Immunotherapy using unconjugated CD19 monoclonal antibodies in animal models for B lymphocyte malignancies and autoimmune disease

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Immunotherapy with unconjugated CD20 monoclonal antibodies has proven effective for treating non-Hodgkin’s lymphoma and autoimmune disease. CD20 immunotherapy depletes mature B cells but does not effectively deplete pre-B or immature B cells, some B cell subpopulations, antibody-producing cells, or their malignant counterparts. Because CD19 is expressed earlier during B cell development, a therapeutic strategy for the treatment of early lymphoblastic leukemias/lymphomas was developed by using CD19-specific monoclonal antibodies in a transgenic mouse expressing human CD19. Pre-B cells and their malignant counterparts were depleted as well as antibody- and autoantibody-producing cells. These results demonstrate clinical utility for the treatment of diverse B cell malignancies, autoimmune disease, and humoral transplant rejection.

B lymphocytes are the origin of humoral immunity, represent a substantial portion of hematopoietic malignancies, and contribute to autoimmunity (1, 2). Consequently, cell surface molecules expressed by B cells and their malignant counterparts represent important targets for immunotherapy. Chimeric or radiolabeled monoclonal antibody (mAb)-based therapies directed against the CD20 cell surface molecule specific for mature B lymphocytes and their malignant counterparts (3) represent an effective in vivo treatment for non-Hodgkin’s lymphoma (4–7). Unconjugated anti-CD20 mAb therapy may also ameliorate the manifestations of rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenic purpura, and hemolytic anemia, as well as other immune-mediated diseases (8–10). Despite the effectiveness of this therapy, most pre-B and immature B lymphoblastic leukemias and many other B cell malignancies do not express CD20, express CD20 at low levels, or lose CD20 expression after CD20 mAb immunotherapy (7). Moreover, only half of non-Hodgkin’s lymphoma patients respond to CD20-directed immunotherapy, and CD20 mAb therapy does not reverse the production of pathogenic autoantibodies. However, CD19 is a structurally distinct cell surface receptor that is expressed from the earliest stages of pre-B cell development until B cell terminal differentiation into plasma cells (11). Thereby, CD19, expressed by most pre-B-acute lymphoblastic leukemias (ALL), common-ALL, null-ALL, non-Hodgkin’s lymphomas, B cell chronic lymphocytic leukemias (CLL), pro-lymphocytic leukemias, and hairy cell leukemias, represents a potentially important new target for unconjugated mAb immunotherapy (12–15).

Developing immunotherapies and carrying out mechanistic studies in humans is challenging. Moreover, human studies primarily focus on changes in blood, which contains only ~2% of the total lymphocyte pool in the normal adult human body (16). Thus, it is difficult to accurately ascertain the effects of immunotherapies on the majority of B cells, which are found in peripheral lymphoid tissues. To overcome this difficulty, we developed a transgenic mouse model for assessing CD19-directed immunotherapies that is amenable to mechanistic studies and genetic manipulation and that may predict the in vivo outcome of human therapies. These mice express the human CD19 gene regulated by its endogenous promoter, which recapitulates the developmental pattern of human CD19 (hCD19) cell surface expression (17–21). Because of CD19 overexpression, hCD19 transgenic (hCD19TG) mice also develop autoimmune disease (11, 19, 21). This preclinical model for immunotherapy thereby allowed the identification, characterization, and mechanistic examination of hCD19-directed therapies for early B lymphoblastic leukemias/lymphomas and autoimmunity.

Materials and Methods

Mice. Transgenic mice expressing hCD19 (TG-1 line) and their wild-type littermates were as described (17). TG-1 mice were generated from the original hi9–1 founders (C57BL/6 × B6/ SJL), and were crossed onto a C57BL/6 background for at least seven generations. Fc receptor common γ chain (FcγRIIIa) mice (B6.129P2-FcγRIIIa<sup>tm1</sup>/mice) from Taconic Farms were crossed and backcrossed with TG-1<sup>+/−</sup> mice to generate hCD19TG<sup>+/−</sup> FcγRIIIa<sup>−/−</sup> and hCD19TG<sup>−/−</sup> FcγRIIIa<sup>−/−</sup> littermates. Mice hemizygous for a c-Myc transgene [cMycTG, C57BL/6J-TgN(IghMyc); The Jackson Laboratory] were crossed with hCD19TG<sup>−/−</sup> mice to generate hCD19TG<sup>−/−</sup> cMycTG<sup>+/−</sup> offspring as determined by PCR screening (22, 23). Rag1<sup>−/−</sup> (B6.129S7-Rag<sup>tm1Mom/J</sup>) mice were from the Jackson Laboratory. Macrophage-deficient mice were generated by tail vein injections of liposome-encapsulated clodronate (0.1 ml per 10 g of body weight; Sigma) on days −2, 1, and 4 as described (24). All mice were housed in a specific pathogen-free barrier facility and first used at 6–9 weeks of age. These studies were approved by the Duke University Animal Care and Use Committee.

