Therapy with Unlabeled and $^{131}I$-labeled Pan-B-Cell Monoclonal Antibodies in Nude Mice Bearing Raji Burkitt’s Lymphoma Xenografts

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ABSTRACT

Clinical trials of radioimmunotherapy (RIT) of lymphoma have produced frequent tumor regressions and remissions, but it has been difficult to determine to what extent these tumor responses have been due to antibody-specific targeted radiation, nontargeted radiation, and/or cytotoxicity mediated by the carrier monoclonal antibody (MoAb). In this report, RIT was studied in athymic nude mice bearing s.c. Raji human Burkitt’s lymphoma xenografts using two different pan-B-cell MoAbs, MB-1 (anti-CD37) and anti-B1 (anti-CD20), which differ in isotype (and thus the potential for interaction with host effector mechanisms) and isotype-matched control antibodies either in the unlabeled state or labeled with $^{131}I$. When a single i.p. injection of 300 $\mu$Ci $^{131}I$-labeled MB-1 (IgG1) was compared to treatment with unlabeled MB-1 or 300 $\mu$Ci $^{131}I$-labeled MYS control IgG1 MoAb, an antibody-specific targeted radiation effect of RIT was seen. $^{131}I$-labeled MB-1 produced a 44 ± 19% (SEM) reduction in tumor size at 3 weeks posttreatment, while unlabeled MB-1 or 300 $\mu$Ci $^{131}I$-labeled MYS control IgG1 antibody treatment resulted in continued tumor growth over this period of time. In vitro studies demonstrated that MB-1 was incapable of mediating antibody-dependent cellular cytotoxicity using Raji tumor cell targets and human peripheral blood mononuclear cells.

Similar to the MB-1 studies, treatment with 300 $\mu$Ci $^{131}I$-labeled anti-B1 produced a 64% reduction in mean tumor size, while 300 $\mu$Ci of control antibody resulted in a 58% increase in tumor size over the same 3-week period. In contrast to MB-1, however, unlabeled anti-B1 (an IgG2a MoAb which in vitro studies showed to be capable of antibody-dependent cellular cytotoxicity) also had a substantial antitumor effect. Indeed, 300 $\mu$Ci $^{131}I$-labeled anti-B1 and unlabeled anti-B1 treatment (using an equivalent amount of total protein in the treatment dose) produced a similar specific reduction in tumor size. Increasing the radioisotope dose of anti-B1 to 450 $\mu$Ci in another experiment did not produce a significant difference in tumor regression compared to a 300-$\mu$Ci dose.

These results suggest that the antitumor effects of $^{131}I$-labeled anti-B1 treatment were dominated by antibody-mediated cytotoxicity mechanisms, such that an antibody-specific targeted radiation effect could not be distinguished. In contrast, antibody-specific targeting of radiation was the dominant mechanism of tumor killing with $^{131}I$-labeled MB-1. These results underscore the importance of investigating non-radiation-related antibody effects as well as radiation effects in ongoing lymphoma RIT trials with pan-B-cell antibodies.

INTRODUCTION

RIT\(^3\) using MoAbs which recognize tumor-associated antigens as a means of targeting cytotoxic radionuclides to tumor cells appears particularly promising for the treatment of the highly radiosensitive lymphatic malignancies. Although tumor responses and remissions have been reported in several clinical trials of RIT in lymphoma (1–8), it has been difficult to determine the relative contributions of various potential mechanisms involved in the observed tumor destruction. These mechanisms include cytotoxicity mediated by antibody-specific targeted radiation versus nonspecifically delivered or nontargeted radiation, and/or effects of the antibody itself. Included in the radiation-associated mechanisms is the potential deposition of cytotoxic radionuclides on tumor cells not targeted by antibody either because of lack of accessibility of the tumor cells to the antibody or because the tumor cells lack target antigen. The antibody-associated mechanisms may involve direct antiproliferative effects on tumor cells through an interaction with a target antigen or indirect Fc-related interactions with host effector mechanisms (such as ADCC or complement-mediated cytolysis) (9–15). A more complete knowledge of the mechanisms of these differing means of tumor cell killing and optimization of these mechanisms will be important to the future success of RIT of lymphomas and other malignancies.

