Monoclonal Antibody SN10 Which Shows a Highly Selective Reactivity with Human B Leukemia-Lymphoma and Is Effectively Internalized into Cells

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ABSTRACT

The monoclonal antibody termed SN10 (IgG1-k) which was generated and characterized in the present study shows a highly selective reactivity with fresh (uncultured) human leukemia-lymphoma cells. The antigen defined by SN10 is a cell surface glycoprotein composed of a single polypeptide chain of Mr 36,000 and designated as gp36.

The primary reactivity of SN10 is against mature B-lineage leukemia-lymphoma cells. For instance, SN10 reacted with all of the 17 B non-Hodgkin's lymphoma specimens, all of the 15 B chronic lymphocytic leukemia specimens, all of the 3 B hairy cell leukemia specimens, and 2 of the 3 B acute lymphoblastic leukemia specimens tested.

Of normal peripheral blood cells, only a marginal reactivity of SN10 was detected with a minor subpopulation (<1-4% among different specimens) of isolated B-cells from healthy donors. No significant reactivity of SN10 was detected against any other isolated normal peripheral blood cells which include T-cells, granulocytes, monocytes, erythrocytes, and platelets. Furthermore, no significant reactivity of SN10 was detected against normal bone marrow specimens.

In immunohistological studies using frozen tissue sections, SN10 reacted well with malignant lymphomas and showed varying patterns of reaction with hyperplastic reactive lymph nodes. Various normal human tissues tested were unreactive with SN10.

In general, glycoprotein 36 was more abundantly expressed on fresh (uncultured) leukemia-lymphoma cells than on cultured leukemia-lymphoma cell lines.

No significant amount of circulating SN10 antigen was detected in the plasma of leukemia-lymphoma patients or normal healthy donors.

Scatchard plot analysis of direct binding of radiolabeled SN10 to a fresh (uncultured) B non-Hodgkin's lymphoma cell specimen, a fresh B chronic lymphocytic leukemia cell specimen, and DND-39 (an American Burkitt's lymphoma cell line) showed equilibrium constants of 5.2, 5.8, and 6.8 x 10^4 liters/mmol, respectively. Thus, SN10 shows a high binding avidity to each of the 3 B leukemia-lymphoma cell specimens tested.

Ricin A chain conjugate of SN10 killed leukemia-lymphoma cells effectively, whereas the same conjugate showed no cytotoxicity against control cells. Thus, SN10 bound to target antigen on the cell surface was effectively internalized into the cell.

The present results suggest the potential of SN10 for therapy as well as for diagnosis of various forms of leukemia-lymphoma, particularly mature B-lineage leukemia-lymphoma.

INTRODUCTION

During the last few decades, considerable improvement in the therapy of various forms of human LL has been achieved primarily because of the successful application of chemo-radiotherapy (e.g., reviewed in Refs. 1 and 2). However, many forms of LL are still associated with poor prognosis. These LL include low-grade NHL, prolymphocytic leukemia, B-ALL, acute myelogenous leukemia, and chronic myelogenous leukemia (2-6). In this regard, there still exists a strong need for the development of a new modality for treating LL.

Recently, several mAbs directed toward LL associated antigens have been shown to be useful for diagnosis and follow-up of LL (7). However, few, if any, anti-LL mAbs were clearly shown to be effective in the in vivo therapy (serotherapy) of LL. Therefore, the generation and characterization of new effective anti-LL mAbs are valuable.

A promising approach to the serotherapeutic utilization of anti-LL mAbs is to target LL cells by conjugating a mAb with an appropriate cytotoxic agent such as chemotherapeutic drugs, toxin subunits, or radioisotopes (reviewed in Refs. 8-12). The tumor specificity of the mAb is of paramount importance in the preparation of an effective immunoconjugate of an anti-LL mAb. In addition, a high binding avidity of the mAb to target antigen and a relatively abundant target antigen on the cells are important. Furthermore, in most cases, the mAb needs to be effectively internalized into the target cells after binding to the cell surface antigen. However, antibody binding to the target antigen should not induce a strong down-regulation of antigen expression (13).

A mAb termed SN10 which was generated and characterized in the present study meets these five important requirements for preparing a potentially effective immunoconjugate. Furthermore, SN10 meets another important requirement for preparing an effective immunoconjugate, i.e., no significant amount of circulating SN10 antigen was detected in the plasma of LL patients and healthy individuals. The lack of circulating antigen will facilitate targeting the tumor cells in vivo by a mAb. In addition, mAb SN10 reacts with a relatively wide range of LL. The availability of a sufficiently large candidate patient population for clinical trials will be important for developing a mAb and an immunoconjugate.