Abs and Immunofluorescence Analysis. The HB12a and HB12b (IgG1) mAbs were generated as described (25, 26). Other mouse anti-hCD19 mAbs included FMC63 (IgG2a, Chemicon; and a generous gift from Heddy Zola, Child Health Research Institute, Adelaide, Australia) (27–29), B4 (IgG1, Beckman Coulter) (12), and HD237 (IgG2b, Fourth International Workshop on Human Leukocyte Differentiation Antigens, Vienna, 1989), an isotype switch variant of the HD37 mAb (30). The mouse anti-mouse CD19 (mCD19) mAb MB19–1 (IgG) was as described (19). Mouse CD20-specific mAbs, MB20–11 and MB20–18, were as described (31). Other mAbs included: B20 mAb RA3–6B2 (provided by Robert Coffman, DNAX); Thyl.1.2 mAb (Caltag, Burlingame, CA); and anti-mouse CD19 (1D3), CD5, CD43, and CD25 mAbs (BD Pharmaning). Isotype-specific and anti-mouse Ig or IgM Abs were from Southern Biotechnology Associates.

For immunofluorescence analysis, single-cell leukocyte suspen-

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Abbreviations: hCD19TG mice, human CD19 transgenic mice; cMycTG, human c-Myc transgenic mice; TNFR, 2,4,6-trinitrophenyl; DNP, 2,4-dinitrophenyl; KLH, keyhole limpet hemocyanin; ADC, Ab-dependent cell cytotoxicity; Fcγ, Fc receptor; FcγRI, Fc receptor for IgG; FcγRII, common γ chain of the FcγRI.

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sions were stained on ice by using predetermined optimal concentrations of each Ab for 20–30 min as described (17). Next, 10,000 cells with the forward and side light-scatter properties of lymphocytes were analyzed on FACScan or FACSCalibur flow cytometers (Becton Dickinson) when possible, with fluorescence intensities shown on a four-decade logarithmic scale. Background staining was determined by using unreactive control mAbs (Caltag) with gates positioned to exclude ≥98% of the cells.

**Immunotherapy.** Sterile endotoxin-free anti-hCD19, anti-mouse CD20 (MB20–11), and unreactive isotype control mAbs (2–250 µg) in 250 µl of PBS were injected into lateral tail veins. Blood leukocyte numbers were quantified by hemocytometer after red cell lysis, with B220+ B cell frequencies in harvested tissues determined by immunofluorescence staining with flow cytometry analysis. Ab doses in humans and mice were compared by using the oncology tool dose calculator (www.fda.gov/cedr/cancer/animalframe.htm).

**Tumor Studies.** A spontaneous tumor in a hCD19TG+/− cMycTG+/− mouse was isolated from lymph node and expanded in vitro. Tumor cells (10⁵ per mouse) were given i.v. to Rag1−/− recipient mice on day 0, with CD19 and isotype-matched control mAbs (250 µg) given i.v. on days 1 and 7. Blood leukocytes from recipient mice were isolated weekly, and the number of circulating mouse CD19−/− B220+ cells was quantified by immunofluorescence staining with flow cytometry analysis.

**Immunizations.** Two-month-old mice were immunized i.p. with 50 µg of 2,4,6-trinitrophenyl (TNP)-conjugated LPS (serotype 026:B6, Sigma) or 25 µg of 2,4-dinitrophenyl (DNP)-conjugated-Ficol (Biosearch) in saline. Mice were also immunized i.p. with 100 µg of DNP-conjugated keyhole limpet hemocyanin (DNP-KLH, Calbiochem) in complete Freund’s adjuvant and were boosted 21 days later with DNP-KLH in incomplete Freund’s adjuvant. DNP- or TNP-specific Ab titers in individual serum samples were measured in duplicate by using ELISA plates coated with DNP-BSA (Calbiochem) or TNP-BSA (Biosearch) as described (18).

