Mediastinal Lymphoma of Clear Cell Type is a Tumor Corresponding to Terminal Steps of B Cell Differentiation

By Peter Möller, Gerhard Moldenhauer, Frank Momburg, Birgit Lämmler, Maria Eberlein-Gonska, Sophie Kiesel, and Bernd Dörken

This article reports eight primary mediastinal tumors occurring in young adults (19 to 43 years, mean 29.4 years), predominantly female (six of eight) adults. Most patients responded badly to aggressive therapy. Progression is presently noted in one patient; five patients died 10, 11, 13, 18, and 22 months after diagnosis. No patient developed leukemia. The tumors were highly proliferative, had a diffuse growth pattern, and comprised clear cells of variable size. They could not be classified histologically, but could, however, be immunohistologically characterized as B cell lymphomas. In all cases, the immunophenotype was LC, cALLA⁺, CD19⁺, CD20⁺, CD21⁺, Ig (surface/cytoplasm)⁺, and PC-1⁺. In addition, the neoplastic cells exhibited variable defects in the expression of HLA-A,B,C and HLA-DR and inconstant expression of other B cell-restricted/associated antigens. This combination of immunophenotypical and clinical features suggests that the mediastinal clear cell lymphoma (MCCL) is a previously undescribed type of B cell lymphoma corresponding to the terminal steps of B cell differentiation.

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NORMAL PERIPHERAL B cells express major histocompatibility (MHC) antigens of class I (HLA-A,B,C) and class II (HLA-D), lineage-restricted differentiation antigens CD19, CD20, CD22 (reviewed by Nadler) and carry immunoglobulin (Ig) on their surface. At the terminal stages of differentiation, the B cell differentiation antigens, HLA-D, and surface Ig disappear; plasma cell-associated antigens such as the PC-1, PCA-1, and the T10 antigen are expressed, and Ig is secreted in large amounts. In neoplastic B cells, however, deficient Ig expression may occur more frequently than initially assumed since most of the so-called “null”-cell lymphomas and common acute lymphocytic leukemias are actually of B cell origin, even chronic B lymphocytic leukemia is reported to lack detectable Ig constituents in a small percentage of cases. Apart from Ig deficiency, cytomorphology of B cell tumors may be so deviant from that of B cells in physiologic development that they occasionally mimic T cell lymphomas, Hodgkin’s disease, and even carcinoma. The mediastinum is known to be the primary site of lymphocytic T cell tumors (“T cell, convoluted” according to Lukes and Collins), Hodgkin’s disease, and epithelial malignomas such as thymic carcinoma, and rarely of sarcoma (reviewed by Otto). Even large series of malignant lymphomas, however, (eg, Brittinger and colleagues, 1,127 cases; Tubbs and colleagues, 2,564 cases), of mediastinal lymphomas (Bernard and colleagues, 350 cases) and extranodal B cell lymphomas (Mohri, 148 cases) fail to contain primary mediastinal tumors of proven B cell origin. Nevertheless, there are three reports on primary non-Hodgkin’s lymphoma of the mediastinum with pronounced aggressiveness and poor prognosis. Conspicuously, these lymphomas have a number of features in common: The patients were mostly young female adults presenting cough, chest pain, dyspnea, and venous obstruction as initial symptoms. The tumors apparently originating in the mediastinum tended to invade adjacent organs and structures and in most cases responded badly to antineoplastic treatment. As to morphology, the tumors were described as being of diffuse large cell type or as poorly differentiated, lymphocytic. In one series, pronounced sclerosis was described as an additional characteristic feature. Concerning the immunophenotype, Perrone and colleagues were able to prove the “lymphoreticular origin” of 53 of their 60 mediastinal tumors by applying a mixture of monoclonal antibodies to leukocyte common (LC) antigen on paraffin sections. Furthermore, immunoreactivity with antisera to κ and λ light chains suggested a B cell origin of five of these tumors. The remaining tumors described by the three groups of authors could be characterized, however, neither as B nor as T cell lymphomas using surface marker techniques available at the time of publication or by using formalin-fixed material.

Here we report that these mediastinal “null”-cell lymphomas are of B cell origin and present an immunophenotype that, although characterized by various defects, is indicative of a very late stage of B cell maturation.