Thus, SN10 appears to have good potential for serotherapy as well as for diagnosis of LL. This mAb was generated by using an antigen preparation which was isolated by our established novel procedure (14, 15).

MATERIALS AND METHODS

Cells. Various established human cell lines were cultured in RPMI 1640 medium supplemented with 4-8% fetal bovine serum, penicillin (100 units/ml), and streptomycin (50 /ug/ml) as described previously (16, 17). Fresh (uncultured) cell and tissue specimens from patients with various LLs were kindly provided by Drs. Tin Han, Edward S. Henderson, and Cameron K. Tebbi and David Chervinsky of our institute. Normal (or nearly normal) bone marrow specimens were from patients who were in remission and had a morphologically normal bone marrow. Mononuclear cells were isolated from the bone marrow aspirates by Ficoll-Hypaque gradient centrifugation.

B-cells, T-cells, granulocytes, monocytes, erythrocytes, and platelets of normal peripheral blood from healthy volunteers were isolated as previously described (16, 18).
Control Murine Mabs, Control Murine IgG, and Reagents. Leu 16 (anti-CD20 mAb; IgG1) and anti-HLA-DR mAb (IgG2) were purchased from Becton Dickinson (Mountain View, CA). Anti-human immunoglobulin λ chain mAb (IgGl) was obtained from AMAC, Inc. (Westbrook, ME). Control murine IgG (MOPC195variant; IgG1) was prepared in our laboratory.

L-[3H]Hepatine and leucine-free medium were purchased from ICN Biomedicals, Inc. (Irvine, CA) and GibCO Laboratories (Grand Island, NY), respectively. Ricin A chain was obtained from Inland Laboratories (Austin, TX).

Antigen Preparation Used for Generating Mabs. Antigen preparation was isolated from the cell membranes of an approximately equal mixture of NHL cells derived from two patients with B-cell type NHLs, i.e., nodular poorly differentiated lymphocytic lymphoma and small lymphocytic lymphoma. The procedures involved are based on our earlier methods for isolating T and non-T ALL antigen preparations (16, 18). A brief description of the present isolation system is given below. Cell membranes were isolated from the NHL cells, and the cell membrane antigens were solubilized by deoxycholate treatment. The solubilized antigens were fractionated by affinity chromatography on serologically connected columns of Lch and Rcinus communis lectin. The Lch-bound and Rcinus communis lectin-bound glycoproteins (mostly glycoproteins) were individually eluted. The Lch-bound and eluted glycoproteins were further subjected to passive immunoaffinity chromatography (14, 15) by passing the glycoproteins through three serologically connected immunoadsorbent columns. These immunoadsorbent columns comprised an MHC class I antigens mAb (B3-3D1) coupled to Sepharose CL-4B, anti-MHC class II mAbs (G4-3A7) coupled to Sepharose CL-4B, and rabbit anti-human peripheral blood lymphocyte antibodies coupled to Sepharose CL-4B. Materials in these pass through fractions were pooled and concentrated.

Generation of Mabs. Monoclonal antibody was generated by immunizing a BALB/c mouse with the isolated antigen preparation. Immunization of the mouse was carried out as previously described (16). Cell fusion, hybridoma screening, cloning, and mAb class determination was carried out as described before (16, 19).

Cellular RIA and FACS Analysis. Details of the cellular RIA which was used for determining reactivity with mAbs and various cultured and uncultured cells were described previously (16, 19). It should be noted that Fc receptors on the target cells are blocked with human IgG during the assay. In selected cases, the reactivity of mAbs with various cell specimens was also determined by FACS analysis. FACS analysis was carried out as described previously (20). Briefly, one million cells were suspended in 10–20 ¿lil of RPMI 1640 medium containing 25 mM HEPES and incubated for 45 min at 37°C with an excess of SNIO or an isotype matching control mouse IgG solution (10Mg/ml) for 90 min at 4°C. After three washes with cold phosphate buffered saline (PBS), the cells were incubated with F(ab'); fragment of sheep anti-mouse immunoglobulin (Sigma Chemical Co.) for 45 min at 4°C. The washed three times with cold phosphate buffered saline and fixed with 1% formaldehyde. The fixed cells were analyzed by FACS using a Becton Dickinson FACS 440.

