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Importance of Cellular Microenvironment and Circulatory Dynamics in B Cell Immunotherapy¹

Qian Gong,* Qinglin Ou,* Shiming Ye,* Wyne P. Lee,* Jennine Cornelius,† Lauri Diehl,** Wei Yu Lin,* Zhilan Hu,* Yanmei Lu,‡ Yongmei Chen,§ Yan Wu,∥ Y. Gloria Meng,∥ Peter Gribling,* Zhonghua Lin,* Kathy Nguyen,* Thanhvien Tran,* Yifan Zhang,* Hugh Rosen,¶ Flavius Martin,* and Andrew C. Chan²*

B cell immunotherapy has emerged as a mainstay in the treatment of lymphomas and autoimmune diseases. Although the microenvironment has recently been demonstrated to play critical roles in B cell homeostasis, its contribution to immunotherapy is unknown. To analyze the in vivo factors that regulate mechanisms involved in B cell immunotherapy, we used a murine model for human CD20 (hCD20) expression in which treatment of hCD20⁺ mice with anti-hCD20 mAbs mimics B cell depletion observed in humans. We demonstrate in this study that factors derived from the microenvironment, including signals from the B cell-activating factor belonging to the TNF family/BLyS survival factor, integrin-regulated homeostasis, and circulatory dynamics of B cells define distinct in vivo mechanism(s) and sensitivities of cells in anti-hCD20 mAb-directed therapies. These findings provide new insights into the mechanisms of immunotherapy and define new opportunities in the treatment of cancers and autoimmune diseases. The Journal of Immunology, 2005, 174: 817–826.

Monoclonal Abs represent a cornerstone in the therapeutic armamentarium for cancers and autoimmune disorders. These therapeutic mAbs neutralize pathogenic cytokines (e.g., anti-TNF mAbs for rheumatoid arthritis), inhibit cellular proliferation (e.g., heceptin for Her-2⁺ breast cancers), induce agonistic or inhibitory receptor activities to regulate cellular functions (e.g., anti-CD3 mAb for transplantation rejection), and eliminate malignant and pathogenic cells by targeting cell surface Ags (1–3). Rituximab, an anti-CD20 mAb is a prototype of the latter category and is efficacious in the treatment of B cell malignancies and autoimmune disorders by depleting malignant and, presumably, pathogenic B cells, respectively (4, 5).

CD20, a four transmembrane glycoprotein expressed on both normal and malignant B cells, is regulated during B cell differentiation and extinguished when B cells undergo terminal differentiation into plasma cells (6–8). Although B cell depletion by rituximab reportedly involves immune effector cells, complement proteins, and proapoptotic mechanisms, the functional consequences of B cell depletion and the in vivo factors that regulate B cell immunotherapy are not well understood (reviewed in Ref. 9). A recent report using a panel of anti-mouse CD20 mAbs demonstrated a role for monocytes as the dominant effector cell through FcRI- and FcRIII-dependent pathways (10). In this study, we demonstrate a greater complexity involving the circulatory dynamics of B cells and survival factors from the microenvironment as critical regulators that determine the mechanistic basis for immunodepletion through complement or the reticuloendothelial system (RES)³. These data provide insights into the effectiveness of B cell immunotherapy and provide additional strategies by which to enhance therapeutic efficacy.

Materials and Methods

Generation of human CD20⁺ mice

Generation of human CD20 (hCD20⁺) mice was accomplished through the use bacterial artificial chromosomes (BAC) incorporating the hCD20 locus. Two independent BACs were injected into blastocysts derived from FVB mice to generate multiple transgenic (Tg) founder lines that expressed hCD20. Two founder mice that transmitted hCD20 expression were subjected to more detailed analysis. Both founder lines demonstrated identical patterns of hCD20 expression and hence data from only one founder line will be presented in this report. The expression level on hCD20 Tg⁺ mice, as determined by mean fluorescence intensity, was ~50% in level when compared with human circulating B cells (data not shown). This difference could be due to differences in transcriptional compatibility between species, the presence of the mouse CD20 gene, enhancer elements required for efficient hCD20 mRNA transcription not found within the BAC or the integration site of the hCD20 BAC. The latter was less likely because all founder lines expressed identical mean fluorescence intensity levels of hCD20 expression (data not shown).

Abs and FACS staining

Two anti-hCD20 mAbs, rituximab and 2H7, were used for immunodepletion studies. All other Abs were purchased from Becton Dickinson and BD Pharmingen. Cell surface expression of molecules was determined using FACSscan and FACSCalibur machines.

