Value of Monoclonal Anti-CD22 (p135) Antibodies for the Detection of Normal and Neoplastic B Lymphoid Cells


Two monoclonal antibodies (To15 and 4KB128) specific for the B cell-associated CD22 antigen (135,000 mol wt) are described. On immunoenzymatic analysis of cryostat tissue sections, these antibodies strongly label both mantle zone and germinal center B lymphoid cells in secondary lymphoid follicles (and also scattered extrafollicular lymphoid cells) but are unreactive with other cell types (with the exception of weak reactivity with some epithelioid histiocytes). These reactions differ from those of monoclonal antibodies B1 and B2 (anti-CD20 and CD21) but are similar to those of the pan-B antibody B4 (anti-CD19). One of the anti-CD22 antibodies (To15) has been tested extensively by immunoenzymatic labeling on >350 neoplastic lymphoid and hematological samples. The CD22 antigen was found in tissue sections in most B cell-derived neoplasms, the major exceptions being myeloma (all cases negative) and a small proportion of high-grade lymphoma (6% of cases negative). In cell smears, the antigen could be found on neoplastic cells in most B cell lymphoproliferative disorders, including common acute lymphoblastic leukemia (ALL) (90% positive) and B cell chronic lymphocytic leukemia (CLL) (89% positive). We conclude that anti-CD22 antibodies are of value for identification of human B cell lymphoproliferative disorders (especially when used in conjunction with anti-CD19 antibodies). Previous reports that the CD22 antigen is absent from many B cell neoplasms are probably due to its being expressed within the cytoplasm of immature B cells rather than on their surface.

Widespread use has been made in recent years of monoclonal antibodies for the immunocytochemical identification of lymphoid cell populations in human tissue and blood samples. The first of these reagents to be characterized were directed against T cell antigens, and at the 1st International Workshop on Human Leucocyte Differentiation Antigens (held in Paris in 1982) eight different T cell-associated molecules were defined. Following the convention established at that Workshop, these molecules are prefixed by the letters CD and listed from 1 to 8.

An equivalent number of B cell-associated markers was not defined at the first Workshop, the only categories emerging at that time being CD9 (a 24,000 mol wt molecule shared between B cells and renal tissue) and CD10, the Common ALL antigen (CALLA). A number of new B cell-associated molecules were, however, defined in the second Workshop (held in Boston in 1984) and numbered CD19 through 24.

Several of these appear to be restricted to B cells, whereas others are shared between B cells and dendritic reticulum cells (the antigen-presenting cell found in B cell follicles).

Although these B cell-associated molecules are clearly defined entities, little information is available on their relative value as markers in the immunocytochemical detection of human B cell neoplasms. In the present article, we describe our experience in using two monoclonal antibodies against the 135,000 mol wt CD22 antigen for the detection of neoplastic B cells in cryostat sections and cell smears. It is evident from this study that anti-CD22 antibodies can be confidently used as pan-B reagents for identification of B cell neoplasms, since they react with most such tumors and are unreactive with T cell-derived neoplasms.

MATERIALS AND METHODS

Tissues

Human tissue biopsies were obtained fresh from the Histopathology Department of the University Hospital, Kiel, and from the John Radcliffe Hospital, Oxford, England. Routine tonsillectomy samples were obtained from the Ear, Nose, and Throat Departments of these hospitals. Histological diagnosis was based on examination of paraffin sections stained with hematoxylin and cosin and/or Giemsa, lymphoid neoplasms being classified according to the Kiel system. Immunohistological phenotyping following immunoenzymatic staining of cryostat sections (described later) was performed as described elsewhere.

Cell Samples

Smears of peripheral blood and bone marrow samples from the Hematology Department of the John Radcliffe Hospital and of normal blood from healthy laboratory personnel were stored at -20°C until they were labeled immunoenzymatically. In addition, cytocentrifuge preparations of Triosil-Ficoll-separated mononuclear cells were stored in the same way. Tonsil cell suspensions were prepared and, in some experiments, enriched in follicular dendritic reticulum cells (FDRCs), as described previously.

In some experiments, cytocentrifuge preparations were made from normal peripheral blood mononuclear cells that had been resorted with neuraminidase-treated sheep RBCs.