Determination of Antigen in the Plasma of LL Patients. A previously reported solid phase RIA (24) was used with a modification. Briefly, 50 µl, in triplicate, of serial dilutions of plasma from healthy donors (control), B NHL patients, and B CLL patients was incubated at 4ºC overnight in the individual wells of 96 well microtiter plates. Then, 50 µl of a 1.6% bovine serum albumen solution in Tris buffer was added and the incubation continued for 1 h at room temperature. The Tris buffer consisted of 20 mM Tris-HCl, pH 7.0, containing 0.1 M NaCl, 2 mM EDTA, 0.03% NaN4 and Trasylol (20 kallikrein units/ml). After the incubation, the wells were washed 3 times with Tris buffer containing 0.25% bovine serum albumen. The wash was followed by adding 100 µl of hybridoma culture fluid, or an appropriate dilution of control mAb or control IgG, and then incubating at 4°C for 1 h. After the wells were washed, 125I labeled F(ab')2 of affinity purified goat anti-mouse IgG antibodies which had been adsorbed with human IgG coupled to Sepharose CL-4B was added and incubated for 1 h at 4°C. The wells were washed and cut out, and the radioactivity was counted in a γ-ray spectrometer.

MOPC 195variant (IgGl) and anti-human immunoglobulin λ chain mAb (IgG1) were included in the test as an isotype matching negative and a positive control, respectively.

A titration experiment showed that in the above described solid phase RIA, we can detect SN10 antigen (gp36), containing as little as 1 µg of total crude cell membrane protein preparation from B NHL cells. However, if SN10 antigen in the plasma is substantially different from the membrane SN10 antigen in their physicochemical properties, the sensitivity of this assay may change significantly.

Direct Binding of 125I Labeled MAb to LL Cells and Scatchard Plot Analysis. The equilibrium constant between 125I labeled SN10 and a fresh (uncultured) B CLL cell specimen, a fresh B NHL cell specimen, or DND-39 (an American Burkitt's lymphoma cell line) cells was determined as described by Trucco el al. (25). The radiolabeling of the purified mAb SN10 was carried out by a method using IODO-GEN (see above). Scatchard analysis of the binding data was carried out as previously described (25, 26). An equilibrium constant and an average maximal number of mAbs bound/cell were estimated by this analysis.

Regulation of Antigen Expression. Antigenic modulation and down-regulation of gp36 was studied by incubating gp36 expressing DND-39 cells for varying times at 37°C with an excess of SN10 or an isotype matching control IgG (MOPC 195 variant; IgG1-x) following a previously described procedure (13, 23). A preliminary titration experiment showed that 10 µg of purified SN10 mAb/1 X 106 DND-39 cells represents antibody excess. The gp36 on the incubated cells was determined by an indirect cellular RIA after appropriate washings of the cells as described previously (13, 23). In the indirect RIA, SN10 was newly added to the SN10 or control IgG treated cells and incubated for 1 h at 4°C, followed by washings and the addition of 125I labeled F(ab')2 of affinity purified goat anti-mouse IgG antibodies (16). The addition and incubation with fresh SN10 was necessary for determining gp36 on the control IgG treated DND-39. Therefore, the same addition and incubation with fresh SN10 was carried out with SN10 treated DND-39. Also, we carried out a control experiment in which fresh
SN10 was not added. In this experiment, the 125I labeled F(ab')2 of affinity purified goat anti-mouse IgG antibodies were added to the DND-39 cells which had been previously incubated with an excess of SN10 and washed. The result of this control test was almost identical to that of the above test in which fresh SN10 was added.

As a positive control, an excess of mAb SN5 defining CD10 (CALLA) was incubated with NALM-6 cells (13).

Preparation of Immunotoxin. The purified mAb and an isotype matching control mouse IgG (IgG1) were individually conjugated to ricin A chain as previously described (27). The conjugates were purified by gel filtration on a calibrated Sephacryl S-300 column (27) followed by affinity chromatography on a Blue Sepharose column (28).

Determination of Activity of Immunotoxin. The in vitro cytotoxic activity of immunotoxin was determined by two different methods, i.e., protein synthesis inhibition assay (29) and a direct test of cytotoxicity against LL cells and control cells (29). The present procedure of the protein synthesis inhibition assay is briefly described below.