MATERIALS AND METHODS

Patients. During the last 2 years, eight unusual primary mediastinal tumors were observed. They occurred in young (19 to 43 years, mean 29.4 years) predominantly female (6 of 8) adults. First clinical symptoms consisted of thoracic pain, thoracic venectasia, and dyspnea; B symptoms were noted in only 1 case. Tumor localization was described as mediastinal in 6 and as (para-)thymic in 2 cases. Initial diagnosis revealed involvement of the lungs in 3, of the pericardium as mediastinal in 6 and as (para-)thymic in 2 cases. Invasive lymphomas (MCCL) is a previously undescribed type of B cell lymphoma corresponding to the terminal steps of B cell differentiation.

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TABLE 1. Clinical Data

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Patient Sex</th>
<th>Age at Diagnosis (years)</th>
<th>First Clinical Symptoms</th>
<th>Local Tumor</th>
<th>Tumor Cell Morphology</th>
<th>Preliminary Diagnosis*</th>
<th>Clinical Course After Surgical Intervention, Radiation and Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B.M.</td>
<td>F</td>
<td>19</td>
<td>(Para-)</td>
<td>Large, polymorphic</td>
<td>Hodgkin's disease</td>
<td>Progression; liver infiltrations and abdominal lymphomas; tracheoesophageal fistula; pneumonia; death 22 months after diagnosis</td>
</tr>
<tr>
<td>2</td>
<td>F.M.</td>
<td>F</td>
<td>21</td>
<td>Mediastinum</td>
<td>Large, polymorphic</td>
<td>Malignant lymphoma</td>
<td>Local progression; death 10 months after diagnosis with lung involvement and pericardic effusion</td>
</tr>
<tr>
<td>3</td>
<td>W.I.</td>
<td>F</td>
<td>31</td>
<td>Mediastinum</td>
<td>Large and medium-sized, polymorphic</td>
<td>Malignant lymphoma</td>
<td>Local progression; death due to respiratory insufficiency 11 months after diagnosis</td>
</tr>
<tr>
<td>4</td>
<td>L.I.</td>
<td>F</td>
<td>35</td>
<td>Mediastinum</td>
<td>Relatively small, nonmonomorphic</td>
<td>Malignant lymphoma</td>
<td>Local progression; abdominal lymph node involvement</td>
</tr>
<tr>
<td>5</td>
<td>G.S.</td>
<td>F</td>
<td>35</td>
<td>Mediastinum</td>
<td>Medium-sized to relatively small, nonmonomorphic</td>
<td>Thymoma</td>
<td>Local progression; lung involvement; retroperitoneal lymphomas; death due to respiratory insufficiency 12 months after diagnosis</td>
</tr>
<tr>
<td>6</td>
<td>S.S.</td>
<td>F</td>
<td>43</td>
<td>Mediastinum</td>
<td>Medium-sized, nonmonomorphic</td>
<td>Malignant lymphoma</td>
<td>Local progression; death due to respiratory insufficiency 18 months after diagnosis</td>
</tr>
<tr>
<td>7</td>
<td>K.M.</td>
<td>M</td>
<td>22</td>
<td>Mediastinum</td>
<td>Medium-sized, polymorphic</td>
<td>Hodgkin's disease</td>
<td>Presently in complete remission after combination chemotherapy</td>
</tr>
<tr>
<td>8</td>
<td>S.G.</td>
<td>M</td>
<td>29</td>
<td>Mediastinum</td>
<td>(Para-) polymorphic</td>
<td>Malignant lymphoma</td>
<td>Progression; liver, kidney, and adrenal gland infiltrations; cervical lymphomas</td>
</tr>
</tbody>
</table>

*Done on the basis of routine hematoxylin and eosin sections for rapid notification.

applied in 7 cases, remission was achieved in only 1 case (patient 7, who received combination chemotherapy comprising endoxan, holoxan, vincristine, and prednisone [CHOP]). Five patients died 10, 11, 13, 18 and 22 months after initial diagnosis of extensive disease and large thoracic tumor masses causing respiratory insufficiency (Table 1).

