involvement may occur, generalized lymphomatous spread or bone marrow involve-

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.1a,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 cm3 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 mm3 and centrifuged. The erythrocytes were lysed with ammonium chloride buffer (0.15 M NH4Cl; 0.01 M KHCO3) 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-
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 Namalwa25 that contains 2 integrated copies of EBV-DNA26 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 BamHI, electrophoresed on a 1% agarose gel and blotted onto a nylon membrane. The EBV BglII-U fragment27 was radiolabeled with 32P 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-DR (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 iso- types) 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 IgGκ 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.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Tumor in situ</th>
<th>Cytoplasm</th>
<th>MedB-1</th>
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<td></td>
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<td>Class I α</td>
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1 mF, mean fluorescence; %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 BglII-U probe of BamHI 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 BamHI-W repeat fragment is indicated by an arrowhead. This blot reveals that both, BJAB and MedB-1 are devoid of EBV-DNA.

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.39 Five hundred ng of DNA were submitted to IgH-gene rearrangement analysis by polymerase chain reaction (PCR). Consensus primers against the FRIII 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 MgCl2, 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. 1c).

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 BamHI digests of MedB-1 DNA was carried out using EBV BglII-U as probe. As
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. Immunocytochemistry, however, revealed IgG/k 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, the original tumor and the MedB-1 cell line completely lacked HLA-A,B,C products and \( \beta_2 \)-m 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.

**Immunoglobulin expression and secretion**

In extension of the flow cytometric immunoprofile, immunocytochemistry 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 \( \lambda \) light chain. IgG/k was detected cytoplasmically in a small minority of MedB-1 cells, at well below 1% under standard culture conditions (Fig. 1d). 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. 4a). In gut-associated lymphoid tissue there was no binding to lymph follicles. A propensity for selectively adhering to the lamina propria was observed (Fig. 4b). Surprisingly, MedB-1 cells showed an extensive binding to thymus that was clearly restricted to the medullary region (Fig. 4c). 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). 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 previously 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, −1, +12(1)(q10) karyotype. Probing mediastinal B-cell lymphomas by molecular cytogenetics we and others have reported frequent overrepresentation of genomic material on chromosomes X and 9, which the consensus region on chromosome 9 has been reported to be derived from a lymphoma with trisomy. Recently, it was shown by Southern blot analysis that the parental tumor of MedB-1 was Case 14 of our series and also the parental tumor of MedB-1, however, contained a CD30-positive subpopulation. The MedB-1 cell line is strongly CD30-negative. 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 IgGκ 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 with immunological features of terminal steps of B-cell development.

MedB-1 cells lack HLA-A,B,C and HLA-DR antigens, which are very often the case in mediastinal B-cell lymphoma. This cell line features the very typical defect in major histocompatibility complex I expression, the molecular mechanism of which remains elusive.

Mediastinal B-cell lymphoma is currently regarded as a primary thymic tumor. As a normal counterpart, the normal thymus has been described in the normal thymic medulla. It is intriguing that MedB-1 cells preferentially adhere to normal thymic medulla early 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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