Endocytosis and Degradation of Monoclonal Antibodies Targeting Human B-Cell Malignancies

Oliver W. Press, Andrew G. Farr, Ingrid Borroz, Susan K. Anderson, and Paul J. Martin


ABSTRACT

Seven murine monoclonal antibodies (MoAbs) recognizing differentiation antigens present on B-lymphocytes were analyzed in preclinical studies for clinical potential use for antibody-targeted therapy of B-cell malignancies. MoAbs HD37 (anti-CD19), IF5 (anti-CD20), HD6 (anti-CD22), MB-1 (anti-CD37), G28-5 (anti-CDw40), 7.2 (anti-class II), and DA4-4 (anti-IgM) were studied for their binding avidities, immunoreactivities, isotypes, endocytosis rates, degradation rates, and number of binding sites on Daudi cells. Lineweaver-Burke analyses of 125I-labeled MoAbs demonstrated immunoreactivities ranging from 59 to 92%. Scatchard analyses of 125I-MoAbs demonstrated that five of the antibodies had binding avidities in excess of 10^5 L/M, whereas MoAbs IF5 and HD37 had avidities of 3.4 × 10^3 L/M. CD20, CD37, μ, and HLA Class II were found to be highly expressed (200,000–400,000 binding sites/cell) on Daudi cells whereas CD19, CD22, and CD40 were less densely expressed (80,000–100,000 sites/cell). DA4-4 (μ), HD6 (CD22), and G28-5 (CDw40) were rapidly internalized by cells, HD37 (CD19) and MB-1 (CD37) underwent endocytosis at an intermediate rate, and 7.2 (class II) and IF5 (CD20) were internalized slowly. Trichloroacetic acid precipitation and high-performance liquid chromatography revealed the following relative rates of 125I-MoAb degradation: DA4-4 (μ) > HD6 (CD22) > HD37 (CD19) > G28-5 (CDw40) > MB-1 (CD37) > IF5 (CD20) > 7.2 (class II).

INTRODUCTION

The identification of tumor-associated antigens has provided an approach by which malignant cells might be eradicated with minimal effects on adjacent normal cells. Preliminary promising results have been obtained in both animal and human tumor models using unmodified MoAbs (1-3), radiolabeled antibodies (4, 5), and immunotoxins (6, 7). Malignancies of the B-lymphocyte lineage are ideal for evaluating the utility of antibody-targeted therapy because (α) B-cell surface antigens have been well characterized (8), (b) numerous high affinity B-cell specific antibodies are available, (c) human anti-mouse antibody formation is minimal in such patients (9), and (d) lymphomas have been shown to be sensitive test systems for new antineoplastic agents (10).

Criteria for selecting an antibody for immunon conjugate trials are poorly defined. Parameters believed to be important include the number of antigen sites per target cell, uniformity of antigen expression on all malignant cells, absence of binding to nontarget tissues, and the immunoreactivity, avidity, isotype, rate of internalization, intracellular routing, and rate of degradation of the targeted MoAb. In an attempt to critically evaluate the relative merits of a panel of MoAbs directed against different B-cell differentiation antigens, we have studied these parameters for MoAbs HD37 (anti-CD19), IF5 (anti-CD20), HD6 (anti-CD22), MB-1 (anti-CD37), G28-5 (anti-CDw40), DA4-4 (anti-IgM), and 7.2 (anti-class II) on a cell line (Daudi) derived from a patient with Burkitt's lymphoma.

MATERIALS AND METHODS

Cell Suspensions. Malignant B-cell (Daudi) lines were maintained in log-phase growth in RPMI 1640 supplemented with 12% FCS, 2 mM glutamine, and 1 mM pyruvate at 37°C in 5% carbon dioxide. Fresh lymphoma cells were obtained from diagnostic biopsies performed on patients with B-cell lymphomas at University Hospital, Seattle after obtaining informed consent. Single cell suspensions were made by mincing lymph node fragments and pressing the cells through wire screens. After removing cell aggregates by sedimentation, cells were stored in 10% dimethyl sulfoxide/90% FCS in liquid nitrogen until analyzed.