Tumor tissue: Fresh tissue was transferred to our laboratory immediately after surgical removal. Representative samples were quick-frozen in liquid nitrogen. Frozen sections (4 to 6 μm) were air-dried overnight, fixed in acetone for 10 minutes at room temperature, and immunostained immediately or stored at −20°C for 1 to 3 weeks. Another sample was fixed in Bouin’s fixative for routine processing purposes; histochemical stains from the paraffin-embedded material were achieved by using hematoxylin and eosin (H & E), periodic-acid Schiff’s (PAS), Giemsa, and the Gomori reticulin method; immunostaining of paraffin sections was preceded by bleaching and destruction of endogenous peroxidase by methanol/H2O2.

Reagents: Monoclonal antibodies (MAbs) to IgM (R1/69) and IgD (IgD26), to the leukocyte common antigen (2B11 and PD7/26), and anti-Ki-I were obtained from Dakopatts, Copenhagen. MAbs against the T4 antigen (anti-Leu-3a (SK2)), the T8 antigen (anti-Leu-2b (SK3)), ε (163-42) and λ (1-155-2) light chains, to the interleukin-2 (IL2) receptor (2A3) and the CD22 antibody anti-Leu-14 were purchased from Becton Dickinson, Mountain View, CA. MAbs to the T11, T6, and T3 antigens were obtained from New England Nuclear, Seattle (Ly3) and Ortho Diagnostics, Raritan, NJ (OKT6, OKT3), respectively. MAbs to common-ALL antigen (cALLa) (J5), to the B cell antigens CD20 (B1), CD21 (B2), CD19 (B4), and MAbs PC-1 and PCA-1, both directed against plasma cell-associated antigens, were furnished by Coulter Immunology, Hialeah, FL. MAbs specific to framework determinants of HLA-A,B,C (W6/32) and HLA-DR (2.06), and Blast-2, reacting with a B cell activation antigen (CD23 antigen) were generous gifts from the producing laboratories (given in references in Table 2); the B cell reagents HD6, HD28, HD37, HD237, HD39, and HD50 are products of some of us (B.D., G.M., 5K.), as is the antibody HEA125 that reacts with a surface glycoprotein (Egpg34) restricted to normal and neoplastic epithelial cells (G.M., F.M.).

A polyclonal biotinylated sheep antibody to mouse Ig and a streptavidin-biotinylated peroxidase complex, both obtained from Amersham Buchler, Braunschweig, FRG, served as detection system for the monoclonal primary antibodies. Unconjugated rabbit-anti-mouse Ig antibody was diluted 1:1,000, and 0.01% H2O2 for 10 minutes), the peroxidase reaction resulted in

Staining procedures: MAbs in culture supernatants were used undiluted; ascites preparations were used as 1:200 dilutions. The anti-mouse Ig antibody was diluted 1:50, and the streptavidin-peroxidase complex was diluted 1:100. Dilutions of primary antisera ranged from 1:50 to 1:1,500; the anti-rabbit Ig antisera was diluted 1:20, and the peroxidase-antiperoxidase complex was diluted 1:100. All dilutions and washing steps were carried out in phosphate-buffered saline (PBS); the secondary antibody solution contained 5% pooled human IgG to inhibit cross-reactions with human surface Ig. Incubation times of tissues were 1 hour at room temperature for the primary antibody (respectively antisera) and 30 minutes for the second-step and third-step reagents. Using AEC as the chromogen (0.4 mg/mL in 0.1 mol/L of acetate buffer pH 5.0 with 5% DMF and 0.01% H2O2 for 10 minutes), the peroxidase reaction resulted in
MHC. major histocompatibility complex.

An intense red precipitate. The sections were counterstained with Harris' hematoxylin and mounted with glycerol gelatin.

Controls. To obtain reliable results, the detection of Ig components was performed twice, using antisera on paraffin sections and mAbs on frozen sections. Positive controls were made at the same time, using paraffin and frozen sections from tonsils to ensure that a negative result on the tumor tissue was reliable and not due to the method. Intrinsic positive controls for the immunoreactivity of the MHC antibodies were interstitial dendritic cells, endothelial cells, and macrophages that, by their staining, indicated the reliability of the reaction and thus excluded false-negative results. Negative controls were performed by omitting the primary antibody (antiserum). No staining was observed, except for the reaction of intermingled granulocytes in frozen sections whose endogenous peroxidase was not destroyed.