**ELISAs.** Serum Ig concentrations were determined by ELISA as described (18, 32). Serum autoantibody levels against dsDNA, ssDNA, and histone were determined by ELISA using calf thymus dsDNA (Sigma–Aldrich), boiled calf thymus DNA (containing dsDNA, and histone) were determined by ELISA using calf thymus DNA (Sigma–Aldrich), and were quantified by hemocytometer after red cell lysis, with B220+ B cell frequencies in harvested tissues determined by immunofluorescence staining with flow cytometry analysis. Numbers indicate the percentage of gated lymphocytes. (C) Circulating B220+ B cell and Thy-1.2+ T cell numbers (±SEM per ml, n = 8 mice) after CD19 (FMC63, ●) or isotype-control (○) mAb treatment. The value shown after time 0 represents data obtained at 1 h. (D) Dose responses for spleen B cell depletion. hCD19TG mice were treated with CD19 or control (CTL) mAbs on day 0 with spleen B cells assessed on day 7 (n = 3). (E) Spleen B cell numbers (±SEM) 7 days after treatment of hCD19TG mice with CD19 (FMC63, ●) or isotype-matched control (○) mAb treatment of hCD19TG−/− mice as determined by immunofluorescence staining with flow cytometry analysis. Numbers indicate the percentage of gated lymphocytes. (C) Circulating B220+ B cell and Thy-1.2+ T cell numbers (±SEM per ml, n = 8 mice) after CD19 (FMC63, ●) or isotype-control (○) mAb treatment. The value shown after time 0 represents data obtained at 1 h. (D) Dose responses for spleen B cell depletion. hCD19TG mice were treated with CD19 or control (CTL) mAbs on day 0 with spleen B cells assessed on day 7 (n = 3). (E) Spleen B cell numbers (±SEM) 7 days after treatment of hCD19TG−/− mice with CD19 (filled bars, 50 µg) or control (open bars, 250 µg) mAb. (F) Bone marrow B cell subset depletion 7 days after CD19 (FMC63) or isotype-matched control mAb (250 µg) treatment of hCD19TG−/− mice as assessed by three-color immunofluorescence staining. IgM+ B220+ pro/pre-B cells were further subdivide based on CD43 expression (Lower). Numbers represent the relative frequencies of each B cell subset within the indicated gates. Bar graphs indicate numbers (±SEM, n = 3) of pro-B, pre-B, and immature and mature B cells within bilateral femurs 7 days after CD19 (filled bars), CD20 (crosshatched bars), or control (open bars) mAb treatment. (G) Peritoneal cavity CD5+ B220+ B1a and CD5− B220− B2 cell depletion 7 days after CD19 (FMC63, filled bars), CD20 (crosshatched bars), or control (open bars) mAb treatment of hCD19TG−/− mice. Numbers represent the relative frequencies of each B cell subset within the indicated gates. Bar graphs indicate the total number (±SEM) of each subset within the peritoneum of mAb-treated mice (n = 3). (C–G) Significant differences between mean results for CD19 or CD20 and isotype-control mAb-treated mice are indicated: *, P < 0.05; **, P < 0.01.
CD19 was expressed at increasing levels by B220 64–95% of splenic and lymph node B cells by day 7. A single 250-μg IgG2b (HD237) isotype depleted 96–99% of blood B cells and circulating and tissue B220 depletion conditions did not affect B cell numbers (Fig. 1). Mice, and isotype-matched control mAbs given under identical mAbs had significant depleting effects when given to wild-type mouse (Fig. 1A) as described (19, 20). Human CD19 was expressed at increasing levels by B220+ B cells in the bone marrow (87% of human blood levels, n = 3), blood (113%), lymph nodes (116%), spleen (134%), and peritoneal cavity (146%). Unconjugated anti-hCD19 mAb treatment effectively depleted hCD19TG+ B cells in vivo when given to mice at 250 μg per mouse (Fig. 1B), single doses 10-fold lower than the 375 mg/m2 dose primarily given four times to humans during anti-CD20 (Rituximab) therapy (4–7). CD19 mAbs depleted the majority of circulating B cells within 1 h of treatment, with potent depletion of spleen and lymph node B cells (Fig. 1 B–E) by day 7 (Table 1). Antibodies of the IgG1 (HB12a, HB12b, and B4), IgG2a (FMC63), and IgG2b (HB237) isotypes depleted 96–99% of blood B cells and 64–95% of splenic and lymph node B cells by day 7. A single 250-μg injection of CD19 mAb depleted circulating B cells for 15 weeks without affecting T cell numbers (Fig. 1C). The bone marrow, blood, spleen, lymph nodes, and peritoneal cavity were also devoid of B cells for up to 11 weeks after mAb treatment (data not shown). After the first appearance of circulating B cells, it took an additional 10–20 weeks for circulating and tissue B cell numbers to reach the normal range at this mAb dose. A single CD19 mAb dose as low as 10 μg per mouse also depleted significant numbers of circulating and spleen B cells (Fig. 1D and data not shown). None of the CD19 mAbs had significant depleting effects when given to wild-type mice, and isotype-matched control mAbs given under identical conditions did not affect B cell numbers (Fig. 1E and data not shown). Thus, CD19 is an effective target for mAb-induced B cell depletion in vivo, with rapid removal of the vast majority of circulating and tissue B220+ B cells in mice.

