Radiolabeled Anti-CD45 Monoclonal Antibodies Target Lymphohematopoietic Tissue in the Macaque

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Despite bone marrow transplantation, many patients with advanced leukemia subsequently relapse. If an additional increment of radiation could be delivered to lymphohematopoietic tissues with relative specificity, the relapse rate may decrease without a marked increase in toxicity. We have examined the biodistribution of two 111I-labeled monoclonal antibodies reactive with the CD45 antigen in Macaca nemestrina. Three animals received 0.5 mg/kg BC8, an IgG1 of low avidity (8 x 10^7 L/mole). Three received 0.5 mg/kg AC8, an IgG2a of moderate avidity (5 x 10^8 L/mole), and two received 4.5 mg/kg AC8. Estimates of radiation absorbed dose demonstrated that these antibodies could deliver up to five times more radiation to lymph nodes, and up to 2.6 times more to bone marrow, than to lung or liver. The higher avidity AC8 antibody at 0.5 mg/kg was cleared more rapidly from blood and resulted in lower antibody uptake in lymph nodes than did BC8 at 0.5 mg/kg. Increasing the dose of AC8 to 4.5 mg/kg resulted in slower blood clearance and higher lymph node uptake. These studies suggest that radiolabeled anti-CD45 antibodies can deliver radiation with relative specificity to lymphohematopoietic tissues. This approach, in combination with marrow transplantation, may improve treatment of hematologic malignancies.

Hematologic malignancies are particularly radio-sensitive, and therefore represent ideal tumors for radiolabeled antibody therapy. While many patients with acute lymphocytic and myelogenous leukemia have been cured by bone marrow transplantation after total body irradiation (TBI) and chemotherapy, relapse remains the major cause of failure of this intensive therapy. In two separate randomized trials, it was shown that it is possible to decrease leukemia relapse rates by increasing the dose of TBI, suggesting that leukemia cells are susceptible to modest increases in radiation dose. However, dose-limiting toxicity of normal organs has prevented improvement in overall disease-free survival. Radiolabeled monoclonal antibodies (MoAbs) may be able to deliver an additional increment of therapy to sites of leukemic involvement in hematopoietic and lymphoid tissues while sparing the normal organs, particularly liver and lung, which are usually the site of dose-limiting toxicity.

Several studies have demonstrated the feasibility of treating lymphoma patients with MoAbs. Tumor-specific reagents such as anti-idiotypic antibodies have the potential disadvantage of failing to target variant antigen-negative cells unless they are immediately surrounded by antigen-positive cells. For certain currently used radionuclides, an effective radiation dose may not be delivered to isolated malignant cells, as such cells require binding of relatively high numbers of radiolabeled antibody molecules to ensure that an adequate number of decay events are directed to the nucleus. The largest clinical experience to date has been with more broadly reactive and readily available reagents, such as anti-CD37 or anti-CD20 antibodies that react with normal B cells and most B-cell lymphomas. Such reagents will target isolated antigen-negative or -negative tumor cells in areas of normal B-cell lymphoid tissue.

In patients with leukemia in remission, and in relapsed patients with subclinical involvement of extramedullary tissues such as lymph nodes, relatively little radiation may be delivered to tumor cells by a truly "leukemia-specific" radiolabeled MoAb. In either clinical situation, isolated malignant cells are surrounded by normal cells. Because the radiation from a radionuclide attached to an antibody molecule bound to the surface of a cell can be emitted in any direction within a geographic area defined by the path length of the radionuclide, the isolated malignant cell may receive a significantly greater absorbed dose if the surrounding normal cells are targeted as well. Thus, these patients may best be treated with an MoAb that is more broadly reactive with cells of lymphohematopoietic lineage. However, such an antibody may result in less specificity of radiation delivery with normal organs receiving a relatively higher dose, and the clinical feasibility of this approach has not been tested.

The CD45 antigen is expressed on virtually all hematopoietic cells, with the main exception of mature erythrocytes and platelets, and is expressed on 85% to 90% of acute myeloid and lymphoid leukemias (and Matthews DC, unpublished observations, September 1990). It is expressed by essentially all white blood cells (WBCs) in lymphoid tissue including spleen and lymph nodes. This antigen is maintained on the cell surface after ligand binding, avoiding the potential problem of deiodination of internalized iodinated antibody (Press OW, unpublished observations, December 1990). It is expressed in relatively high levels (approximately 200,000 copies on the average circulating mononuclear WBC). We have previously studied the biodistribution of radiolabeled MoAbs reactive with the CD45 antigen in mice and demonstrated that such antibodies can deliver radiation to tissues of hematopoietic and lymphoid tissue.