DND-39, a gp36 expressing lymphoma cell line, or MOLT-4, a gp36 negative T ALL cell line, was incubated, in triplicate, with varying concentrations of SN10-RA or control IgG-RA for 24 h. The incubated cells were centrifuged, washed, and resuspended in leucine-free medium containing 1 μCi of [3H]leucine. The cell suspension was incubated for 4 h and centrifuged, and the pelleted cells were washed. The cells were harvested on glass fiber filters using a multiple semiautomatic cell harvester (type 7010; Skatron Inc., Sterling, VA), and the 3H radioactivity was determined in a liquid scintillation spectrometer. Protein synthesis in the conjugate treated cells is expressed as the percentage of [3H]leucine incorporated into control cells not exposed to conjugate.

RESULTS

Initial Characterization of mAb. Reactivity of culture supernatants of 48 hybridoma primary cultures and hybridoma clones derived from the selected primary cultures were initially characterized using a cellular RIA with various cultured and uncultured cells.

Based on these test results, the mAb termed SN10, which was produced by hybridoma clone 2B-4G9-E7, was chosen for further studies. The properties of SN10 are described in this report. SN10 was found to be an IgG1-κ antibody. The reactivity of SN10 with various human LL cell lines and EB virus transformed nonmalignant cell lines is summarized in Table 1. SN10 reacted with all of the 10 mature B LL cell lines tested. In addition, SN10 reacted with 2 (i.e., REH and KM-3) of the 3 immature non-T/non-B (B-cell lineage) LL cell lines, one (i.e., NALM-1) of the 2 immature pre-B LL cell lines, and two (i.e., ARH-77 and HS) of the 3 plasma cell lines tested. However, SN10 did not react significantly with T, myelo/monocytic, and myeloid/lymphoid LL cell lines (Table 1).

SN10 showed a weak but definite reactivity against 3 EB virus transformed nonmalignant B-cell lines. It should be noted, however, that EB virus is absent in many of the SN10 reactive LL cell lines, e.g., REH, KM-3, NALM-1, and SU-DHL-4 (30).

Reactivity of SN10 with Fresh (Uncultured) LL Cells. Reactivity of SN10 with uncultured LL cells was routinely determined by a cellular RIA and, in selected cases, also by FACS analysis. The results of a cellular RIA with uncultured LL specimens from 68 different LL patients are summarized in Fig. 1. The test results are consistent with those obtained using cultured LL cell lines (Table 1). SN10 reacted with all but one of mature B LL cell specimens tested. SN10 reacted with all of the 17 B NHL, all of the 15 B CLL, both of the 2 B prolymphocytic leukemia (PLL), the 2 eosinophilic leukemia (EO), and the 2 myelomonocytic leukemia (M5).