Antibodies. MoAbs used in these experiments included anti-HLA class II MoAb 7.2 (IgG3), anti-human IgM (μ chain) MoAb DA-4 (IgG1, from the American Type Culture Collection), anti-CD19 MoAb HD37, and anti-CD22 MoAb HD6 (both IgG2a, gifts from Dr. Jeffrey Ledbetter, University of Texas at Dallas, and Dr. Moldenhauer, German Cancer Research Center, Heidelberg), anti-CD20 MoAb IF5 and anti-CDw40 MoAb G28-5 (both IgG2a, gifts from Dr. Jeffrey Ledbetter, Oncogen Corporation, Seattle), anti-CD37 MoAb MB-1 (IgG2, gift from Dr. Ron Levy, Stanford University), and control MoAbs 9E8 (IgG2a), and MOPC-21 (IgG1). The seven pan-B MoAbs studied were chosen because of the reliability of their expression on human B-cell malignancies (Table 1). MoAbs were purified from ascitic fluid by affinity chromatography on Sepharose-Staphylococcal protein A (11) or by ion-exchange chromatography. Protein concentrations were determined using the BCA assay following the recommendations of the manufacturer (Pierce Chemical Company, Rockford, IL).

Radioiodination. MoAbs were labeled with 125I-Na by the Iodo-Gen method. MoAb (100 μg) was incubated with 0.5 mCi of 125I-Na in glass tubes coated with 10 μg of iodogen (Pierce Chemical Co., Rockford, IL) for 10 min at room temperature. Free 125I-Na was removed by chromatography on a Pharmacia PD-10 column (Pharmacia, Piscataway, NJ). Eluted fractions containing 125I-labeled antibody were pooled and stored at 4°C. The immunoreactivity (proportion of antibody molecules in a preparation which were capable of binding to antigen) and avidity of radiolabeled MoAbs were determined by modifications of previously described methods (12-14).

Determinations of Number of Cell Binding Sites. Two methods were used to estimate the number of binding sites for B MoAbs on Daudi cells. In the first method, serial dilutions of 125I-labeled MoAbs were bound to a fixed number of Daudi cells (200,000) at room temperature (22°C) for 2 h. Cell suspensions were then centrifuged and the number of bound and free cpm quantitated by gamma counting. The ratio of bound/free cpm was plotted versus the concentration of antibody, and the X-intercept was taken as the number of antigen binding sites per cell (12-14). Data were corrected for the immunoreactivity of the MoAb (see above) and for nonspecific binding using irrelevant cell lines and irrelevant 125I-labeled MoAbs as described by Badger et al. (13). Reanalysis of the data using the LIGAND program to model nonspecific binding as a fitted parameter yielded similar avidity constants (14).

In the second method, the numbers of cell surface binding sites were estimated by flow cytometry using fluorescent microbead standards.

Received 1/10/89; revised 4/5/89, 5/22/89; accepted 6/8/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.1This work was supported by NIH Grants CA-28149, CA-44991, and CA-41081 and by DOE Grant DE-FG06-88ER60719. During portions of this research, Dr. Press was the recipient of a First Independent Research and Training Award from the NIH (CA-46134).

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3The abbreviations used are: MoAb, monoclonal antibody; FCS, fetal calf serum; CAME, cytosine-arabinoside-conjugated goat anti-mouse immunoglobulin; IT, immunotoxin; SN, supernatant; TCA, trichloroacetic acid; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase.

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were harvested from microwells with cotton swabs. Cell pellets were
subjected to a second acid/papain wash. The two acid washes were
pooled and the radioactivity in the eluted $^{125}\text{I}$-MoAb was determined
as described. To facilitate comparison with the other antibodies, data for HD6 were normalized so that the
endocytosis assay. Daudi cells were washed in cold serum-free RPMI
1640 and pelleted by centrifugation. $^{125}\text{I}$-labeled MoAbs were added to
the cell pellets in a ratio of 1 ng protein (200,000 cpm) per 10$^6$ cells in
a volume of 0.5 ml. Jurkat cells or irrelevant MoAbs 9E8 or MOPC21
were used as controls to assess nonspecific binding. Cells were incubated with
$^{125}\text{I}$-MoAbs at 4°C for 1 h and then washed twice with cold RPMI
1640. Aliquots containing 1 x 10$^6$ cells were plated in 200 µl RPMI
1640 in microtiter plates (Flow Laboratories), warmed to 37°C (in a
huminified CO$_2$ incubator) for various time intervals, and then assayed
for radioactivity in the SN, surface membrane, and intracellular compo-
3.0, Sigma, St. Louis, MO) was conjugated to Fab’ fragments of
monovalent Fab’-HRP-GAMIg reagent was performed. HRP (RZ =
3.0, Sigma, St. Louis, MO) was conjugated to Fab fragments of
GAM Ig by the metaperiodate method (15, 17). Immunoperoxidase
degradation of the $^{125}\text{I}$-MoAbs
Assay. Daudi cells were stained to saturation with each of the
study antibodies, washed twice, stained with 1:20 FITC-GAMIg,
washed twice, and analyzed by flow cytometry. The fluorescence inten-
sity per cell was interpolated onto a standard fluorescence calibration
curve constructed using a Quantitative Fluorescein Microbead Standards Kit. Results were normalized using the fluorescence to protein (F/P)
ratio determined empirically for each MoAb using Simply Cellular
Microbeads (Flow Cytometry Standards Corp.) as described by the
manufacturer for indirect immunofluorescence.