RESULTS

Histomorphology. Most of the tumors showed a diffuse growth pattern (Fig 1). The cells had clear and abundant cytoplasm in common but varied considerably in size. The nuclei were roundish to very irregular; one tumor contained cells with multilobated nuclei, and a second revealed giant cells with extremely pleomorphic nuclei resembling Reed-Sternberg cells. The nucleoli were predominantly small and mostly singular. In cases 1 and 2, the tumor cells are best described as large and pleomorphic; in case 3 as mixed, large and medium-sized and pleomorphic; in cases 6 and 8 as medium-sized monomorphic; and in case 5 (Fig 1) as mixed, medium-sized to relatively small but monomorphic. The smallest tumor cells, however, were twice as large as the diffusely infiltrating normal lymphocytes that were a regular constituent of the lesion. Tumor necroses, mostly ill-defined and small but never confluent, were observed in six cases. Mitoses were numerous and always typical. Even more frequent were single-cell degenerations. The tumors were further characterized by an alveolar to microalveolar reticular fiber scaffold; case 1 showed pronounced sclerosis. In conclusion, the histologic picture of these tumors was not compatible with any of the morphologically defined B cell lymphomas listed in the current classifications. The histomorphology of these lymphomas resembles either pleomorphic peripheral T cell lymphoma or thymoma; in two cases, it even resembled Hodgkin's disease.

Immunophenotype. (Table 3) The tumors were LC' and HEA125' and thus clearly defined as nonepithelial in nature. They all expressed the B cell-restricted antigens CD19 and CD20, as could be shown by the binding of mAbs HD37, HD237, B4, and B1, respectively. The first three mAbs showed minor differences in binding intensity in five cases; B4 failed to react with tumors 3 and 7. CD22 was expressed on the tumor cells of six cases but was undetectable.
in cases 3 and 8. CD23, a putative B cell activation antigen, was present on tumor cells of cases 1 and 4 (Fig 2) and on a minor part of the neoplastic cells of cases 7 and 8. Two other activation antigens, Ki-1 and the IL 2 receptor, could not be detected. Immunoglobulin constituents (heavy and light chains, J chain) were not detectable in the whole tumor series in either the cytoplasm or on the surface of the tumor cells. Ig of various light and heavy chain classes was present only in mature, morphologically typical plasma cells, which, however, were very scarce. In addition, one case contained minute islands of small, round lymphoid cells carrying surface IgM (Fig 3). The neoplastic cells of every case strongly bound PC-1, a mAb recognizing a plasma cell-associated antigen (Fig 4). PCA-1, another mAb of this category, reacted in cases 5 and 6 with the entire neoplastic population and in a varying number of tumor cells of cases 1, 2, and 4. MAb OKT10, also known to bind to normal plasma cells, reacted with tumor 2 and with parts of the neoplastic population of case 4 CALLA and CD21 could not be detected in either case. The tumors showed variable deficiencies in MHC antigen expression: at least two cases completely lacked class I and class II antigens (Fig 5); a third case (case 6) contained only a very small number of scattered HLA-ABC* and HLA-DR* large cells among the unreactive tumor cells (Fig 6); although probably of histiocytic origin, it cannot be completely ruled out that those cells were part of the neoplastic population. Four other tumors contained varying proportions of MHC antigen-deficient neoplastic cells. The coordinate expression of class I and class II

Table 3. Antigen-Expression of Tumor Cells in Eight Cases of Ig-Mediastinal Clear Cell Lymphoma of B Cell Type

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>1, B.M.</th>
<th>2, F.M.</th>
<th>3, W.I.</th>
<th>4, L.I.</th>
<th>5, G.S.</th>
<th>6, S.S.</th>
<th>7, K.M.</th>
<th>8, S.G.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-ABC</td>
<td>W6/32</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>2.06</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>PD7/26 and 2B11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CALLA (CD 10)</td>
<td>J5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CD 19</td>
<td>HD37</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD237</td>
<td>(+)</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CD 20</td>
<td>B4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CD 21</td>
<td>B2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CD 22</td>
<td>HD6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+ (+)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD39</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leu14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD 23</td>
<td>Blast-2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD60</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cell-associated antigen</td>
<td>PC-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PCA-1</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OKT10</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ig (cytoplasmic/surface)</td>
<td>Ki-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL2 receptor</td>
<td>2A3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell antigens T6, T11, T3, T4, T8</td>
<td>OKT6, Lyt3, OKT3, Leu3a, Leu2b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Egp34</td>
<td>HEA125</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* -, Negative; +, positive reaction on the entire tumor cell population; (+), weakly positive; ±, positive and negative tumor cells in equal parts, +, negative tumor cells clearly outnumber positive ones; — > +, very few scattered positive cells, putative tumor cells.
Fig 2. Case 4. Monoclonal antibody (MAb) HD50 recognizing the activation-associated CD23 antigen bound in varying intensity to the tumor cell population but not to the stromal cell component of the lymphoma. Immunoperoxidase; original magnification ×122.