Table 1 Reactivity of SN10 with LL cell lines and EB virus transformed nonmalignant cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin of cell line</th>
<th>SN10</th>
<th>Control IgG Antigen</th>
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<tbody>
<tr>
<td>LL non-T/non-B*</td>
<td>ALL</td>
<td>1354 ± 137</td>
<td>256 ± 54</td>
</tr>
<tr>
<td>REH</td>
<td>ALL</td>
<td>1308 ± 122</td>
<td>356 ± 72</td>
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<tr>
<td>KM-3</td>
<td>ALL</td>
<td>346 ± 48</td>
<td>238 ± 88</td>
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<tr>
<td>NALM-6</td>
<td>ALL</td>
<td>943 ± 112</td>
<td>323 ± 38</td>
</tr>
<tr>
<td>NALM-6</td>
<td>ALL</td>
<td>390 ± 69</td>
<td>253 ± 28</td>
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<tr>
<td>LL B</td>
<td>BALL-1</td>
<td>780 ± 109</td>
<td>316 ± 36</td>
</tr>
<tr>
<td>B</td>
<td>BALL-2</td>
<td>1602 ± 57</td>
<td>282 ± 15</td>
</tr>
<tr>
<td>B</td>
<td>BALL-3</td>
<td>741 ± 54</td>
<td>356 ± 1</td>
</tr>
<tr>
<td>B</td>
<td>BALL-5</td>
<td>780 ± 208</td>
<td>277 ± 12</td>
</tr>
<tr>
<td>B</td>
<td>SU-DHL-4</td>
<td>1697 ± 166</td>
<td>272 ± 51</td>
</tr>
<tr>
<td>B</td>
<td>DND-39</td>
<td>1554 ± 102</td>
<td>174 ± 20</td>
</tr>
<tr>
<td>B</td>
<td>Daudi</td>
<td>1920 ± 351</td>
<td>305 ± 14</td>
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<tr>
<td>B</td>
<td>Raji</td>
<td>796 ± 149</td>
<td>269 ± 39</td>
</tr>
<tr>
<td>B</td>
<td>Ogun</td>
<td>443 ± 65</td>
<td>143 ± 12</td>
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<tr>
<td>B</td>
<td>Chevalier</td>
<td>1823 ± 105</td>
<td>450 ± 166</td>
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<tr>
<td>Plasma</td>
<td>ARH-77</td>
<td>961 ± 18</td>
<td>132 ± 40</td>
</tr>
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<td></td>
<td>RPMI 8226</td>
<td>285 ± 51</td>
<td>419 ± 120</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>581 ± 54</td>
<td>238 ± 31</td>
</tr>
<tr>
<td>LL T</td>
<td>MOLT-4</td>
<td>271 ± 42</td>
<td>256 ± 54</td>
</tr>
<tr>
<td></td>
<td>JM</td>
<td>311 ± 91</td>
<td>223 ± 64</td>
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<tr>
<td></td>
<td>CCRF-HSB-2</td>
<td>221 ± 31</td>
<td>203 ± 30</td>
</tr>
<tr>
<td></td>
<td>HPB-MLT</td>
<td>322 ± 35</td>
<td>216 ± 29</td>
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<tr>
<td>LL myeloid/myelomonocytic</td>
<td>HL-60</td>
<td>327 ± 22</td>
<td>244 ± 30</td>
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<tr>
<td></td>
<td>U937</td>
<td>339 ± 18</td>
<td>200 ± 12</td>
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<tr>
<td>LL myeloid/lymphoid</td>
<td>K562</td>
<td>196 ± 12</td>
<td>156 ± 34</td>
</tr>
<tr>
<td>EB virus transformed</td>
<td>CCRF-SB</td>
<td>2703 ± 162</td>
<td>205 ± 26</td>
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<tr>
<td></td>
<td>RPMI 1788</td>
<td>693 ± 84</td>
<td>261 ± 95</td>
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<tr>
<td></td>
<td>RPMI 8057</td>
<td>692 ± 77</td>
<td>184 ± 13</td>
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* Purified mouse plasmacytoma IgG1 (MOPC 195 variant) was dissolved in the hybridoma culture medium at a concentration of 10 μg/ml.

** B-cell lineage.

† CML-BC.
phocytic leukemia, all of the 3 B hairy cell leukemia, and 2 of the 3 B ALL specimens tested.

However, it should be noted that generally the reactivity of SN10 with uncultured B LL cell specimens is stronger than with cultured B LL cell lines.

SN10 did not react significantly with T NHL, T CLL, or T ALL specimens. With a few exceptions, non-T/non-B (including pre-B) ALL specimens did not react or reacted only marginally with SN10. SN10 reacted with 3 of the 6 acute myelocytic, monocytic, or myelomonocytic leukemia specimens tested.

An example of FACS analysis with a B NHL and a B CLL specimen is shown in Fig. 2. The majority (77 and 72%, respectively) of the B NHL and B CLL specimens reacted with SN10.

Reactivity with Uncultured Normal Cells. B-cells, T-cells, monocytes, granulocytes, erythrocytes, and platelets were isolated from peripheral blood of three healthy donors and tested for reactivity with SN10 by a cellular RIA. SN10 showed a weak marginal reactivity with B-cell specimens but no significant reactivity with other cell specimens. Therefore, the reactivity of SN10 with B-cells was further tested by FACS analysis. The FACS analysis results of 2 of the 3 specimens tested are shown in Fig. 2. A weak marginal reactivity of SN10 was detected with a minor subpopulation (1, 2.7, and 3.9%) of the B-cell preparations derived from the three different donors. One of these B-cell preparations was tested for its reactivity with an anti-HLA-DR mAb (monomorphic; Becton Dickinson) by FACS analysis. The test showed that 77.5% of the B-cell preparation reacted with the anti-HLA-DR mAb.