Microfluorimetry. The binding of MoAbs to target cells was deter-
mixed by indirect immunofluorescence using a FITC-GAM Ig (TAGO
Inc., Burlingame, CA). Cells were incubated for 30 min on ice with
MoAbs at saturating concentrations, washed, incubated with a 1:20
dilution of FITC-GAM Ig, washed, and fixed with 1% paraformal-
dehyde. Cells were analyzed on a cell sorter (Facsar; Becton-Dickinson
Inc., Sunnyvale, CA).

immunoconjugate internalization (16).

RESULTS

Characterization of MoAbs. The binding avidities, immuno-
reactivities, and the number of surface binding sites for the
MoAbs on Daudi cells are summarized in Table 2. The anti-
odies segregated into two groups with respect to surface anti-
gen density: CD20, CD37, $\mu$, and HLA class II molecules were
highly expressed (200,000–400,000 binding sites/cell) whereas
CD19, CD22, and CDW40 were less densely expressed (80,000–
100,000 sites/cell). For comparison purposes, the expression of B-cell antigens on fresh lymphoma cells were quantified for five patients with low and intermediate grade B-cell lympho-
mas. Large numbers of binding sites were found for MoAbs
1F5 (CD20; 128,000 ± 33,000 sites) and MB-1 (CD37; 59,000
± 12,000 sites) with lower antigen densities for the other
MoAbs [DA4-4 ($\mu$), 50,000 ± 27,000 sites; 7.2 (class II), 28,000
± 6000 sites; G28-5 (CDW40), 19,000 ± 3000 sites; HD37

Table 1 Immunophenotypes of human B cell malignancies

<table>
<thead>
<tr>
<th>Antigen expression</th>
<th>Non-Hodgkin’s lymphoma</th>
<th>B-chronic lymphocytic leukemia</th>
<th>Hairy cell leukemia</th>
<th>Non-T, Non-B acute lymphocytic leukemia</th>
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<tbody>
<tr>
<td>CD19</td>
<td>352/419</td>
<td>123/125</td>
<td>18/30</td>
<td>314/323</td>
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<tr>
<td>CD20</td>
<td>401/441</td>
<td>85/92</td>
<td>23/23</td>
<td>20/46</td>
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<tr>
<td>CD22</td>
<td>327/421</td>
<td>21/42</td>
<td>6/6</td>
<td>3/11</td>
</tr>
<tr>
<td>CD37</td>
<td>212/228</td>
<td>52/67</td>
<td>11/11</td>
<td>3/15</td>
</tr>
<tr>
<td>CDw=40</td>
<td>22/23</td>
<td>24/31</td>
<td>4/4</td>
<td>N.A.</td>
</tr>
<tr>
<td>DR</td>
<td>427/438</td>
<td>50/50</td>
<td>7/9</td>
<td>323/325</td>
</tr>
<tr>
<td>Ig</td>
<td>336/349</td>
<td>115/137</td>
<td>26/27</td>
<td>0/35</td>
</tr>
</tbody>
</table>
| * Compiled from references (8, 19–26).
Table 2  Characterization of murine mAbs recognizing human B cell antigens