Immunocytochemical Reagents

Details of the monoclonal antibodies used in this study and of the immunoenzymatic reagents (eg, PAP, APAAP, peroxidase conjugates) are given in previous publications. Antibodies B1, B2 and B3 were obtained from Dr L. Nadler and from Coulter.

Immunoenzymatic Labeling Procedures

Tissue sections and cell smears were labeled by a two-stage or three-stage indirect immunoperoxidase procedure, by the PAP immunoperoxidase technique, or by the APAAP immunokaline phosphatase technique.
Production of Monoclonal Antibodies to the CD22 B Cell-Associated Antigen

Two antibodies of this specificity were prepared and used in this study.

Antibody To15. A cell fusion experiment was performed using spleen cells from a Balb-c mouse immunized with a potassium iodide extract of normal human tonsil cell membranes. Culture supernatants were screened by immunoperoxidase labeling of tonsil cryostat sections, and one clone (To15) reacting selectively with B cell follicles was cloned and grown by conventional procedures.

Antibody 4KB128. Antibody 4KB128 was obtained (by the same hybridoma technique) using spleen cells from a mouse previously immunized with neoplastic cells from a case of hairy cell leukemia.

Immunoprecipitation

Immunoprecipitation experiments were performed by conventional procedures, using surface-iodinated human tonsil cells and rabbit anti-mouse Ig bound to Staphyloccal protein A as precipitating reagent. Electrophoresis in sodium dodecyl sulfate (SDS) polyacrylamide gel and autoradiography were performed by conventional techniques.

Purification of Antigen

Membranes extracted from frozen hairy cell leukemia spleen in 2.5% Tween 40 were solubilized in 2% Nonidet P40, 20 mmol/L of Tris-HCl pH 7.5 containing protease inhibitors. The detergent extract was precleared by passage over bovine serum albumin (BSA) coupled to Sepharose 4B, and 25 mL (equivalent to 20 g of original tissue) was then passed down a 5 mL Sepharose 4B:4KB128 antibody column (antibody 4 mg/mL of Sepharose) at 1 mL/h. After being washed with 50 mL of 250 mmol/L of NaCl in 1% Nonidet P40, 20 mmol/L of Tris-HCl pH 7.5 at 2 mL/h, bound antigen was eluted with a 40-mL pH 7.5 linear gradient at 2 mL/h. Fractions were precipitated with 10% trichloroacetic acid (TCA), washed with acetone, solubilized in SDS-urea, and analyzed by SDS polyacrylamide gel electrophoresis (PAGE) using a 7.5% gel and Coomassie blue staining.

RESULTS

Production of Monoclonal Antibodies To15 and 4KB128

Supernatant from two hybridoma cell cultures, To15 and 4KB128, reacted selectively with B cell follicles in lymphoid tissue, and the same pattern was maintained during cloning and subsequent growth of these cell lines.

Mol Wt of Antigen Detected by Antibodies To15 and 4KB128

Antibodies To15 and 4KB128 both precipitated a polypeptide chain with a mol wt under reducing conditions of 125,000 to 135,000. In some experiments, this material appeared as a doublet of two bands of only slightly different mol wts. It was also possible to isolate the CD22 antigen from human tissue using Sepharose antibody immunoabsorants (Fig 1). This material migrated with the same mol wt as that of the radiolabeled band detected by immunoprecipitation.

Immunocytochemical Labeling of Peripheral Blood Cells

Immunoenzymatic staining of cell smears. Antibody To15 labeled 15% of lymphoid cells (range 10% to 25%) in blood smears from 15 normal subjects. Antibody 4KB128 (tested on a smaller number of samples) labeled essentially the same number of lymphoid cells. Granulocytes, monocytes, RBCs, and platelets were unstained. In cytocentrifuge preparations of peripheral mononuclear cells rosetted with sheep erythrocytes, antibodies To15 and 4KB128 reacted selectively with nonrosetting lymphocytes.

Immunohistological Labeling of Human Tissue Sections

Normal tissue. The two anti-CD22 antibodies labeled B cell follicles with moderate to strong intensity (depending on the labeling procedure used) staining both mantle zone lymphocytes and germinal center cells. In centrifuged preparations of tonsil-cell suspensions enriched for FDRCs, antibody To15 labeled these cells weakly, although this could not
be discerned (because of lymphoid cell reactivity) in tissue sections.