To further characterize the specificity of the mAb, SN10 was tested for its reactivity with normal (or nearly normal) bone marrow specimens by FACS analysis; these bone marrow specimens were obtained from 5 different LL (4 ALL and one acute myelocytic leukemia) patients in remission. FACS analysis results of 2 of the 5 bone marrow specimens are shown in Fig.

Reactivity of SN10 with two of these NHL lymph node samples are illustrated in Fig. 3: these two are lymph nodes involved by follicular small cleaved cell lymphoma (Fig. 3e) and diffuse small cleaved cell lymphoma (Fig. 3c). An isotype matching control murine IgG (IgG1) did not show significant reaction with the NHL samples except for a focal reaction due to endogenous peroxidase; an example is shown in Fig. 3b. SN10 showed varying patterns of reactivity with hyperplastic reactive lymph nodes from three donors. An example is shown in Fig. 3d; sparse to moderate numbers of the germinal center and interfollicular cells are SN10 positive, while the majority of follicular mantle zone cells are SN10 positive. G4-3A7 (anti-HLA-DR; Ref. 20), an isotype matching positive control mAb, reacted strongly with both germinal center and mantle zone cells and moderately with the interfollicular cells of the same reactive lymph node.

No significant reactivity with SN10 was noted in normal epidermis, lung, kidney, or small intestinal mucosa.

**Determination of Circulating Antigen in the Plasma of LL Patients.** Circulating antigen in the plasma of patients may bind an administered mAb and thereby inhibit the therapeutic efficacy of the administered mAb and immunoconjugate. Therefore, we tested for circulating SN10 antigen in the plasma of LL patients and healthy individuals (control) by using a solid phase RIA. A titration experiment showed that this RIA allows us to detect SN10 antigen contained in as little as 1 µg of total crude cell membrane protein preparation from B NHL cells. No significant amount of SN10 antigen was detected in any of the plasma samples derived from 7 different B NHL patients and 5 different healthy individuals (Fig. 4). Similarly, no significant SN10 antigen was detected in the plasma samples derived from 7 different B CLL patients.

**Molecular Nature of Antigen.** Cell membranes were prepared from a mixture of malignant cells from two B NHL patients. Cell membranes were also prepared from malignant cells of a B CLL patient as well as of a B prolymphocytic leukemia patient. Glycoproteins were isolated separately from each of the three cell membrane preparations and radiolabeled individually with 125I.

The immunoprecipitates obtained by using the radiolabeled samples and SN10 (IgG1-κ), an isotype matching control mouse IgG, or control mAbs were analyzed by SDS-PAGE and autoradiographs were prepared. Some of the results are shown in Fig. 5.

The results of the NHL sample are shown in Fig. 5, lanes A–E. Under reduced conditions, the SN10 immunoprecipitate showed a single component of approximately M, 36,000 (lane A), whereas no significant component was immunoprecipitated by an isotype matching mAb Leu 16 (anti-CD20 mAb; lane B) or by an isotype matching control mouse IgG (MOPC 195 variant; lane C). Under unreduced conditions, the SN10 immunoprecipitate showed a single component with a molecular size similar to that of the reduced antigen (lane D), whereas
Fig. 3. Reactivity of SN10 with lymphomatous and reactive lymph nodes as determined by immunohistochemical staining. a, follicular small cleaved cell lymphoma: SN10 reactivity is confined to the majority of the lymphocytes within the neoplastic follicles. The interfollicular areas are essentially negative. b, negative isotype matching control IgG with the same lymph node (as a) focal reaction due to endogenous peroxidase in the interfollicular histiocytes is noted. c, diffuse small cleaved cell lymphoma: the majority of the lymphocytes are SN10 positive. d, reactive lymphoid hyperplasia: the majority of follicular mantle zone lymphocytes are SN10 positive. Sparse to moderate numbers of SN10 positive cells are also noted within the germinal center and interfollicular areas (frozen sections; x 240).

Fig. 4. Absence of gp36 in the plasma of LL patients and healthy individuals. A solid phase RIA was used to determine circulating gp36 in the plasma of 7 B NHL patients, 7 B CLL patients, and 5 healthy individuals. An isotype matching control IgG (MOPC 195-variant) and anti-human immunoglobulin A chain mAb (IgGl) were used along with SN10 as a negative and a positive control. gp36 was not detectable in any of the tested specimens. Therefore, only the results of NHL and healthy control specimens are shown. A titration experiment was carried out separately to determine the sensitivity of the RIA for detecting gp36 (see "Materials and Methods").