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen*</th>
<th>Isotype</th>
<th>Binding sites/cell*</th>
<th>Avidity* (L/M)</th>
<th>Immunoreactivity (%)a</th>
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<tbody>
<tr>
<td>DA4-4</td>
<td>IgG1</td>
<td>IgG1</td>
<td>330,772</td>
<td>4.34 x 10⁹</td>
<td>92</td>
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<tr>
<td>HD6</td>
<td>CD22</td>
<td>IgG1</td>
<td>86,683</td>
<td>2.88 x 10⁹</td>
<td>69</td>
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<tr>
<td>G28-5</td>
<td>CDw40</td>
<td>IgG2a</td>
<td>86,835</td>
<td>4.50 x 10⁹</td>
<td>86</td>
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<td>HD37</td>
<td>CD19</td>
<td>IgG1</td>
<td>91,131</td>
<td>0.30 x 10⁹</td>
<td>76</td>
</tr>
<tr>
<td>MB-1</td>
<td>CD37</td>
<td>IgG1</td>
<td>234,311</td>
<td>2.99 x 10⁹</td>
<td>92</td>
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<tr>
<td>IFS</td>
<td>CD20</td>
<td>IgG2a</td>
<td>306,097</td>
<td>0.37 x 10⁹</td>
<td>59</td>
</tr>
<tr>
<td>F5</td>
<td>Class II</td>
<td>IgG2b</td>
<td>393,163</td>
<td>1.71 x 10⁹</td>
<td>74</td>
</tr>
</tbody>
</table>

a  CD, cluster designation as assigned by the Third International Workshop on Monoclonal Antibodies (8).

b  Means of 5 experiments (two performed with radiolabeled antibodies and three with the fluorescent microbead method). There was good correlation between the results obtained with the two different types of assays. (Standard errors of the means were <15% except for DA4-4 [±27%] and HD37 [±30%].)

c  Avidities determined by Scatchard analyses of ¹²⁵I-MoAb binding to Daudi cells (14).

d  Immunoreactivity determined by Lineweaver-Burk analyses of ¹²⁵I-MoAb binding to Daudi cells. (Average of 4 experiments [Standard Errors ±8% except 7.2 = 15%].)

(CD19), 19,000 ± 200 sites; and HD6 (CD22), 8000 ± 4000 sites. All seven MoAbs bound to 90–100% of Daudi cells with narrow, unimodal peaks on flow cytometry. Binding sites were also fairly uniformly present on fresh lymphoma B-cells for MoAbs IFS (CD20), 7.2 (class II), and MB-1 (CD37) (with 97–100%, 85–100%, and 76–99%, respectively, of the B-cells from the patients expressing the relevant antigen). However, there was more variability in antigen expression for MoAbs DA4-4 (µ), HD6 (CD22), HD37 (CD19), and G28-5 (CDw40) with 20–92%, 52–89%, 77–89%, and 77–93%, respectively, of fresh lymphoma B-cells bearing the relevant antigens.

Endocytosis and Degradation of Pan-B MoAbs. MoAbs differed substantially in their behavior after cell binding. DA4-4 (µ), HD6 (CD22), and HD37 (CD19) were rapidly internalized by cells (Fig. 1, a, b, and d) resulting in the disappearance of radioactivity from the cell surface and appearance of label in the intracellular compartment. Peak levels of intracellular ¹²⁵I-MoAb were detected at 4 h of culture with subsequent declines attributable to degradation and exocytosis of ¹²⁵I-MoAbs (see below). SN radioactivity appeared rapidly with these antibodies and accounted for 70–80% of total culture cpm at 24 h. G28-5 (CDw40) was also rapidly internalized, but intracellular retention of this MoAb was more prolonged, with 40% of the label remaining in an intracellular compartment between 4 and 24 h, and with a plateau in SN cpm at 30% (Fig. 1c). MoAbs MB-1 (CD37), 7.2 (class II), and IFS (CD20) were characterized by more prolonged retention on the cell surface with slower intracellular accumulation of label (Fig. 1, e, f, and g).

Fig. 2 shows that ¹²⁵I-DA4-4 (µ) was the MoAb most extensively degraded by Daudi cells, with 40–50% of all culture radioactivity being TCA soluble after 24 h of culture. ¹²⁵I-IFS (CD20) and ¹²⁵I-7.2 (class II) showed the least degradation with <10% of the label being TCA-soluble after 24 h. These experiments demonstrated that the rapid disappearance of 40–45% of surface-bound IFS over the first 4 h of culture seen in Fig. 1f was primarily due to dissociation, a finding consistent with its low avidity.