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origin with relative specificity (Matthews DC, unpublished observations, January 1989 through March 1991). Estimates of radiation absorbed dose in these experiments suggest that the marrow received two to three times more and the lymph nodes three to eight times more irradiation than the lungs, the normal organ receiving the highest dose.

In this study we have examined the ability of anti-CD45 antibodies to deliver radiation with relative specificity to lymphohematopoietic tissues in a primate species, *Macaca nemestrina*, by studying the biodistribution of two antibodies reactive with the CD45 antigen. Normal animals were infused with one of two 131I-labeled anti-CD45 antibodies that differ with respect to isotype and avidity for the antigen. The results of these studies indicate that relative specificity of radiation delivery to hematolymphoid tissues is achievable with radiolabeled anti-CD45 MoAb in normal nonhuman primates. Our findings also suggest that both antibody avidity and dose are important in determining the biodistribution of antibody and affect the relative antibody concentrations achievable in the various target lymphohematopoietic organs.

**MATERIALS AND METHODS**

*Animals.* Juvenile male *M. nemestrina* animals, between 4 and 6 years old (5.5 to 8.8 kg) were obtained from and housed at the Regional Primate Research Center at the University of Washington. They were maintained in accordance with guidelines of the Animal Care Committee of the University and those of the Committee on Care and Use of Laboratory Animals of the University.
Institute of Laboratory Animal Resources of the National Research Council.

MoAbs. Hybridoma cells secreting MoAb BC8, a murine IgG1 that recognizes the CD45 antigen of both humans and macaques, were the gift of Claudio Anasetti (Fred Hutchinson Cancer Research Center, Seattle, WA). Hybridoma cells secreting MoAb AC8, a murine IgG2a recognizing the CD45 antigen of nonhuman primates, were kindly provided by W. Michael Gallatin (Fred Hutchinson Cancer Research Center). 1A14 is a murine IgG2a that recognizes the murine Thy 1.1 antigen, and served as a negative control antibody for AC8. Cell lines were inoculated in traperitoneally in specific pathogen-free (SPF) mice for the production of ascites. Antibodies were purified from ascites by batch extraction and high-pressure liquid chromatography (HPLC) using ABX exchange resin (J.T. Baker, Phillipsburg, NJ). Fractions containing eluted antibody were pooled, concentrated, and dialyzed into phosphate-buffered saline (PBS, 40 mmol/L NaCl/8 mmol/L NaH_{2}PO_{4}/2.7 mmol/L KCl/1.5 mmol/L KH_{2}PO_{4}, pH 7.2) using a PM-10 ultrafiltration membrane (Amicon Corp, Danvers, MA). Concentrated antibody was aliquoted and stored at −70°C until use. Antibody concentration was determined using a modification of the BCA protein assay (Pierce Chemical Co, Rockford, IL) standardized with bovine serum albumin (Sigma, St Louis, MO). DT, a murine IgG1 reactive with an idiotypic determinant on a human lymphoma sample, provided by IDEC Pharmaceuticals Corporation (Mountain View, CA), was used as an isotype-matched negative control for BC8.

Iodination and characterization. MoAbs BC8 and AC8 were iodinated with Na^{111}I (specific activity 8.0 Ci/mg, New England Nuclear, Boston, MA; or to 50 Ci/mg, ICN, Irvine, CA), using the Chloramine-T method. Sodium thiosulfate and carrier NaI added after 5 minutes quenched the reaction. The labeled MoAb was separated from low molecular weight reactants by Sephadex G-10 column chromatography (Pharmacia, Piscataway, NJ). The isotype-matched negative control MoAbs DT and 1A14 were labeled with ^{125}I using the same technique. After pooling of the eluted fractions containing ^{125}I MoAb, the antibodies were tested for immunoreactivity (percent of counts able to bind at antigen excess) with a modified cell binding assay, and for avidity to viable macaque peripheral blood lymphocytes as described. Briefly, known quantities of antibody were diluted in tissue culture medium (RPMI-1640 and 1% bovine serum albumin) and incubated with 2 to 5 × 10^{6} cells in microcentrifuge tubes (Costar, Cambridge, MA) for 1 hour at room temperature. Cells were washed two times and bound radioactivity counted. Antibodies routinely retained more than 80% of their baseline immunoreactivity after iodination and were more than 98% pure as determined by cellulose acetate electrophoresis.