This conclusion was supported by the following results obtained with a B CLL sample (lanes F-K) and a B prolymphocytic leukemia sample (lanes L and M). Under reduced conditions with the CLL sample, SN10 immunoprecipitated a single component of M, 36,000 (lane F), whereas anti-HLA DR mAb, a control mAb, immunoprecipitated two components of 33,000 (α chain) and 28,000 daltons (β chain) (lane G). The control IgG immunoprecipitated no significant component (lane H). Essentially, the same results were obtained with the CLL sample under unreduced conditions (lanes I-K).

With a B prolymphocytic leukemia sample, SN10 immunoprecipitated an M, 36,000 component (lane L), whereas the control IgG precipitated only a minor nonspecific component (lane M).

Thus, an M, 36,000 antigen was specifically immunoprecipitated by SN10 from each of the three samples tested, i.e., a B NHL, a B CLL, and a B prolymphocytic leukemia sample. The SN10 antigen was designated gp36.

It should be noted that the reduced SN10 antigen and unreduced SN10 antigen are in slightly different positions relative to the molecular weight markers; the marker proteins used were reduced before the gel analysis. Such small differences between reduced and unreduced proteins were previously observed for other proteins (31). The molecular weight of the SN10 antigen was estimated using the relative mobility of the reduced antigen...
because it was compared to molecular weight marker proteins that had been reduced before the gel analysis.

CD20 antigen has a molecular weight similar to that of the SN10 antigen, although its specificity is substantially different from that of SN10 (32, 33). Therefore, we compared the SN10 antigen with the CD20 antigen (Fig. 5). No significant component was immunoprecipitated from our glycoprotein mixtures by Leu 16, an anti-CD20 mAb from Becton Dickinson, whereas an M, 36,000 component was specifically immunoprecipitated from the same sample by SN10. This finding is consistent with the fact that the CD20 antigen is not a glycoprotein (33), whereas the SN10 antigen is a glycoprotein. To further compare the SN10 antigen with the CD20 antigen, we carried out a sequential immunoprecipitation experiment using the glycoprotein mixtures, Leu 16 and SN10. Leu 16 did not preclear SN10 antigen at all. Taken together, the above results indicate that the SN10 antigen is different from the CD20 antigen.

Biochemical Nature of Epitope. Daudi, a Burkitt’s lymphoma cell line (Table 1), which expresses gp36 on the cell surface, was treated with trypsin and mixed glycosidases, and the antigenic determinant remaining on the treated cells was tested for by means of a cellular RIA. As a control, a mAb L1-1E5-2C8 which defines an epitope containing sialic acid(s) of the CD24 antigen (34) are included. For L1-1E5-2C8, KM-3 cells did not show significant inhibitory activity against DND-39 at concentrations >5 nM but not against gp36 negative MOLT-4. The control IgG-RA conjugate showed no significant inhibitory activity against either DND-39 or MOLT-4. In the above test, no immunotoxin potentiator such as NH4Cl
DISCUSSION

In the past several years, a number of investigators reported many mAbs which reacted with human normal and malignant B-cells (reviewed in Refs. 37 and 38). However, only a small number of these mAbs show a highly selective reactivity with malignant B-cells over normal B-cells (39–44).

A new mAb SN10 which was generated and characterized in this study shows a highly selective reactivity with malignant B-cells. Furthermore, SN10 appears to be different from those previously reported mAbs in the antibody specificity and/or in the molecular nature of the antigen defined. The antigen defined by SN10 appears to be different from any of the reported CD series (1–78) antigens (45). However, it is prudent that any definite conclusions about the novelty of SN10 antigen be withheld until either SN10 is compared with other mAbs in an International Workshop or the chemical structure (e.g., amino acid sequence) of the antigens defined by these mAbs is determined.

The antigen defined by SN10 is a cell surface glycoprotein composed of a single polypeptide chain of 36,000 and designated gp36. The epitope defined by SN10 appears to be primarily composed of the protein moiety of gp36 on Daudi lymphoma cells. In general, gp36 is expressed to a greater degree on fresh (uncultured) LL cells than on cultured LL cell lines. At present, we do not know the origin of gp36. However, it may be a transformation associated antigen because (a) SN10 strongly reacts with malignant B LL cells, (b) SN10 shows a weak marginal reaction with a few percent (e.g., < 1, 2.7, and 3.9%) of normal peripheral blood B-cells, (c) SN10 does not react significantly with normal bone marrow cells, (d) SN10 reacts with EB virus transformed nonmalignant B-cell lines as well as EB virus negative LL cell lines, and (e) SN10 shows varying patterns of reactivity with the hyperplastic reactive lymph nodes.