We have recently been studying two different MoAbs, MB-1 and anti-B1, labeled with $^{131}I$ in clinical RIT trials in B-cell lymphoma patients and have observed significant tumor responses with both radiolabeled antibodies (6, 16). These antibodies recognize different surface antigens present on normal and malignant B-cells. MB-1 recognizes the CD37 antigen, while anti-B1 recognizes the CD20 antigen. They also differ in their isotype. MB-1 is of the IgG1 subclass, while anti-B1 is of the IgG2a subclass. These differences raise the potential of different relative contributions to tumor destruction via antibody-mediated antitumor effects between the two antibodies. While animal models have their limitations, they offer the opportunity to study RIT under controlled conditions difficult to replicate in human studies. To further examine the mechanisms of RIT-induced antitumor effects produced by the above two antibodies in our clinical studies, we studied these antibodies in a preclinical athymic nude mouse model in which we have previously demonstrated specific targeting of MB-1 and anti-B1 to xenografted human B-cell lymphomas (17–21). In this report we show that the antitumor effects of $^{131}I$-labeled MB-1 appeared to be mainly due to antibody-specific targeted radiation. In contrast, the antitumor effects of $^{131}I$-labeled anti-B1 appeared to be mainly due to antibody-mediated cytotoxicity mechanisms. These results emphasize the potentially important role of non-radiation-related antibody effects in tumor responses in clinical RIT trials and the necessity of studying such effects in such trials utilizing different MoAbs.

MATERIALS AND METHODS

Monoclonal Antibodies. MB-1 is a mouse IgG1 anti-CD37 MoAb, which immunoprecipitates a Mr 40,000 cell surface protein present on all surface immunoglobulin-bearing human tumors tested (22). The MB-1 antibody was provided in purified form by IDEC Pharmaceuticals (Mountain View, CA). The mouse MoAb, anti-B1, is an IgG2a that

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3 The abbreviations used are: RIT, radioimmunotherapy; MoAb, monoclonal antibody; ADCC, antibody-dependent cellular cytotoxicity; BCS, bovine calf serum.

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immunoprecipitates a $\alpha$, 35,000 cell surface phosphoprotein (CD20) (23). The anti-B1 antibody was provided in purified form by Coulter immunoprecipitates a $\alpha$, 35,000 cell surface phosphoprotein (CD20). S3H5/2a was produced and purified from mouse ascites. It is reactive with a mouse immunoglobulin idiotype (24). MoAbs MS (antigen specificity unknown) and MYS (anti-human immunoglobulin idiotype) were bioreactor produced.

Cell Lines. The Raji Burkitt's lymphoma cell line was maintained in suspension culture at 37°C in a humidified 5% CO$_2$ atmosphere using RPMI 1640 supplemented with 10% BCS (Hyclone Laboratories, Inc., Logan, UT). The Raji cell line was obtained from the American Type Culture Collection. HT-1080 cells were grown in monolayer culture in Eagle's minimal essential medium with Earles' salts and nonessential amino acids (Whittaker M.A. Bioproducts) supplemented with 10% BCS.

Cell-mediated Cytotoxicity. Raji target cells were labeled with $^{51}$Cr (250–500 mCi/mg; Amersham, Arlington Heights, IL) by incubating them with 100 μCi $^{51}$Cr for 1 h at 37°C in complete medium. The cells were washed twice, incubated in 50 ml of complete medium for 30 min, and then washed again. Viability was always greater than 95%. Labeled target cells (1 x 10$^6$) were added to human effector cells at the desired effector to target ratios in V-bottomed microtiter plates (Corning Glass Works, Corning, NY). Human effector cells (lymphocytes and other mononuclear cells) were obtained after informed consent from normal volunteers by venipuncture. Blood was collected in sterile tubes containing preservative-free heparin and layered over Histopaque 1077 (Sigma Chemical Co., St. Louis, MO). After density centrifugation at room temperature over 30 min, mononuclear cells were harvested and washed three times. Viability was always greater than 95%.