Antibody localization. The double isotope labeling method of Pressman was used along with external body scanning. Most animals received SSKI 1 to 2 drops/day starting 2 days before study. On day 0 the animals were anesthetized with Ketamine Hydrochloride (Parke-Davis, Morris Plains, NJ), placed under a gamma camera (GE 400AT gamma camera [GE, Milwaukee, WI] with a high energy collimator interfaced to a dedicated ADAC 3300 computer [ADAC, Milpitas, CA]), and infused with a mixture of ^{125}I-labeled anti-CD45 antibody (3 to 4 mCi) and ^{131}I-labeled control antibody (1 to 2 mCi). Three animals received 0.5 mg/kg ^{131}I-BC8 with 0.5 mg/kg ^{131}I-DT intravenously over 10 minutes; one of these was killed at 92 hours, and the other two at 45 hours. Three animals received 0.5 mg/kg ^{131}I-AC8 with 0.5 mg/kg ^{125}I-1A14 intravenously over 10 minutes; two were killed at 45 hours, and one at 92 hours. Two animals received 4.5 mg/kg ^{131}I-AC8 with 0.5 mg/kg ^{125}I-1A14 intravenously over 45 minutes, with one killed at 45 hours and one at 92 hours. All infusions were tolerated well, with the only observed side effect being mild shivering in one of the animals receiving 4.5 mg/kg AC8; this resolved at the end of the infusion and was not accompanied by respiratory distress.

^{131}I images were obtained with a GE 400AT gamma camera interfaced to a dedicated ADAC 3300 computer. Dynamic images at one frame per 5 minutes were collected for the first hour during and after the infusion, and static scans were performed at 1, 2, 21, and 45 hours and, in three animals, at 70 and 92 hours after infusion. Images acquired over liver, lung, marrow, axillary nodes, and heart (blood pool) were identified, and time-activity curves for these organs were generated using counts for regions of interest, corrected for decay.

Blood samples were drawn at hours 0 (end of infusion), 0.5, 1, 2, 21, and 45 (and 70 and 92 in three animals). Complete blood counts (CBC) were performed on most samples. Bone marrow biopsies using a Jamshidi needle (Baxter, Valencia, CA) were obtained at hours 21, 45. Inguinal lymph node biopsies were performed at hour 21 (or 45 for the 5-day animals). Five of the animals were euthanized with thiopental at hour 46, and three at hour 92. Organ weights for lung, liver, spleen, and kidney were obtained for all but the first animal. Core (50 to 300 mg) samples of these and other tissues were removed, blotted of excess blood, and weighed. These tissue and blood samples were counted in a multichannel gamma cell counter (Packard 5000 Autogamma Counter, Meriden, CT) for ^{131}I and ^{125}I contents. Data were corrected for decay of ^{125}I and ^{131}I and adjusted for crossover from the ^{131}I channel to ^{125}I. Results were expressed as percent of injected dose per gram of tissue based on comparison with a standard aliquot of the infusion dose in each animal. Biodistribution curves for organs of interest were determined by correlating the time-activity curves generated from the

![Fig 2. Concentration of radiolabeled antibodies in tissues (±SEM), expressed as percent of injected dose per gram, for the two monkeys in each group receiving the 0.5 mg/kg dose of BC8 or AC8 which were necropsied at 45 hours. (A) Anti-CD45 antibodies. (B) Negative control antibodies.](from www.bloodjournal.org)
gamma camera scans with the absolute percent injected dose per gram tissue obtained by sampling and counting tissue.

Radiation dosimetry. The time-activity curves were used to estimate organ residence times and integral absorbed doses to selected tissues. The long-term (infinite-time) component to the time-activity curves was represented by an exponential function, estimated by least-squares regression.

Absorbed doses (rad per millicurie administered) to organs and tissues were estimated from the residence times, taking into account the contributions due to both penetrating photon and nonpenetrating beta radiation components of radioiodine emissions. The cross-organ photon absorbed doses were estimated by the MIRD (Medical Internal Radiation Dose Committee of the Society of Nuclear Medicine) technique; the organ models used to represent the macaque (5.5 to 8.8 kg) were the MIRD 1 year (9.7 kg) and newborn (3.6 kg) human pediatric models. Masses of each organ or estimated organ masses for macaques of specified weight were used, and absorbed doses were calculated by interpolation using the pediatric human models.

Estimates of absorbed dose to marrow involved the assumption that the total mass of biopsy specimens consisted of 50% hematopoietic tissue and 50% bony trabeculae and fat.