Fig 3. Case 2. Demonstration of IgM by monoclonal antibody (MAb) R1/69. The large tumor cells were devoid of detectable IgM whereas scattered reactive small lymphocytes stained strongly, thus excluding a false-negative result. Immunoperoxidase; original magnification ×305.

Fig 4. Case 8. Monoclonal antibody (MAb) PC-1 recognizing a plasma cell-associated antigen bound strongly to the entire tumor cell population. Negative connective tissue fibers are apparent. Immunoperoxidase; original magnification ×133.

Fig 5. Case 5. Monoclonal antibody (MAb) W6/32 strongly stained dendritic interstitial cells and small infiltrating lymphocytes but did not bind to the tumor cell population, thus indicating its abnormal loss of HLA-A,B,C molecules. Immunoperoxidase; original magnification ×122.
Dyspnea, and venous obstruction were common initial signs. The colleagues' group were aged ≤ 35 years. Cough, chest pain, and pleural effusion were frequent initial symptoms. Eight of Trump and Mann's, and 9 of those in our series were female. Nine of those of Levitt and colleagues showed reactivity of the tumor cells and occasionally crowded around blood vessels (Fig 1). The epithelium-specific mAb HEA125 showed reactivity in neither case.

In sum, this series of eight polymorphic mediastinal tumors is characterized by variable defects in MHC antigen expression, by the constant phenotype CD10⁻, CD19⁺, CD20⁺, CD21⁺, PC-1⁻ and complete Ig deficiency, suggesting that they represent tumors of terminal B lymphocyte differentiation.

DISCUSSION

In several aspects, the clinical features of our patients with primary non-Hodgkin's lymphoma of the mediastinum resemble those of the patients described by Levitt and colleagues, Trump and Mann, and Perrone and colleagues. As compared with mediastinal T lymphoblastic lymphoma, which has pronounced male predominance (eg, Brittinger and colleagues, ratio of males to females 9:1), there seems to be a clear female predominance for mediastinal "null"-cell lymphoma: 6 of 12 of the patients of Levitt and colleagues, 7 of 11 of the patients of Trump and Mann, 43 of 60 of the patients of Perrone and colleagues and 6 of 8 of those in our series were female. Nine of those of Levitt and colleagues, 8 of Trump and Mann's, and 7 of our patients were younger adults (19 to 37 years); 85% of Perrone and colleagues' group were aged ≤ 35 years. Cough, chest pain, dyspnea, and venous obstruction were common initial signs. Apart from mediastinal mass, there was additional tumor involvement of pericardium at initial examination in three of the patients of Levitt and colleagues, 1 of the patients of Trump and Mann, and in 2 of the patients of our series; lung involvement was noted twice in Levitt and colleagues' group, 3 times in Trump and Mann's group, and 3 times in our group of patients. The chest wall was the most frequent site of extrathoracic involvement in Perrone's series. When given aggressive antineoplastic treatment, 10 of the patients of Levitt and colleagues reached a transient complete remission, but neither of Trump and Mann's patients and only one of our patients was regarded as disease-free afterwards. No patient developed leukemia. Progressive disease, relapse, and chemotherapeutic failure was commonly noted. Four of the patients of Levitt and colleagues, 8 of Trump and Mann's, 20 of the patients of Perrone and colleagues, and 5 of our patients were dead at time of publication, with a survival time ranging from 4 to 22 months; median survival given by Trump and Mann was 16 months. To conclude, we feel that we are dealing with a lymphoma type with a rather distinct clinical picture.