Recently, SN10 was further tested for reactivity with concanavalin A activated T-cells. In this test, we included an anti-transferrin receptor mAb (OKT9) as a positive control as previously described (23). SN10 did not react significantly with either activated or preactivated T-cells, while OKT9 showed a strongly enhanced reactivity with the activated T-cells as compared to its reactivity with preactivated T-cells.

Characterization of the molecular properties, particularly amino acid sequence, of gp36 will be valuable for understanding the origin of the antigen. Currently, we are contemplating a study to isolate sufficient quantities of purified gp36 for determining the partial amino acid sequence of the antigen.

We would like to point out that mAb SN10 was generated using an unconventional approach, i.e., immunizing animals (mice) with an LL antigen preparation rather than intact LL cells. Previously, we developed a novel system for isolating immunologically active LL associated cell membrane antigen mixtures (14, 15). Using such antigen preparations isolated from T and non-T/non-B (immature B-lineage including pre-B) LL cells to immunize mice, we previously generated several mAbs directed to T or non-T LL associated cell surface antigens (16, 18, 19, 23, 46). In the present study, we extended these studies and prepared a B LL associated cell membrane antigen preparation from mononuclear cells derived from malignant spleens of two patients with B NHL (see “Materials and Methods”). SN10 was generated by immunizing mice with this antigen preparation.

Recently, several mAbs reactive with normal and malignant B-cells have been widely used for diagnosis and follow-up of LL (reviewed in Ref. 7). SN10 will be particularly useful for such purposes because it reacts strongly with many malignant cells and tissues of B-lineage but shows little reactivity with normal counterparts, i.e., normal peripheral blood B-cells and normal bone marrow B-cells. SN10 showed no significant reactivity with a variety of other normal cells and normal solid tissues tested. The normal cells include T-cells, granulocytes, monocytes, erythrocytes, and platelets. The normal solid tissues include epidermis, kidney, lung, and small intestine. Thus, SN10 shows a remarkably high tumor specificity.

SN10 may be useful for studying the tumorigenic mechanisms in LL. Another important application of antitumor mAbs will be utilization of mAbs as a specific delivery vehicle of a
cytotoxic agent(s) to tumor targets. However, only those mAbs which meet several fundamental criteria (see “Introduction” and below) may have potential for an effective delivery vehicle. These criteria include relatively high tumor specificity, high antigen binding affinity, high antigen density, effective internalization into the target cells, noninduction of down-regulation of antigen expression, and lack of circulating antigen in the plasma of patients (see “Introduction”). SN10 appears to meet these fundamental requirements to serve as an effective delivery vehicle.

As an initial test for the usefulness of SN10 for preparing immunoconjugates, SN10 was conjugated with RA, and the in vitro cytotoxic activity of SN10-RA was determined. SN10-RA killed LL cells effectively, whereas the same conjugate showed no cytotoxicity against control cells (Fig. 7). Thus, SN10 bound to the target antigen (gp36) on LL cells was effectively internalized into the cells. However, the binding of mAb SN10 to LL cells did not cause significant down-regulation of gp36 expression as assayed by our test procedure (Fig. 6; “Materials and Methods”).

Although RA conjugate of SN10 is effective in the in vitro killing of cultured LL cells, its activity is not particularly strong. However, it is likely that SN10-RA conjugates show stronger cytotoxic activity against fresh (uncultured) LL cells compared to the target antigen (gp36) on LL cells was effectively internalized into the cells. However, the binding of mAb SN10 to LL cells did not cause significant down-regulation of gp36 expression as assayed by our test procedure (Fig. 6; “Materials and Methods”).

Another important use of SN10 will be radioimmunodetection and radioimmunotherapy of NHL after SN10 is conjugated to an appropriate radioisotope because SN10 displays a remarkably high tumor specificity and is an IgG1 antibody. IgG1 antibodies show less nonspecific binding (e.g., binding to Fc receptors) than IgG2a, IgG3, and IgM antibodies.

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Monoclonal Antibody SN10 Which Shows a Highly Selective Reactivity with Human B Leukemia-Lymphoma and Is Effectively Internalized into Cells

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