Fluorescence-activated cell sorter (FACS) analysis of bound antibody. The FACS II (Becton-Dickinson, Mountain View, CA) was used to determine antigen and bound antibody on target cells obtained both before and after infusions. Samples included peripheral blood leukocytes obtained before MoAb infusion and at 2, 21, and 45 hours, and in some cases at 92 hours. Cell suspensions teased from lymph nodes obtained at 21 and/or 45 hour biopsies, and cell suspensions of tissues obtained at autopsy were also studied, including a “peripheral” node (femoral) and a “central” node (mesenteric). For some animals, cell suspensions of spleen and marrow were also analyzed.

Samples (5 x 10^6 cells) were incubated for 30 minutes at 4°C with AC8 or BC8 at supersaturating concentrations (40 μg/mL) in staining medium (PBS with 2% fetal calf serum, 2% human AB serum, and 0.1% sodium azide), or with staining medium only. After three washes, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated goat F(ab')2 fragments specific for murine IgG and IgM (Tago, Inc, Burlingame, CA) at a ratio of 1:50 in staining medium for 20 minutes. After two washes, cells were resuspended in 250 μL of staining medium or fixative (1% paraformaldehyde in PBS) and analyzed with a FACS II calibrated with fixed chicken erythrocytes for forward and 90° scatter. Cells were analyzed for forward scatter, 90° scatter, and green fluorescence after gating out small events (red blood cells, platelets) on forward scatter. The preinfusion peripheral blood leukocytes

Fig 3. Anterior gamma camera images obtained 45 hours after infusion of radiolabeled antibodies. (A) After BC8 0.5 mg/kg (macaque No. 1). (B) After AC8 0.5 mg/kg (macaque No. 4). a, Axillary nodes; f, distal femur; h, humeral head; i, inguinal nodes overlying femoral head; t, proximal tibia.
incubated with second stage only (ie, no specific antibody for first stage) were used as a negative control.

**Autoradiography.** Small pieces of lymph nodes, spleen, and thymus from autopsies and biopsies were quick-frozen in OCT compound (Miles Laboratories, Naperville, IL) by immersion in alcohol supercooled by dry ice. Tissue sections (6 to 8 μm) on slides precoated with albumin were dipped in a 2.5:1 NTB-2 photographic emulsion (Eastman Kodak, Rochester, NY):0.33% Dreft detergent (Proctor and Gamble, Cincinnati, OH) using an automated dipper (Pelco 33000; Ted Pella Inc, Redding, CA). The slides were incubated for 1 week to obtain emulsion tracts from the I^131 activity, and a matching set of slides was incubated for 2 weeks starting 6 weeks after necropsy, for tracks from the I^131 activity, then developed using Dektol developer (Eastman Kodak), fixed for 10 minutes and counterstained with water-sensitive hematoxylin and eosin.

**RESULTS**

**Cell binding.** The results of antibody immunoreactivity and avidity studies of BC8 and AC8 with macaque and human peripheral blood lymphocytes (PBLs) are shown in Table 1. BC8 displayed an association constant (avidity) of 6 x 10^7 (L/mol) for macaque cells and 5 x 10^6 (L/mol) for human cells, with the number of molecules bound per cell being very similar (2 x 10^6) between species. For AC8 the association constant was 5 x 10^7 (L/mol) for macaque cells, and the number of molecules bound per cell 2 x 10^6. Thus, the avidity of AC8 for macaque cells was almost 10-fold greater than that of BC8 for macaque cells, and was approximately equivalent to the avidity of BC8 for human cells.

**Biodistribution in normal macaques.** Blood clearance curves for both the specific and nonspecific antibodies following infusion of 0.5 mg/kg BC8 (three animals), 0.5 mg/kg AC8 (three animals), or 4.5 mg/kg AC8 (two animals) are shown in Fig 1, A and B. Mean blood levels of the two negative control antibodies DT (n = 3) and 1A14 (n = 5) did not differ, and their clearance was predictably slower than for either specific antibody. After infusion of 0.5 mg/kg of specific antibody, blood clearance was biphasic with a shorter initial t½ for the higher avidity AC8 antibody (0.6 ± 0.1 hours) than for the less avid BC8 (2.7 ± 0.31 hours) (P = .05 by Wilcoxon Rank Sum Test). Both antibodies had a similar second phase t½ (56.5 ± 25.4 hours, 50.2 ± 16.4 hours, and 51.4 ± 20.2 hours for DT, 1A14, and BC8, respectively).