Radiolabeled MB-1. Animals treated with unlabeled MB-1 showed a 44 ± 19% reduction in tumor size at 3 weeks after injection. In vivo effect of MB-1 and control MoAb on Raji B-Cell Tumor Growth. Groups of 10 nude mice bearing Raji tumors were treated with a single dose of 100 μg unlabeled MB-1 or 300 μCi $^{131}$I-labeled MB-1 (40 μg labeled plus 60 μg unlabeled) or 300 μCi $^{131}$I-labeled MB-1 (27 μg labeled plus 73 μg unlabeled) control MYS antibody. As shown in Fig. 1, at 3 weeks after injection, there was a 37 ± 67% increase in tumor size over this period of time. In a comparison of all three groups, there was a significant difference (P = 0.007) in mean tumor size demonstrated by $^{131}$I-labeled MB-1. In a comparison of $^{131}$I-labeled MB-1 and $^{131}$I-labeled MYS, the difference in mean tumor size was significant at P < 0.01. The difference in mean tumor size at 3 weeks after injection was used for the evaluation because at this time one would expect to see the effects of the RIT treatment and because in this study the animals were evaluated for only 3.5 weeks. The results indicate that 300 μCi $^{131}$I-labeled MB-1 inhibited tumor growth due to antibody-specific targeted radiation.
received 100 μg unlabeled MB-1, 300 μCi (40 μg labeled plus 60 μg unlabeled) 131I-labeled MB-1, or 300 μCi (27 μg labeled plus 73 μg unlabeled) 131I-labeled MYS antibody. Change in tumor size (bidimensional product) was then assessed at varying times after injection. Data are expressed as the average of 10 animals per group ± SEM (bars). Statistical differences among the groups are discussed in the text.

Because of the differences in the administered protein doses given to the experimental groups in the preceding experiment, the next experiment was carried out with the total protein dose adjusted to 100 μg (consisting of a mixture of radiolabeled and unlabeled antibody) for all groups. The MS bioreactor-produced antibody was used as a control. Groups of 10 nude mice bearing s.c. Raji xenografts received a single i.p. injection of 100 μg unlabeled anti-B1, 300 μCi 131I-labeled anti-B1 (39 μg labeled plus 61 μg unlabeled), or 300 μCi 131I-labeled (41 μg labeled plus 59 μg unlabeled) MS control antibody. As shown in Fig. 3, there was a significant difference in tumor size at 6 weeks posttreatment in animals treated with 300 μCi 131I-labeled MS antibody versus those treated with unlabeled or 300 μCi 131I-labeled anti-B1 antibody (P = 0.0006). At 4 weeks after injection, the level of significance was P = 0.06, while at 3 weeks and earlier after injection the differences between these three groups of animals were not significant. There was no significant difference in the reduction in tumor size produced by unlabeled or 300 μCi of 131I-labeled anti-B1 throughout the experiment, and the level of significance at 41 days was P = 0.307. As shown in Fig. 4, 70% of tumors completely regressed after treatment with 300 μCi 131I-labeled anti-B1, compared to only 20% with 300 μCi 131I-labeled MS antibody (P = 0.02). However, there was no difference in the proportion of animals with tumors that completely regressed after injection with unlabeled or 300 μCi 131I-labeled anti-B1 antibody.

In another study, with the total protein dose adjusted to 100 μg, no differences in tumor size reduction or rate of complete regressions were observed among animals treated with unlabeled or 300 and 450 μCi 131I-labeled anti-B1 (data not shown).

isotype-matched control antibody. The protein dose of unlabeled anti-B1 was 36 μg. The protein doses of 131I-labeled anti-B1 MoAb in this experiment were 36 μg (300 μCi) and 18 μg (150 μCi), while the protein doses of 131I-labeled S3H5/2a were 66 μg (300 μCi) and 33 μg (150 μCi), respectively. The differences in protein doses for the two radiolabeled antibodies were due to their different specific activities. As shown in Fig. 2, a rapid decrease in tumor size was evident at 1 week after injection of unlabeled or 300 μCi 131I-labeled anti-B1. The differences in mean tumor size of these two groups of animals were significantly different from those of the other groups of animals (P < 0.01). At 14 days after antibody injection, the difference between mean tumor size in the groups of animals treated with 150 or 300 μCi 131I-labeled anti-B1 were significant at a level of P = 0.025, while the level of significance for 300 μCi 131I-labeled anti-B1 versus 300 μCi 131I-labeled S3H5/2a was P = 0.014. At 3 weeks after injection, the decrease in tumor size with 300 μCi 131I-labeled anti-B1 and unlabeled anti-B1 was 64 and 93%, respectively. With 300 μCi control S3H5/2a antibody, there was a 58% increase in tumor size over 3 weeks. The difference in mean tumor size between unlabeled and 300 μCi 131I-labeled anti-B1 at 3 weeks after injection was significant (P = 0.019). The difference in tumor size between 300 μCi 131I-labeled anti-B1 and 300 μCi 131I-labeled S3H5/2a at 3 weeks after injection was also significant (P = 0.02). No significant difference was observed at the 150 μCi level between specific and control antibody throughout the 3-week period.
Toxicity. Peripheral leukocyte counts from animals treated with $^{131}$I-labeled anti-B1 and SHS/2a were obtained in the experiment described in Fig. 2. Three hundred $\mu$Ci $^{131}$I-labeled anti-B1 produced a minor (30%) but significant ($P = 0.0001$) decrease in peripheral WBC at 6 days after injection relative to saline-treated controls (not shown in Fig. 2) while 300 $\mu$Ci $^{131}$I-labeled SHS/2a produced no change. Complete recovery was seen at 20 days. Changes in the average body weights of the animals in the different treatment groups varied by less than 10% from the starting body weight for the duration of the experiment.