Our central finding is that these tumors are B cell lymphomas. Referring to the B cell differentiation scheme given by Nadler, the immunophenotype LC⁺, CD10⁻, CD19⁺, CD20⁺, CD21⁺, Ki1⁻, T cell antigens, common to all the tumors of our series, suggests a terminal B cell differentiation. This view is supported by the presence of the plasma cell-associated antigens PC-1 in 8 of 8, PCA-1 in 5 of 8, and T10 in 2 of 8 tumors, indicating that at least a partial plasma cell phenotype is expressed although cytology is never plasmocytic. On the basis of the PCA-1 reactivity of neoplastic cells, Anderson and co-workers characterize hairy cell leukemia as a tumor of the pre-plasma cell. Plasmacytoma, plasma cell leukemia, and myeloma are reported to express PCA-1, PC-1, and (OK)T10. On the other hand, the diffuse large cell lymphomas of B cell type analyzed by Freedmann and co-workers failed to express PCA-1 and PC-1, a finding that caused them to argue that these tumors are the neoplastic counterparts of normal B cells at the midstage of differentiation. Based on these observations the immunophenotype of the aleukemic lymphomas described herein indicates that it is probably a novel B lymphoma type. It might be placed between tumors of midstage differentiation and hairy cell leukemia/plasma cell tumors. This tumor cell variant might alternatively be regarded as the malignant counterpart of the final stage of an alternative pathway of B cell development: the monocytoid B cell, also known as the cell of the so-called "immature sinus histiocytes," which is a B cell that cannot be located in the current physiological differentiation scheme. In this context, it is noteworthy that monocytoid B cells have clear cell morphology and are PCA-1⁺ (P. Möller, unpublished observations).

Nevertheless, as compared with the normal mature B cell, the tumor cells of MCCL exhibit considerable lacks in antigen expression. There are no Ig constitutents. This complete loss of Ig has been observed by several authors (discussed previously) to occur in large cell B lymphomas of unknown or follicular origin. Two of our tumors failed to express the CD22 antigen, as could be verified by staining.
with three different mAbs. This may be interpreted in two ways. CD22 expression may either have been impaired by a defect in gene expression or abrogated in the course of terminal differentiation. Furthermore, there are minor irregularities in the binding of CD19 mAbs: HD237 failed to react with a subset of neoplastic cells of case 3; B4 did not recognize tumors 3 and 7. Whether this is due to inaccessibility or defect of the corresponding epitope or just to a lesser affinity of these antibodies cannot be answered. Because the CD23 antigen seems to be closely related to activation, the partial or complete nonexpression of the CD23 antigen in six of eight tumors is probably of functional nature. Severe defects, however, must be suspected because of the partial or complete nonexpression of MHC class I molecule (HLA-A,B,C) observed in all tumors but one. To our knowledge, very few reports exist describing lack of class I antigen expression in malignant lymphoma: Woda and co-workers observed this lack in three cases of large "histiocytic" B cell lymphomas, and the Daudi cell line is known to be devoid of β2-microglobulin, which is part of the heterodimeric class I molecule. Because class I antigens serve as restriction elements of T cell-mediated cytotoxicity, HLA-A,B,C-deficient tumor cells possibly escape immune attack by cytotoxic T cells. This may account for the clinical aggressiveness of the primary mediastinal B cell lymphomas.

Six of eight of the tumors had either partial or complete defects in expression of the HLA-DR framework determinant recognized by mAb 2.06. Defects in class II antigen expression in B cell lymphomas were sporadically noted by several authors. Our evaluation of the staining for HLA-DR probably underestimates the real extent of nonreactivity within the neoplastic population since intermingled small negative cells were regarded as reactive T cells, as was suggested by parallel sections stained with mAbs to T cell antigens. For multiple myeloma, it was assumed that the neoplastic plasma cells lost their class II antigens while entering the terminal stage of differentiation. Although we discuss this stage of development for our tumor group, a down-regulation of MHC class II antigen expression seems more unlikely to us than assumption of a pathological loss, ie, a further defect in antigen expression since all tumors lacking class II molecules were also class I antigen-deficient. Class II antigens are transducers for B cell activation and maturation. Thus, a defective expression of these important trigger molecules might contribute to the incomplete differentiation of the neoplastic B cells in the primary mediastinal lymphoma of clear cell type.

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