**Fig 4.** Time-activity curves (± SD) for organs of interest, expressed as percent of injected dose per gram tissue. Antibodies are BC8 0.5 mg/kg (---) (n = 3), AC8 0.5 mg/kg (-----) (n = 3), and AC8 4.5 mg/kg (-----) (n = 2). Data for the 70- and 92-hour time points reflect one animal in each group.
Table 2. Estimated Radiation Absorbed Dose (rad/mCi) in Macaque With ^131I-Labeled Anti-CD45 Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Animal</th>
<th>Organ</th>
<th>MNo.1</th>
<th>MNo.2</th>
<th>MNo.6</th>
<th>Average</th>
<th>SD</th>
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<tbody>
<tr>
<td>BC8 0.5 mg/kg</td>
<td>M</td>
<td>Marrow</td>
<td>47.8</td>
<td>91.3</td>
<td>30.3</td>
<td>56.5</td>
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<td>102.0</td>
<td>126.0</td>
<td>36.5</td>
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<td></td>
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<td>Spleen</td>
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<td>226.0</td>
<td>175.0</td>
<td>174.3</td>
<td>52.0</td>
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<td></td>
<td></td>
<td>Liver</td>
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<td>24.8</td>
<td>15.2</td>
<td>19.8</td>
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<td></td>
<td></td>
<td>Kidney</td>
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<td>11.1</td>
<td>11.9</td>
<td>2.3</td>
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<td></td>
<td></td>
<td>Lung</td>
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<td>32.4</td>
<td>15.1</td>
<td>25.2</td>
<td>9.0</td>
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<td>82.1</td>
<td>60.8</td>
<td>64.5</td>
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<td>224.0</td>
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<td>8.3</td>
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<td>Lung</td>
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<td>57.2</td>
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36.2 ± 13.9 hours). These differences may reflect the more rapid specific uptake of the higher avidity AC8 by cells to which it has ready access, such as circulating leukocytes, and cells in the spleen and bone marrow. In contrast, the higher (4.5 mg/kg) dose of AC8 resulted in a much longer early t½ (10.2 ± 0.2 hours), presumably as a result of saturation of antigen on the most accessible cells.

The biodistribution data as percent injected dose per gram of the radiolabeled anti-CD45 antibodies are presented in Fig 2A (with corresponding nonspecific control antibody data in Fig 2B) for the four animals receiving low-dose BC8 or AC8 and autopsied 45 hours after infusion. The concentration of radiolabel was highest in spleen and nodes with low-dose BC8, while low-dose AC8 resulted in higher radiolabel concentrations in spleen and almost equal levels in nodes and marrow. There was some degree of specific localization to liver and lung, most likely reflecting binding to tissue macrophages. The sequestration of circulating, antibody-coated cells in liver and lung could also account for some of this activity because there was a moderate (30% to 40%) decrease in circulating WBCs at 1 hour after the infusion compared to preinfusion count, with no obvious differences between antibodies or doses (data not shown). Despite some uptake in the liver and lung, greater amounts of relative binding was seen in lymphohematopoietic tissue compared with other organs. Neither antibody was found in significant amounts in the thymus.

The specific uptake and retention of radioiodine in lymph nodes, spleen, and marrow is shown in the gamma camera images reproduced in Fig 3. Figure 3A shows axillary nodes at 45 hours in a macaque receiving low-dose BC8. Figure 3B shows the better marrow visualization with less obvious node activity 45 hours after infusion of low-dose AC8.

Time-activity curves for organs of interest are shown in Fig 4. The spleen and nodes had similar uptake with lower avidity antibody BC8 at 0.5 mg/kg, with relatively slow clearance of the bound antibody. However, with low-dose AC8 there was very high and prompt splenic uptake of antibody, with the level in spleen almost a log greater than that in the lymph nodes. The high uptake of AC8 by the large pool of antigen-positive cells in the spleen resulted in a rapid decrease of the circulating level of antibody. There was a significantly lower uptake of low-dose AC8, as compared with BC8, in lymph nodes (P = .05 by Wilcoxon Rank Sum test for the difference in lymph node antibody concentration between the 0.5 mg/kg dose of BC8 and