In Vitro Effects of Unlabeled MB-1 and Anti-B1 on Raji Tumor Cells. ADCC assays using $^{51}$Cr-labeled Raji cells as targets showed that MB-1 did not cause lysis of tumor cells in the presence of human peripheral mononuclear cells (Fig. 5). Anti-B1, however, was capable of ADCC. Similar assays have shown no lysis of tumor cells by anti-B1 in the presence of varying numbers of nude mouse spleen effector cells, but other effector cells were not tested (data not shown). No direct antiproliferative effect on Raji cells was detected in $^{3}H$thyidine incorporation experiments with the anti-B1 antibody (data not shown) as well as with the MB-1 antibody.

DISCUSSION

In this study two clinically promising pan-B-cell antibodies, MB-1 and anti-B1, which have different specificities and are of different isotype subclasses were compared in a controlled experimental system of human Raji B-cell tumor xenografts in athymic nude mice to investigate the mechanisms by which RIT with these antibodies can cause tumor growth inhibition. Parallel experiments were performed with radiolabeled and unlabeled specific and control nonspecific antibodies of the same isotype subclass.

With the MB-1 antibody, an antibody-specific targeted radiation effect appeared to be the dominant antitumor mechanism of radiolabeled MB-1. A single injection of 300 $\mu$Ci $^{131}$I-labeled MB-1 produced a reduction in tumor size at 3 weeks posttreatment. By contrast, an increase in tumor size over this period of time was seen with unlabeled MB-1 or 300 $\mu$Ci $^{131}$I-labeled MYS control antibody at the same total protein dose of 100 $\mu$g. Although 300 $\mu$Ci $^{131}$I-labeled anti-B1 also produced a reduction in mean tumor size at 3 weeks posttreatment (while 300 $\mu$g of control antibody resulted in an increase in tumor size over the same period), unlabeled anti-B1 also had a substantial antitumor effect. In fact, in none of our controlled studies with anti-B1 were we able to distinguish any additional antitumor effect of radiolabeled antibody over unlabeled antibody. This suggested that the dominant antitumor mechanism of $^{131}$I-labeled anti-B1 was related to effects mediated by the antibody moiety of the radioconjugate. Indeed, the greater reduction in tumor size produced by unlabeled and 300 $\mu$Ci $^{131}$I-labeled anti-B1 compared to 150 $\mu$Ci $^{131}$I-labeled anti-B1 (shown in Fig. 2) may have been a consequence of the higher anti-B1 protein dose in the preparations given the groups treated with 300 $\mu$Ci $^{131}$I-labeled anti-B1 and unlabeled anti-B1 (36 $\mu$g) versus those given to the group treated with 150 $\mu$Ci $^{131}$I-labeled anti-B1 (18 $\mu$g). Also, tumor regression was actually greater with unlabeled anti-B1 than radiolabeled anti-B1 in the study shown in Figs. 3 and 4. A potential explanation for this is that radiolabeling resulted in damage to the antibody moiety sufficient to partially reduce the ability of the antibody to carry out its radiation-independent antitumor mechanisms. This is supported by the results shown in Figs. 3 and 4 where antitumor effects of labeled and unlabeled antibody were not significantly different when a larger excess of unlabeled antibody was added to the labeled preparation. Whether smaller protein doses than those used in these studies would allow one to distinguish any antibody-specific targeted radiation effect on tumors remains to be determined. It is also of interest that increasing the dose of $^{131}$I-labeled anti-B1 to 450 $\mu$Ci did not result in any further enhancement of tumor regression over 300 $\mu$Ci (data not shown). Whether still higher radionuclide doses would finally permit targeted radiation effects on tumors to be seen also remains to be investigated.