CD19 mAb Depletes Pre-B and B1 Cells in Vivo. Within the bone marrow, CD19 expression is heterogeneous in humans and hCD19TG+/− mice (12, 13, 33). A small fraction of pro-B cells (20%, CD43+ IgM− B220+) expressed hCD19 in hCD19TG+/− mice, whereas the majority of pre-B cells and mature B cells in the bone marrow expressed hCD19 at relatively high levels as described (17). Consistent with this finding, the vast majority of hCD19+ cells in the bone marrow of hCD19TG+/− mice were depleted by CD19 mAb treatment, including the majority of CD25+ CD43lo IgM− B220+ pre-B cells and IgM+ B220+ immature B cells (Fig. 1F). By contrast, CD20 mAb treatment had no significant effect on these cells. The peripheral B cells remaining in CD19 mAb-treated mice primarily represented phenotypically immature pro/pre-B cells emigrating from the bone marrow (data not shown). Peritoneal B cells in hCD19TG+ mice express hCD19 at higher levels than other tissue B cells (Fig. 1A) because of higher hCD19 expression by CD5+ IgM− B220+ B1a cells (19). As a result, CD19 mAb treatment depleted a significant portion of peritoneal B1 and conventional B2 cells by day 7 (Fig. 1G). Other CD19 mAbs (HB12a, HB12b, B4, and HD237) also depleted the majority of bone marrow and peritoneal B220+ cells (data not shown). By contrast, CD20 mAb treatment did not significantly affect peritoneal B cells at this time point (Fig. 1G), as described (34). Thus, most hCD19+ cells were depleted from bone marrow and the peritoneal cavity by CD19 mAb treatment but not CD20 mAb treatment.

Monocytes Deplete CD19+ B Cells Through Fcγ Receptor for IgG (FcγR)-Dependent Processes. The innate immune system mediates B cell depletion after CD20 mAb treatment through FcγR and monocyte-dependent processes (34–36). Because FcRγ expression is required for FcγRI (CD64) and FcγRII (CD16) assembly (37), FcRγ−/− mice were used to assess the role of FcγR in tissue B cell depletion. CD19 mAb treatment depleted most B cells in heterozygous FcRγ+/− littermates (Fig. 2A). However, there were no significant differences between means. The Student t test was used to determine the significance of differences between sample means.