Fig 5. Flow cytometric analysis of peripheral blood leukocytes for presence of bound antibody and for expression of CD45 antigen after infusion of BC8 0.5 mg/kg, AC8 0.5 mg/kg, or AC8 4.5 mg/kg. Histograms are of fluorescence on a five-log scale. Samples are preinfusion peripheral blood leukocytes incubated with FITC-conjugated GAMlgG + M alone (-----), and 92-hour postinfusion peripheral blood leukocytes incubated with FITC-conjugated GAMlgG + M alone (-----), or with FITC-conjugated GAMlgG + M after incubation with additional BC8 or AC8 (----).
AC8). This difference in antibody uptake in lymph nodes may be explained by the rapid clearance of the AC8 antibody from circulation. Lymph node lymphocytes presumably were less accessible to circulating antibody than were splenocytes, and therefore required prolonged exposure to a sustained level of antibody in the blood to achieve high uptake of antibody. Such a sustained level of circulating antibody was seen with the 0.5 mg/kg dose of BC8 (which was cleared more slowly from circulation than the 0.5 mg/kg dose of AC8). When the dose of AC8 was increased to 4.5 mg/kg, resulting in a prolonged higher blood level, the relative percent injected dose per gram in the spleen decreased, and the percent injected dose per gram in nodes increased to the level seen with low-dose BC8.

For marrow, the highest initial percent injected dose/gram was seen with low-dose AC8. Both low-dose BC8 and high-dose AC8 resulted in similar, relatively lower levels at early time points. The levels at 45 hours were not appreciably different between antibodies or doses. The liver and lung time-activity curves were similar for both antibodies, without a major effect of dose of AC8.

Radiation absorbed dose estimates. The estimated radiation absorbed doses received by target and critical normal organs with each of the three antibody regimens, expressed in units of rad per millicurie $^{131}$I, are given in Table 2. In each case the lung was the normal organ estimated to receive the highest absorbed radiation dose per millicurie administered. The lower avidity antibody BC8 at 0.5 mg/kg delivered fivefold more absorbed dose to nodes, and two-fold more absorbed dose to marrow, as compared with lung. Low-dose AC8 delivered a similar absorbed dose to marrow, but a significantly lower dose to nodes ($P = .05$ by Wilcoxon Rank Sum test), but 1.3-fold more than lung. When the dose of AC8 was increased to 4.5 mg/kg, the dose to nodes increased markedly, achieving a four to one ratio compared with lung, while the marrow to lung ratio fell slightly.

FACS analysis of in vivo bound antibody. Flow microfluorimetry of blood, lymph nodes, and in some cases spleen and marrow was used to determine the extent of antibody binding (ie, saturating vs nonsaturating), its pattern (homogeneous vs heterogeneous) as well as the effect of antibody administration on CD45 expression. The CD45 antigen did not modulate on binding with either BC8 or AC8 in vivo, because expression of CD45 determined by incubation of cells with supersaturating amounts of additional BC8 or AC8 resulted in the same intensity of fluorescent signal as seen with the preinfusion sample.

Samples of peripheral blood leukocytes obtained 2 hours after antibody infusion showed virtual saturation of antigen sites with administered antibody at all doses (data not shown). For both low-dose BC8 and AC8 the levels of bound antibody decreased slowly thereafter, although appreciable amounts of bound antibody were still present at 92 hours (Fig 5). After administration of high-dose AC8, the levels of bound antibody on circulating leukocytes were appreciably higher at 92 hours.

Differences between low-dose BC8, low-dose AC8, and high-dose AC8 were seen in the pattern of bound antibody in lymph nodes at all time points and node sites (peripheral and central) studied. As shown in Fig 6, the administration of low-dose BC8 resulted in homogeneous but nonsaturating antibody distribution on lymphocytes; in most nodes, more than 85% of cells demonstrated bound antibody. The 0.5 mg/kg dose of the higher avidity AC8 antibody resulted in a very heterogeneous staining pattern, with 8% to 16% of cells having nearly saturating levels of surface antibody while the remaining lymphocytes had little or no bound antibody. High-dose AC8 saturated CD45 sites on virtually all of the lymphocytes in the nodes.