These differences in the behavior of radiolabeled MB-1 and anti-B1 prompted us to further investigate potential differences in the capabilities of these antibodies to carry out antibody-mediated antitumor functions. Importantly, we found that while neither antibody was directly cytostatic or cytotoxic, only anti-B1 could mediate specific cytolsis in the presence of human immune effector cells (Fig. 5). This is likely due to the difference in isotype subclass of the two antibodies. Antibodies of the IgG2a subclass (the subclass of anti-B1) have been found to be superior in conducting ADCC and/or complement-mediated cytolysis than those of the IgG1 subclass (the subclass of

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4 R. Levy (Stanford University, Palo Alto, CA), personal communication.
MB-1) (24, 31–33). To ideally demonstrate that subclass differences accounted for differences in the in vivo effects of MB-1 and anti-B1, would require comparing subclass switch-variants of these antibodies. Unfortunately, such switch-variants are not available for testing. Although the study of F(ab')2 fragments may be of some use in this regard, the analysis of results may be complicated by potential differences in biodistribution and clearance of the fragments compared to the intact antibodies.

In other studies, Badger et al. (34, 35) using a murine IgG2b MoAb against the Thy-1.1 differentiation antigen demonstrated that the antibody labeled with $^{131}$I was superior to unmodified antibody or $^{131}$I-labeled control antibody in its therapeutic effect against a murine T-cell lymphoma. This is similar to our findings with $^{131}$I-labeled MB-1. It is interesting to note that the unmodified IgG2b effect seen by Badger et al. was not as prominent as the unmodified IgG2a effect in our system. The difference may be due to many possible factors including a potential greater sensitivity of Raji cells to ADCC or complement-mediated cytolsis, a greater ADCC potential of anti-B1 over the anti-Thy-1.1 antibody, differences in antibody affinity or dose, host differences, or tumor delivery. Nouriag et al. (36) using the same T-cell lymphoma model found that $^{131}$I-labeled anti-Thy 1.1 MoAb produced 92% complete regression of SL2 lymphoma nodules containing 0.3 to 1% variant lymphoma cells that did not express the Thy-1.1 antigen. This study suggested that emitted radiation from radiolabeled antibody bound to antigen-positive tumor cells could kill adjacent tumor cells that did not express the target antigen and that this was a potential advantage of radiolabeled antibodies over unlabeled, drug-conjugated, or toxin-conjugated antibodies which would require binding to all cells to be therapeutically effective, a difficult task due to heterogeneous distribution of antibodies within tumors and the presence of antigen-negative tumor cells. Another study reported the successful treatment of Raji Burt-kitt's lymphoma xenografts in athymic nude mice with a single injection of about 300 μCi $^{131}$I-labeled Lym-1 pan-B-cell MoAb reactive with this tumor but did not include the controls we used (37).

There are some limitations of mouse data that may make extrapolation to the human system difficult. The mouse pan-B-cell-reactive MoAbs we used did not cross-react with normal mouse cells, whereas they did show cross-reactivity with normal B-cells in human peripheral blood and well vascularized organs. Because of the small size of the mouse, the whole-body contribution of absorbed dose to tumor in mice due to γ-irradiation would also be expected to be substantially less compared to the situation in humans (where self-absorption of the γ rays would be more likely). It is also possible that nonspecific $^{131}$I-labeled MoAb might have greater effects in humans due to these considerations.

The results of the present study indicate that although antibody-specific targeted radiation may play a significant role in the therapeutic effects of radiolabeled antibodies, the effects of the antibody moiety of the radioconjugate may also be substantial depending on the antibody chosen for the conjugate. These results also suggest that a careful evaluation of antibody effects should be performed in RIT trials in patients with non-Hodgkin's lymphoma. Indeed, tumor responses have been seen in patients using not only high radionuclide doses of radiolabeled antibody (3) but also low radionuclide doses (5), which is consistent with a potential involvement of the antibody itself in the observed tumor responses. Also, considering that unlabeled pan-B-cell antibodies have been infused prior to or simultaneously with radiolabeled antibody in an effort to optimize the biodistribution and tumor delivery of radiolabeled antibody (1–4, 6, 16), this issue becomes even more relevant. Finally, these results have implications for the design of radioimmunoconjugates. Although, radiolabeled antibody fragments have been reported to have superior tumor localization abilities and more favorable tumor dosimetry than radiolabeled intact antibodies, the use of such fragments may potentially nullify important additional therapeutic effects mediated by the Fc portion of antibodies (38, 39).

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MoAb THERAPY OF LYMPHOMA


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