**Table 1. CD19 mAb-induced B cell depletion in hCD19TG+/− mice**

| Tissue subsets | Control mAb | CD19 mAb | Depletion, % |
|---------------|-------------|----------|--------------|
| BM: B220+    | 34.1 ± 5.7 (11) | 8.2 ± 1.3 (11) | 76±          |
| BM: Pro-B    | 7.5 ± 1.0 (5)   | 9.7 ± 2.2 (5)  | 0            |
| BM: Pre-B    | 17.4 ± 5.0 (5)  | 1.0 ± 0.1 (5)  | 94±          |
| BM: immature | 7.0 ± 1.6 (5)   | 0.4 ± 0.1 (5)  | 93±          |
| BM: mature   | 8.6 ± 1.4 (5)   | 0.04 ± 0.01 (5) | 99±          |
| Blood: B220+ | 8.2 ± 1.4 (11)  | 0.04 ± 0.01 (11) | 99±          |
| Spleen: B220 | 252 ± 22 (11)   | 17 ± 2 (11)    | 93±          |
| LN: B220     | 8.9 ± 1.1 (11)  | 0.6 ± 0.1 (11) | 93±          |
| PC: B220     | 11.6 ± 1.1 (11) | 3.7 ± 0.3 (11) | 68±          |
| PC: B1a      | 8.6 ± 1.2 (5)   | 3.1 ± 0.6 (5)  | 61±          |
| PC: B2       | 3.4 ± 0.6 (5)   | 0.8 ± 0.2 (5)  | 73±          |

†Values (±SEM) indicate cell numbers present in mice 7 days after mAb treatment (250 μg) with the percentage (%) of cell depletion indicated. BM numbers are per ml. LN numbers are for bilateral inguinal and axillary nodes. Numbers of mice are indicated in parentheses. Significant differences between means are indicated: *, P < 0.05; **, P < 0.01.
significant changes in bone marrow, blood, spleen, lymph node, or peritoneal cavity B cell numbers in FcRγ−/− mice after CD19 mAb treatment when compared with control mAb-treated littermates. Likewise, hCD19TG+/+ mice were rendered macrophage-deficient by treatment with liposome-encapsulated clodronate. Although liposome-encapsulated clodronate is not able to deplete all macrophages (24, 35), CD19 or CD20 mAb treatment did not significantly deplete circulating B cells after 1 day, whereas mAb treatment eliminated circulating B cells in mice treated with saline-loaded liposomes (Fig. 2B). After 7 days, CD19 mAb treatment was significantly less effective for blood (P < 0.05) and tissue (P < 0.01) B cell depletion in clodronate-treated mice than in PBS-treated littermates. In blood and spleen, CD19 mAb treatment also had a more significant effect on B cell numbers in clodronate-treated mice compared with CD20 mAb treatment. These findings implicate macrophage-mediated Ab-dependent cellular cytotoxicity (ADCC) as the major effector mechanism for CD19+ B cell depletion in vivo.

CD19 and CD20 mAb-Mediated B Cell Depletion Is Additive in Vivo. Because CD19 and CD20 mAbs mediate B cell depletion through ADCC, whether simultaneous CD19 and CD20 mAb treatments enhanced B cell depletion was assessed in vivo by using minimally effective mAb doses. Mice were treated with suboptimal 2-μg doses of each mAb individually or a combination of both mAbs. B cell depletion in mice treated with both CD19 and CD20 mAbs led to significantly more B cell depletion than was observed with either mAb alone (Fig. 3). Thus, combined CD19 and CD20 mAb therapies had additive effects that enhanced B cell depletion.

CD19 mAb Treatment Depletes Malignant B Cells in Vivo. Eμ-cMycTG mice, in which the c-Myc protooncogene is controlled by the Ig heavy-chain enhancer, primarily develop aggressive, monoclonal B cell-derived lymphomas at an early age, have an ∼90% mortality rate by 20 weeks of age, and have an ∼12-week median age of survival (22, 23). To assess the efficacy of CD19-directed immunotherapy in vivo, hCD19TG+/+ cMycTG mice were generated that developed aggressive B cell-derived lymphomas at an early age. Tumor cells from one mouse were isolated, expanded in vitro, and characterized to be hCD19+ and mouse CD19+ CD20− CD43− IgM− IgD− B220+ lymphoblasts (Fig. 4A), which are typical of the pre-B/immature B cell tumors of cMycTG mice (22, 23). Transplantation of these tumor cells into 20 Rag1−/− mice resulted in circulating CD19+ B220+ lymphoblasts by 2 weeks in 10 randomly selected recipients that were treated with a control mAb, with death by 3.5 weeks (Fig. 4B–D). By contrast, CD19 mAb treatment 1 and 7 days after tumor transplantation prevented the appearance of circulating tumor cells in all 10 randomly selected recipients for up to 7 weeks (Fig. 4C). One CD19 mAb-treated mouse died during blood harvest at week 3 and three CD19 mAb-treated mice were killed at week 7, but none of the mice displayed tumor cells. Two CD19 mAb-treated mice died after 8 weeks, consistent with the short longevity of Rag1−/− mice, and the remaining four mice were killed at 21 weeks, but all were without obvious leukemia/lymphoma. Thus, CD19 mAb treatment dramatically inhibited malignant B cell growth in vivo.