The pattern of antibody distribution in the spleen also differed between antibodies and doses (Fig 7). Low-dose BC8 again produced a homogeneous, nonsaturating distribution of bound antibody, while with low-dose AC8 there were two populations of cells: a small population that was almost saturated, and a larger population with a small amount of antibody on the cell surface. High-dose AC8 saturated antigen sites on all splenocytes. High-dose AC8 also saturated the marrow leukocytes (Fig 8), with low-dose

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**Fig 6. Flow cytometric analysis of femoral node lymphocytes for presence of bound antibody and for expression of CD45 antigen 45 hours after infusion of BC8 0.5 mg/kg, AC8 0.5 mg/kg, or AC8 4.5 mg/kg. Histograms are of fluorescence on a five-log scale. Samples are preinfusion peripheral blood leukocytes (negative control) incubated with FITC-conjugated GAMlgG + M alone (--), and 45 hour postinfusion lymphocytes incubated with FITC-conjugated GAMlgG + M after incubation with additional BC8 or AC8 (-----).**
TARGETED LYMPHOCYTIC IRRADIATION

A BC8 0.5 mg/kg

B AC8 0.5 mg/kg

C AC8 4.5 mg/kg

Fig 7. Flow cytometric analysis of splenocytes for presence of bound antibody and for expression of CD45 antigen 92 hours after infusion of BC8 0.5 mg/kg, AC8 0.5 mg/kg, or AC8 4.5 mg/kg. Histograms are of fluorescence on a five-log scale. Samples are preinfusion peripheral blood leukocytes (negative control) incubated with FITC-conjugated GAMlgG + M alone (-----), and 92-hour postinfusion splenocytes incubated with FITC-conjugated GAMlgG + M alone (--), or with FITC-conjugated GAMlgG + M after incubation with additional BC8 or AC8 (--).

BC8 resulting in fairly homogeneous but lower absolute levels of cell-bound antibody. Low-dose AC8 marrow distribution was also relatively homogeneous, with lower levels of bound antibody than the 4.5 mg/kg dose of AC8.

 Autoradiography. The differences in microscopic lymph node antibody localization between low-dose BC8, low-dose AC8, and high-dose AC8 are shown in Fig 9. Radiiodine in the node from a low-dose BC8 animal was distributed almost homogeneously throughout the node, although the track emulsion grains appeared slightly less dense over the germinal centers, which may reflect in part the greater cell diameters (and therefore less surface area/tissue volume) of the germinal center B-cell "blasts." The radiiodine appeared far more scattered and patchy in the low-dose AC8 node, with no discernible pattern to its distribution. Specifically, the antibody did not appear localized to perivascular or perilymphatic areas, as might have been expected from the FACS analysis that showed a small percentage of saturated cells and the majority of cells with little or no bound antibody. High-dose AC8 resulted in a homogeneous distribution of radionuclide. The differences in splenic microdistribution were similar, although not as easily appreciated given the anatomy of the spleen (data not shown). Thymuses contained very little activity, consistent with their uniformly low uptake as determined by percent injected dose per gram tissue.

DISCUSSION

These studies have demonstrated the ability of radiolabeled MoAbs reactive with the CD45 antigen to deliver radiation relatively specifically to hematopoietic and lymphoid tissues. Our results support the feasibility of using such a broadly reactive reagent to direct radiation to these organs in patients with hematologic malignancies.

The biodistribution of two antibodies of different isotype and avidity for the target antigen was studied. Both antibodies were found to localize to hematopoietic and lymphoid tissue, although with some differences in relative specificity of distribution. In particular, higher concentrations of the

Fig 8. Flow cytometric analysis of bone marrow nucleated cells for presence of bound antibody and for expression of CD45 antigen 92 hours after infusion of BC8 0.5 mg/kg, AC8 0.5 mg/kg, or AC8 4.5 mg/kg. Histograms are of fluorescence on a five-log scale. Samples are preinfusion peripheral blood leukocytes (negative control) incubated with FITC-conjugated GAMlgG + M alone (-----), and 92-hour postinfusion marrow nucleated cells incubated with FITC-conjugated GAMlgG + M alone (--), or with FITC-conjugated GAMlgG + M after incubation with additional BC8 or AC8 (--).
lower avidity IgG1 antibody BC8 were reached in lymph nodes compared with the IgG2a antibody AC8 when administered at a dose of 0.5 mg/kg. Not only did the lower avidity BC8 antibody achieve a higher percent injected dose per gram in lymph nodes than did AC8, but its distribution was far more homogeneous in nodes. Only when AC8 was administered at a high enough dose to achieve a sustained serum level did its relative concentration in nodes match that of the lower avidity antibody BC8. This dose also produced homogeneous antibody distribution at saturating levels.

As these two antibodies differ with respect to both isotype and avidity, either or both of these factors could contribute to the observed differences in distribution. However, there was no difference in the blood clearance or in tissue localization between negative control antibodies of each isotype. Therefore, the difference in clearance of the two anti-CD45 antibodies was likely due to differences in their avidities, with the higher avidity AC8 antibody being cleared most rapidly because of its avid binding to accessible antigen positive cells (particularly circulating cells, splenocytes, and marrow cells). Thus, by 2 hours after
infusion, there was a fivefold greater amount of the low-avidity BC8 in the circulation than AC8.