Analysis of ¹²⁵I-MoAbs in Culture Supernatants using HPLC. Culture SNs were analyzed by HPLC using a size exclusion column to determine the size of molecules bearing ¹²⁵I. In cultures containing rapidly internalized and degraded antibodies, relatively little radioactivity in the SN was on intact IgG molecules after 24 h (e.g., 32% for ¹²⁵I-DA4-4 (anti-µ)). In contrast, the majority of SN cpm in cultures containing slowly degraded antibodies was present on intact IgG after 24 h of incubation (e.g., 67% for MoAb 7.2). HPLC confirmed the efficacy of TCA in precipitating intact ¹²⁵I-MoAb molecules; all TCA soluble radioactivity in SNs was present on molecular species ≤2,000 Daltons (data not shown).

Immunoelectron Microscopy of MoAb Internalization. Endocytosis and intracellular trafficking of MoAbs was demonstrated directly by immunoperoxidase electron microscopy. All cells showed circumferential labeling at time 0 [shown for MoAb HD6 (CD22) in Fig. 3a]. MoAbs DA4-4 (µ), HD-6
Fig. 3. Immunoperoxidase electron microscopy of Daudi cells labeled with MoAb HD6 (CD22). Cells were labeled with MoAb HD6 and peroxidase-conjugated Fab' -GAM Ig at 4°C before warming cells to 37°C to initiate endocytosis. At time 0, cells displayed circumferential surface staining (a) (bar, 1 μm). Within minutes of warming at 37°C extensive endocytosis of HD6 via "coated pits" (arrows in b and c) and "coated" vesicles (arrowhead in b) was apparent (bars, 0.1 μm). Within 30 min cells contained numerous peroxidase-labeled endocytic vesicles or "receptosomes" (arrows in d) and tubular cisternae (arrowheads in d) (bar, 0.5 μm). Between 2 and 18 h after warming to 37°C, progressive accumulation of peroxidase in lysosomes was observed (e) (bar, 0.5 μm).

(CD22), and G28-5 (CDw40) were rapidly internalized via coated pits (shown for MoAb HD6 (CD22) in Fig. 3, b and c), which subsequently pinched off, giving rise to labeled endocytic vesicles (Fig. 3d). Within 30 min at 37°C, 65-70% of cells treated with these three MoAbs had detectable label in endosomes (Fig. 4, a–c). Subsequently, there was a slowly progressive increase in the percentage of cells containing labeled lysosomes [up to 67% for DA4-4 (μ), 40% for HD6 (CD22), and 37% for G28-5 (CDw40) after 18 h] (Figs. 3e and 4) accompanied by a slight decline in the percentage of cells containing labeled endosomes. MoAbs 1F5 (CD20) and 7.2 (class II) were much more slowly internalized as demonstrated by the prolonged persistence of surface label and the more gradual appearance of peroxidase label in endosomes and lysosomes (>93% of cells retained surface label even after 18 h of culture (Fig. 4, f and g). Of note was the fact that cells labeled with MoAbs HD37 (CD19), MB-1 (CD37), 1F5 (CD20), and 7.2 (class II) showed progressive accumulation of label in the endosomes over the full 18-h time period studied (Fig. 4, d–g), in contrast to the plateau and decline seen with MoAbs DA4-4 (μ), HD6 (CD22), and G28-5 (CDw40) (Fig. 4, a–c). Furthermore, MoAbs DA4-4 (μ), HD6 (CD22), and G28-5 (CDw40) were found to enter a prominent tubular endocytic compartment (arrowheads in Fig. 3d), whereas the other four antibodies had very limited transport to this compartment (data not shown). These tubular cisternae were frequently seen in close proximity to the trans-Golgi apparatus, which has been reported to be important in translocation of toxins such as ricin and diphtheria toxin to the cytosol (27). The main Golgi apparatus itself was not prominently involved in the intracellular trafficking of any of the seven MoAbs studied.