CD19 mAb Treatment Eliminates Humoral Immune Responses and Autoantibodies. Because CD19 mAb treatment depleted most B cells, the effect of CD19 mAb treatment on serum Ab levels was assessed. Two weeks after a single injection of CD19 mAb, serum IgM, IgG, and IgA Ab levels were significantly reduced, and they remained reduced for at least 10 weeks (Fig. 5A). The influence of B cell depletion on T cell-independent type 1 (TI-1) and type 2 (TI-2) Ab responses was therefore assessed by immunizing hCD19TG+/+ mice with TNP-LPS, DNP-Ficoll, or DNP-KLH, respectively, 7 days after CD19 or control mAb treatment. Significant hapten-specific IgM, IgG, and IgA Ab responses were not observed in CD19 mAb-treated mice, whereas control mAb-treated littermates generated significant Ab responses (Fig. 5B). CD19 mAb-treated mice immunized with DNP-KLH
also failed to generate Ab responses after antigen rechallenge on day 21. To selectively assess the effect of B cell depletion on secondary Ab responses, mice were immunized with DNP-KLH on day 21. To selectively assess the effect of B cell depletion on secondary Ab responses, mice were immunized with DNP-KLH on day 21. To selectively assess the effect of B cell depletion on secondary Ab responses, mice were immunized with DNP-KLH on day 21. To selectively assess the effect of B cell depletion on secondary Ab responses, mice were immunized with DNP-KLH on day 21. To selectively assess the effect of B cell depletion on secondary Ab responses, mice were immunized with DNP-KLH on day 21.

**Discussion**

These studies reveal that cell surface hCD19 is an effective target for mAb-induced B cell depletion in vivo, with CD19 mAb treatment rapidly removing the vast majority of circulating and tissue B220+ B cells in hCD19TG mice. Although CD19 and CD20 mAbs share a common effector mechanism (Fig. 2), they may also offer several unique advantages in relation to currently available CD20-directed immunotherapies such as Rituximab. First, CD19 permits the targeted depletion of pre-B and immature B cells from the bone marrow in addition to mature B cells in the periphery (Fig. 1). CD19 therapy may therefore offer a treatment for early B lymphoblastic leukemias/lymphomas and other malignancies that derive from early B cell precursors. Second, CD19-directed mAbs effectively deplete peritoneal cavity B cells (Fig. 1G), which are more resistant to CD20-directed therapy (34). Third, because CD19 mAb treatment eliminates early B cell precursors, the depletion of peripheral B cells was almost 2-fold more durable (Fig. 1C) than CD20 therapy, which depletes only mature B cells (35). Fourth, CD19-directed therapies may deplete pathogenic B cells before antigen receptor selection and thereby have significant advantages in treating autoimmune disease. Recently developed mouse models for CD20 immunotherapy have provided considerable insight into the mechanism and utility of that treatment strategy (34–36). Thus, based on the successful use of anti-CD20 mAb therapy for B cell depletion in humans, the current results forecast successful B cell depletion in humans by using unconjugated CD19 mAbs.

CD19 mAb-induced B cell depletion depended on FeRy expression (Fig. 2F) and was reduced when macrophage numbers were reduced (Fig. 2B). This finding implicates macrophage-mediated ADCC as the likely mechanism for B cell elimination. Thus, target antigen density and the numbers of mAb molecules that are able to bind B cells and mediate FcR-mediated depletion may be important factors influencing mAb therapeutic efficacy. Consistent with this finding, combination treatments including CD19 and CD20 mAbs were more effective than either treatment alone (Fig. 3), which may be clinically important in cases where CD20 expression is limiting, yet CD19 is expressed at high densities. Thereby, the increased CD19 density on peritoneal B1a cells (19) may explain their sensitivity to CD19 mAb treatment (Fig. 1G) relative to CD20 mAb-mediated depletion (34). CD19 mAb engagement of CD19 alone does not explain the degree of B cell depletion observed in the current studies because signal-inducing IgA anti-mouse CD19 mAbs (19) do not deplete B cells in vivo (S. Sato and T.F.T., unpublished observations). Likewise, a rat IgG2a anti-mouse CD19 mAb (1D5) down-regulates CD19 expression and reduces peritoneal B1a cell numbers by inhibiting replication rather than accelerating turnover, without affecting conventional spleen B cells. Thus, the induction of ADCC by unconjugated IgG anti-CD19 mAbs is likely to contribute most significantly to B cell depletion.