We postulate that the slower clearance of BC8 compared with AC8 was a result of its lower avidity, with the higher avidity antibody binding rapidly to circulating cells. However, it is possible that cells with surface-bound AC8 (IgG2a) are more rapidly cleared by Fc receptor bearing cells in spleen, liver, and lymph nodes than those with BC8 (IgG1) on their surface, in part accounting for these differences in clearance rate. Nonetheless, the sustained level of circulating BC8 antibody may well have led to the higher concentration over time in lymph nodes compared with AC8. For well-vascularized tissues such as the spleen and marrow, antibody uptake is presumably prompt, and prolonged exposure to high blood levels of antibody may not be necessary to allow binding of antibody to the majority of antigen-positive cells. For tissues such as lymph nodes, where many cells are several cell diameters distant from the capillary lining, it may be important to maintain higher circulating levels of antibody so that a continuous concentration gradient remains to allow diffusion of antibody to the most target cells. The lack of localization of either antibody to the thymus is consistent with other studies of the biodistribution of anti-T-cell antibodies, and suggests the potential of a relative “blood-thymus” barrier limiting access of the relatively large antibody molecules to the antigen-positive cells in this organ. It is possible that smaller molecules such as F(ab’), or F(ab’) antibody fragments would lead to higher concentrations of antibody in this tissue.

Even more striking than the difference in overall lymph node content of infused antibody was the marked heterogeneity of antibody distribution in lymph node cells with AC8, the more rapidly cleared and higher avidity antibody. Fujimori et al have argued that antibodies of the highest avidity are least likely to distribute homogeneously through a mass of tissue because they bind avidly to the first cells encountered after diffusion into the parenchyma from the circulating blood. This concept is supported by the FACS analysis of bound antibody in animals infused with the 0.5 mg/kg dose of AC8, demonstrating near saturation of 8% to 16% of nodal lymphocytes with the remaining cells having little to no bound antibody. Alternatively, the heterogeneity could reflect the significantly lower circulating blood levels of antibody, providing a lower “driving force” to push antibody further into the tissue. In support of this, we found that animals receiving an almost 10-fold higher dose of the same AC8 antibody had higher sustained blood levels and all cells in the lymph node were saturated with antibody. While increasing the dose of this higher avidity antibody can overcome inhomogeneous distribution in lymph nodes, it may supersaturate cells in marrow resulting in decreased relative uptake. Thus, the ideal antibody dose for targeting both marrow and lymph nodes may be somewhere between 0.5 and 4.5 mg/kg. It should be noted that for a radionuclide of relatively long path length, such as I131, the irregular distribution seen with the higher avidity antibody may be of little clinical significance, and does not provide a clear indication of the local absorbed dose at the multicellular level. However, such differences could be critical for a radionuclide of shorter path length or for an immunotoxin.

Overall, despite differences in the biodistribution of the two anti-CD45 antibodies that we studied, results with both support this approach as a promising means of delivering radiation to all hematopoietic tissues with relative sparing of other, nonhematopoietic tissues. We have also examined the biodistribution of two rat antimurine CD45 antibodies in normal mice, and confirmed the finding that radiolabeled anti-CD45 antibodies can radiate lymphohematopoietic....
tissues with relative selectivity in rodents as well as in primates (Matthews DC, unpublished observations, January 1989 through March 1991). In these murine studies, estimates of radiation absorbed dose calculated from groups of animals receiving antibody doses between 0.2 and 4 mg/kg suggested that lymph nodes would receive 3- to 8-fold more, the bone marrow 2- to 3-fold more, and the spleen 5- to 16-fold more irradiation than would the lung. These “therapeutic ratios” of radiation received by target organs, as compared with the critical normal organ receiving the highest dose, were similar to those seen in the macaque studies.

Radiolabeled anti-CD45 MoAbs thus are novel reagents that can be used to deliver radiation relatively selectively to hematopoietic and lymphoid organs. This approach may improve treatment of hematologic malignancies, in combination with marrow transplantation, and may provide a novel strategy for achieving marrow ablation and immunosuppression when marrow transplantation is used as a method for treating genetic diseases.

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Radiolabeled anti-CD45 monoclonal antibodies target lymphohematopoietic tissue in the macaque

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