The two anti-CD22 antibodies were compared with the pan-B reagents B1, B2, and B4 (anti-CD20, 21, and 19, respectively). Neither B1 nor B2 labeled B cell follicles as extensively as, or gave reactions of comparable intensity to, the two anti-CD22 reagents. Both B1 and B2 stained follicle mantle zone lymphocytes more weakly than germinal centers, and much of the germinal center staining was in a meshwork pattern in germinal centers characteristic of FDCs (particularly marked with antibody B2). In contrast, antibody B4 was closely similar in its staining reactions to the anti-CD22 reagents, labeling both mantle zones and germinal centers.

In addition to B cell follicles, antibodies To15 and 4KB128 stained scattered small cells in interfollicular and paracortical regions. The two antibodies were unreactive with all nonlymphoid tissue and organs tested (skin, lung, stomach, small and large intestine, kidney, liver, salivary and thyroid gland, urinary bladder, testis, and central and peripheral nervous tissue), with the exception of weak staining of epithelioid histiocytes in granulomatous lesions. Antibody B1 also reacted with epithelioid histiocytes, usually more intensely than the anti-CD22 reagents.

**Neoplastic tissues**. One of the two anti-CD22 antibodies (To15) was tested against a wide range of neoplasms (Table 1). It labeled most human B cell neoplasms, the principal exceptions being myeloma (consistently negative) and a few high-grade B cell lymphomas (4 negatives in 64 cases). The strength of labeling was usually of moderate intensity and could be enhanced markedly by use of the three-stage immunoperoxidase technique or the “enhanced” APAAP procedure. The strongest reactions were seen in hairy cell leukemia and B cell prolymphocytic leukemia. The intensity of labeling of cases of common acute lymphoblastic leukemia (common ALL) and B cell chronic lymphocytic leukemia (CLL) varied widely; a number of positive cases showed only weak labeling. Nevertheless, most cases in each of these two disorders gave unequivocally positive reactions.

Other neoplasms analyzed with antibody To15 (Table 1), were unreactive (including 56 cases of T cell lymphoproliferative disease) with the exception of a minority of cases of acute myeloid leukemia. These reactions were usually weak; in one case of acute monocytic leukemia, however, the neoplastic cells were relatively strongly labeled. The other anti-CD22 antibody (4KB128) was tested on a smaller number of neoplasms and gave reactions closely similar to those of antibody To15.

**DISCUSSION**

Both of the antibodies used in this study appear to recognize the B cell-associated molecule designated CD22. This is evidenced both by the results of immunoprecipitation and affinity chromatography experiments from this laboratory and by similar experiments and statistical analysis of FACS data in the 3rd International Workshop on Human Leucocyte Differentiation Antigens. The observation that in some experiments two polypeptide chains of similar mol wt were immunoprecipitated is in keeping with a similar report from Moldenhauer and colleagues on two other anti-CD22 antibodies.

The two anti-CD22 antibodies clearly delineated B cell follicles in cryostat sections of lymphoid tissue and did so with greater clarity than did either B1 (anti-CD20) or B2 (anti-CD21). Hofman and co-workers also found that antibody B1 labeled germinal center cells but only a proportion of mantle zone lymphocytes; in contrast, Bhan and co-workers reported labeling of all cells in lymphoid folli-

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**Table 1. Immunocytochemical Staining of Human Neoplasms With Antibody To15 (Anti-CD22)**