In addition to eliminating the majority of normal B cells, CD19 mAb treatment prevented leukemia and death in mice transplanted with CD19+CD20- immature B cell tumors (Fig. 4). Indeed, a chimeric version of the FMC63 mAb used in the current study is not...
cytotoxic in vitro in the presence of complement or cells capable of
mediating ADCC (29), but reduces tumor size by ~30% in human B
lymphoma cell line xenotransplantants (28). The CLB-CD19 and
HD37 mAbs have also exhibited variable tumoricidal effects in
different mouse xenotransplantation studies (38–41), especially
when given in combination with interleukin-2 (42). Non-Hodgkin's
lymphoma patients have also been treated with an unconjugated
mouse anti-CD19 mAb (43, 44). Circulating tumor cells were
temporarily reduced in two of six patients after CLB-CD19 mAb
infusion. One patient achieved partial remissions after two periods
of mAb treatment, whereas one patient showed a minor response.
In a subsequent trial carried out in combination with interleukin-2,
a partial remission occurred in one patient, with a >50% reduction
of circulating B cells in a leukemic patient (44). However, circu-
lating B cell numbers where not changed in four of five remaining
patients. A mouse IgG1 CD19 mAb-based immunotoxin has also shown
safety and some efficacy in vivo during phase I/II clinical trials for B cell malignancies (45, 46). Thus, although studies
using CD19 mAbs for human therapy first appeared promising, this
treatment strategy has not been pursued further. However, in the
above cases, it is possible that the immune status of the patients
might have compromised their ability to generate adequate ADCC-
mediated tumor removal, or the therapeutic benefit of CD19 mAb
treatment could have been obscured by the mouse origins of most
CD19 mAbs used, dosing, irradiation of recipient mice, or the
unknown ability of each mAb to interact with FcR in vivo. Regardless, CD19 mAb therapy had no unexpected toxicities,
while the current study suggests that CD19 mAb treatment offers
an effective therapy for treating B cell lineage malignancies, especially
in patients with tumors that lack or express only low-level CD20.
CD19 is expressed at high levels on nearly all malignant B cells [80%
of acute lymphoblastic leukemias, 88% of B cell lymphomas, and
100% of B cell leukemias (13, 14)], whereas there is more heter-
ogeneity in CD20 expression among lymphoma types (7, 47).
CD19 mAb treatment also reduced humoral immune responses
and autoantibody production in hCD19TG mice (Fig. 5). These
dramatic reductions are likely to reflect the elimination of both
immature and mature B cells from bone marrow and tissues. CD19
dexpression by some normal human plasma cells has also been
reported (48–50), which may also be true in hCD19TG mice based
on the results of this study. These data suggest that primary and
recall Ab responses can be attenuated by effective B cell depletion
after CD19 mAb therapy. By contrast, CD20 mAb treatment of mice
does not reduce serum Ig levels or early IgM responses to
antigens, which may reflect the continued generation of immature
bone marrow B cells or the durability of B1a cells after CD20 mAb
treatment (34). The ability of CD19 mAb treatment to reduce
immune responses may also represent a treatment strategy when
humoral immunity should be avoided such as with transplantation,
immunotherapy with chimeric mAbs, or with immunotoxin thera-
pies. Moreover, the ability of CD19-directed therapies to deplete B
cells before antigen receptor selection may allow the effective
depletion of pathogenic B cell clones and their autoantibody
products and thereby reestablish normal tolerance mecha-
isms. Regardless, the current studies demonstrate that CD19-
directed therapies are effective for B cell depletion in these mouse
models of leukemia/lymphoma and autoimmunity.

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