It is important to note that the two assays used in Figs. 1 and 4 measure different parameters, and therefore the contours of the curves obtained differ. The radioimmunoassays depicted in Fig. 1 give a quantitative measure of the relative proportions of antibody on the cell surface, inside cells, and in the culture supernatant at various time points. For any given time in Fig. 1, the sum of the values in these three compartments is 100%. In contrast, the immunoelectron microscopy studies in Fig. 4 measure the percentage of cells at each time point which have any amount of antibody on their surface, in endosomes, and in lysosomes. This technique does not measure the relative distribution of antibody in the various compartments, hence, the values for time points later than time 0 in Fig. 4 do not add up to 100%. Thus, after 2 h of incubation, almost 50% of the 125I-DA4-4 cpm had been cleared from the surface membrane (Fig. 1a), but 90% of cells examined ultrastructurally still retained small amounts of surface bound antibody (Fig. 4a).
Fig. 4. Endocytosis of MoAbs by Daudi cells as determined by immunoelectron microscopy. Cells were treated with MoAbs and peroxidase-conjugated Fab'-GAMig at 4°C as described in the legend for Fig. 3. At 0, 0.5, 2, and 18 h cells were washed, fixed and processed for electron microscopy. Cells were scored for peroxidase-positive surface membranes (○), endocytic vesicles (△), and lysosomes (□) morphometrically.

Electron microscopy revealed that the density of surface label remaining on cells after 18 h varied greatly. MoAb DA4-4 (μ) was distributed in small surface patches (Fig. 5a), whereas cells labeled with MoAbs 1F5 (CD20) and 7.2 (class II) often showed dense circumferential distributions even after 18 h (Fig. 5b). The slow rate of internalization of MoAb 7.2 (Class II) by Daudi cells (Figs. 1 and 4) was quite a surprise, since it had previously been shown that normal B-cells spontaneously internalize Class II molecules (28), and we expected crosslinking with MoAb 7.2 to accelerate this process. However, MoAb 7.2 proved to be the slowest of the seven MoAbs cleared from the surface of Daudi cells. This finding may reflect rapid recycling of intact MoAb 7.2 back to the surface membrane after endocytosis (28), or slow modulation of class II antigen, which has been reported to occur with some malignant B-cells (29).

DISCUSSION

Several groups have assessed the merits of various pan-B antibodies for therapy of B-cell malignancies (1, 4, 5, 19, 30, 31), but MoAb selection has remained arbitrary because the relative importance of the various parameters influencing efficacy remains uncertain. This report compares the in vitro immunobiological properties of seven MoAbs which have potential clinical utility. Table 3 summarizes the salient features of the seven MoAbs studied. Since different MoAbs targeting the same surface antigen can vary greatly in their rates of endocytosis and subsequent intracellular routing (16, 32), caution should be exerted in extrapolating the findings reported for the MoAbs studied in this report to other MoAbs or to other cell types, even if the same surface antigen is targeted.

Of the parameters considered desirable for antibody-directed immunotherapy, high surface antigen density is perhaps least controversial since it permits a high concentration of effector molecules to be focused on target cells. High antibody avidity also appears desirable based on in vitro IT studies (31), though moderate or low avidity MoAbs may “percolate” deeper into solid tumor masses in vivo (33). High immunoreactivity may maximize targeting and minimize toxicity in IT and radioimmunotherapy trials, but MoAbs with moderately low immunoreactivity may suffice for unmodified antibody trials (3) since toxicity is minimal and dose escalation can compensate for a reduced immunoreactive fraction. Antibody isotype appears to be of primary importance for trials using unmodified antibodies, where the murine IgG1, and IgG2a isotypes are most effective at interacting with human effector cells (34, 35). Rapid MoAb internalization into target cells is deleterious for unmodified antibody trials [because interactions with effector cells are abrogated (36-38)], advantageous for immunotoxin studies [since the reagents must reach ribosomes (6)], and isotype-dependent for radioimmunoconjugate protocols [since intracellular dehal-
Endocytosis of $^{131}$I-MoAbs is detrimental (4), but intracellular localization of short acting isotopes (e.g., $\alpha$-emitters) is beneficial. Intracellular routing of ITs to the trans-Golgi reticulum (27) and prolonged retention in prelysosomal compartments may maximize IT efficacy (16, 27), whereas rapid delivery of ITs and radioimmunoconjugates to lysosomes may attenuate their clinical efficacy (16, 39).