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>No. Tested</th>
<th>No. Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B cell derived</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>53</td>
<td>49</td>
</tr>
<tr>
<td>Prolymphocytic leukemia</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
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<td>28</td>
</tr>
<tr>
<td>Lymphoplasmacytoid lymphoma/Waldenstrom’s</td>
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<td>8</td>
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<tr>
<td>Multiple myeloma</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Centroblastic-centrocytic lymphoma*</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Centrocytic lymphoma</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Centroblastic lymphoma†</td>
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<td>21</td>
</tr>
<tr>
<td>Lymphoblastic lymphoma, Burkitt-type</td>
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<td>9</td>
</tr>
<tr>
<td>Lymphoblastic lymphoma, non-T, CALLA-positive</td>
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<td></td>
</tr>
<tr>
<td>Immunoblastic lymphoma†</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Common acute lymphoblastic leukemia</td>
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<tr>
<td>Acute lymphoblastic leukemia</td>
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<td>1</td>
</tr>
<tr>
<td>Total</td>
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<td>230</td>
</tr>
<tr>
<td><strong>Other leukemias</strong></td>
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</tr>
<tr>
<td>Acute myelomonocytic leukemia</td>
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</tr>
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<tr>
<td>Acute megakaryoblastic leukemia</td>
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<td>0</td>
</tr>
<tr>
<td>Total</td>
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<td>3</td>
</tr>
<tr>
<td><strong>T cell derived</strong></td>
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<tr>
<td>Chronic lymphocytic leukemia (T cell)</td>
<td>11</td>
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</tr>
<tr>
<td>Sézary syndrome/mycosis fungoides</td>
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<tr>
<td>Peripheral T cell lymphoma</td>
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<tr>
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<tr>
<td>Total</td>
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<tr>
<td><strong>Miscellaneous</strong></td>
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<tr>
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<tr>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>0</td>
</tr>
</tbody>
</table>

Staining was performed on cryostat tissue sections as described in the text, or by the APAAP immunoenzymatic procedure on blood or bone marrow smears, or on cytocentrifuge preparations. In all cases scored as positive, the majority (>70%) of neoplastic cells gave a positive reaction.

*Centroblastic-centrocytic lymphoma is approximately equivalent to follicular lymphoma in other classifications.
†Centroblastic and immunoblastic lymphoma are equivalent to "histiocytic lymphoma, diffuse" in the Rappaport classification.
CD22 ANTIGEN ON NORMAL AND NEOPLASTIC B CELLS

ancy may rebated to the relative sensitivities of labeling methods. When adjacent lymphoid tissue sections were stained with antibody B1 and anti-CD22 antibodies, however, the former reagent consistently gave weaker reactions. In this context, the initial detection of the two anti-CD22 antibodies by screening on tissue sections (rather than by binding to cell suspensions, used in screening for antibody B1) may account for their better reactivity as immunohistological reagents.

Anti-CD22 antibodies thus appear to be valuable reagents for labeling B cells in tissue sections and also in cell smears, and are comparable in our experience to antibodies against the 95,000 mol wt B cell-associated marker CD19 (first detected by antibody B4). They appear to be specific for B cells, with the exceptions of weak reactions with FDRCs and epithelioid histiocytes. Furthermore, the reaction of anti-CD22 with epithelioid histiocytes and FDRCs was less pronounced than the labeling of these cells by antibodies B1 and B2, respectively.

As shown in Table 1, positive labeling with the anti-CD22 antibodies was obtained in most B cell neoplasms in tissue sections or cell smears. The negative reactions of cases of myeloma is in keeping with observations that a number of other human B cell antigens are lost during terminal differentiation to plasma cells. The only positive reactions against neoplastic B cells other than B cells were those occasionally observed with cells from myeloid leukemia (Table 1). This may be related to the observation that CD22 is weakly expressed by epithelioid histiocytes (ie, by another cell type of myeloid/monocytic origin).

The broad spectrum of reactivity of anti-CD22 antibodies against neoplastic B cells observed in the present study contrasts with other reports that the CD22 antigen is undetectable on many B cell neoplasms. This discrepancy may be related to reports that the CD22 antigen is initially found during B cell maturation in the cell cytoplasm, and only subsequently emerges onto the cell surface. In consequence, blast cells in cases of common ALL contain cytoplasmic CD22 but lack this antigen on their surface. Phorbol ester stimulation of common ALL and pre-B cell lines can induce membrane expression of CD22, however. The practical implication of these observations is that immunocytochemical staining methods that allow antibodies to gain access to the cell interior (ie, in fixed cell smears or tissue sections) will reveal cytoplasmic CD22, whereas staining of cells in suspension will only reveal surface membrane antigen.

Whatever the explanation of the higher positivity rate for CD22 antigen on neoplastic B cells in the present study as compared with previous reports, it is evident that these monoclonal anti-CD22 antibodies will detect most B cell neoplasms studied in tissue sections or cell smears. Because identification of neoplastic cell lineage on the basis of at least two independent markers is advisable, we believe that in routine diagnostic practice a combination of anti-CD19 and CD22 antibodies constitutes a reliable pair of reagents for detecting B cell neoplasms.

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