Cross-reactivity of MoAbs with nonmalignant tissues increases the potential toxicity of immunotherapy, though temporary elimination of normal, circulating B-cells by pan-B MoAbs has been well tolerated (3, 4). Cross-reactivity of MoAbs with nonlymphoid cells or tissues (e.g., kidney, nerve axons) may lead to unsuspected toxicities (40), although some degree of normal tissue cross-reactivity is tolerable as shown by trials using anti-CD37, antiferritin, and anti-class II antibodies (4, 29, 41). Interestingly, cross-reactivity of anti-CDw40 antibodies such as G28-5 with carcinomas may actually increase their clinical utility (8).

Since many B-cell antigens are receptors for naturally occurring ligands (8, 19), it is possible that MoAbs targeting these molecules may induce or suppress differentiation, activation or mitogenesis of tumor B-cells. However, we have seen no convincing evidence to support this concern in the patients we have treated so far with anti-B-cell MoAbs (3, 4). Antigen shedding is an undesirable feature since circulating antigen impedes MoAb targeting to tumor sites (1) and may result in immune complex disease.

On the basis of the parameter analysis above, we speculate that MoAbs 1F5 (CD20) and MB-1 (CD37) may be well suited for in vitro applications as unmodified antibodies since they are cleared from the surface of cells less rapidly than HD6 (CD22), DA4-4 ($\mu$), and G28-5 (CDw40), have more surface binding sites than HD37 (CD19), HD6 (CD22), and G28-5 (CDw40), and will not cross-react with nontarget cells as extensively as MoAb 7.2 (class II). MoAb 1F5 (CD20) might be somewhat preferable for its higher specificity for B-cells, higher number of surface binding sites, slower endocytosis and degradation rates, and IgG2a isotype. Conversely, MoAb MB-1 (CD37) has a higher immunoreactivity (4) and possibly less chance of inducing activation and proliferation of B-cells (8).

Selection criteria for MoAbs used as carriers of drugs or toxins differ from those for antibodies used in unmodified form. Ideally, MoAbs to be used as drug or toxin conjugates should be efficiently delivered to target cells, rapidly internalized, and delivered to endocytic compartments [e.g., receptosomes or trans-Golgi reticulum (27)]. We would predict that HD6 (CD22) and G28-5 (CDw40) might make effective immunotoxins because of their rapid internalization by malignant B-cells. Anti-$\mu$ MoAbs (e.g., DA4-4) are less desirable for in vivo IT trials because of the high concentrations of IgM in blood, even though they are rapidly endocytosed by cells and make effective ITs in vitro. MoAb 1F5 (CD20) is poorly internalized, and would not be expected to make a good immunotoxin reagent.

MoAb MB-1 (CD37) is a good candidate for radioimmunotherapy trials because of its high avidity, high immunoreactivity, large number of surface binding sites, and intermediate rate of dehalogenation. MoAb 1F5 (CD20) may also suffice for radioimmunotherapy because of its slow dehalogenation and the high number of binding sites on B-cell tumors, though its low immunoreactivity is unfavorable. The rapid degradation rates for MoAbs DA4-4 ($\mu$) and HD6 (CD22) make these antibodies less attractive as candidates for trials using radiolabeled MoAbs. The reactivity of MoAb 7.2 (class II) with many non-B-cells is undesirable, though animal and human experiments suggest that this may not be an absolute impediment (5, 29).

These predictions have not yet been prospectively tested, but the available evidence is supportive for our hypotheses. MoAb 1F5 (CD20) in unmodified (or trace-labeled) form induced temporary responses in three of five lymphoma patients [one minor, one partial, and one complete response (3, 4)], MoAb HD6 (CD22) made a much more potent ricin A-chain IT than did HD37 (CD19) (32, 42), anti-CD20 MoAbs made totally ineffective ITs (43), and $^{131}$I-labeled MoAb MB-1 (CD37) induced major responses in all five patients with refractory lymphomas treated [four complete responses and one partial response (4)].

ACKNOWLEDGMENTS

We are grateful to Drs. E. Vitetta, J. Ledbetter, G. Moldenhauer, and R. Levy for providing antibodies, to Dr. E. Vitetta for reviewing the manuscript, and to Judy Groombridge and Liz Caldwell for expert technical assistance.

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Endocytosis and Degradation of Monoclonal Antibodies Targeting Human B-Cell Malignancies