Mobilization of marginal zone (MZ) compartment B cells

Mice were pretreated with control IgG2a 3 days before the initiation of the study (day 3) to minimize nonspecific effects of IgG on cellular trafficking. At day 0, mice were treated with 0.2 mg control IgG2a or anti-hCD20 mAb. Mice were injected i.v. on day 2 with 0.1 mg anti-CD11a (M17) and 3 Abbreviations used in this paper: RES, reticuloendothelial system; hCD20, human CD20; BAC, bacterial artificial chromosome; Tg, transgenic; GC, germinal center; MZ, marginal zone; CVF, cobra venom factor; FO, follicular; NHL, non-Hodgkin’s lymphoma.

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anti-αi integrin (PS2) mAbs and blood analyzed 1.5 and 6 h following the administration of the anti-integrin mAbs. For studies with LPS, the anti-integrin mixture was substituted with 25 μg of LPS treatment and mice analyzed 6 h following LPS treatment.

**Inhibition of lymphocyte egress**

Human CD20 Tg+ mice were treated by oral gavage with vehicle control or compound SEW2871 (10 mg/kg every 6 h). A single dose of control or anti-hCD20 mAb (0.5 mg i.p.) was administered 2 h after the first dose of compound SEW2871. B and T lymphocytes isolated from lymph nodes 20 h following treatment.

**Generation of chimeric mice**

Chimeras were generated by transferring 15 × 10⁶ bone marrow-derived cells from hCD20- (100.0 chimeras) or 7.5 × 10⁶ bone marrow-derived cells each from hCD20+ and hCD20- littermates (50:50 chimeras) into irradiated hCD20- littermates (2 doses of 525 rads). The latter mixture resulted in 35–60% hCD20+ Tg+ B cells in three independent experiments. Five weeks following transfer, mice were treated with 0.2 mg of control IgG2a or anti-hCD20 mAb and analyzed 7 days following treatment.

**Inhibition of complement by cobra venom factor (CVF)**

Mice were treated with daily doses of CVF (2 μg/mouse, i.p.) for three consecutive days. One hour following the second dose of CVF, mice were treated with either 0.1 mg of control IgG or anti-hCD20 mAb. Serum C3 levels were monitored using a cell-based FACS assay (Sigma-Aldrich).

**Depletion of NK, polymorphonuclear neutrophils, and macrophages**

Mice were treated with anti-NK1.1 (0.2 mg/mouse, i.p.) or anti-Gr-1 (0.25 mg/mouse, i.v.) mAbs to deplete NK and neutrophils, respectively. Treatment with control IgG2a (0.1 mg) or anti-hCD20 mAb (0.1 mg) on day 2 was followed by FACS analysis of circulating or splenic B220- MC, MZ or follicular (FO) B cells on day 4.

For macrophage depletion, mice were pretreated with buffer or clodronate-loaded liposomes. One day later, mice were then treated with either 0.1 mg of control IgG2a or anti-hCD20 mAb.

**Results**

**Hierarchy of B cell subset susceptibilities**

To understand the in vivo mechanisms by which therapeutic mAbs eliminate cells by targeting cell surface Ags, we used a murine model with an integrated BAC encoding hCD20 that recapitulates the developmental expression of hCD20 in the mouse B cell lineage under its own genetic regulatory elements. Expression of hCD20 was readily detected at the immature B cell stage in the bone marrow and was highly regulated during differentiation (Fig. 1A). Only B220+ peripheral blood cells expressed hCD20 (Fig. 1B). Immunohistochemical analysis of splenic tissue derived from these hCD20 Tg+ mice revealed colocalization of hCD20 staining with IgM among the B cell zones (Fig. 1C). Paralleling human B lineage cells, hCD20 was not colocalized with IgMhigh staining plasma cells by immunohistochemical analysis (Fig. 1C, white arrows) nor on syndecan-1+ plasma cells by FACS analysis (data not shown). This model also preserved the Ag epitopes of hCD20 and Ab specificities of two anti-hCD20 mAbs, rituximab and 2H7. Rituximab is a chimeric anti-hCD20 mAb approved in the treatment of non-Hodgkin’s lymphoma (NHL) and 2H7 is a humanized anti-hCD20 mAb in clinical development in autoimmune disorders (reviewed in Ref. 9).

Similar to the depletion of peripheral B cells observed in patients treated with rituximab or 2H7, administration of a mouse anti-hCD20 (2H7) mAb resulted in complete and reversible depletion of peripheral blood B cells (Fig. 2A, top row, Fig. 2B, and data not shown). Because hlgG1 demonstrates substantially different binding to human and murine FcγRs, the remaining studies used an anti-hCD20 mAb (2H7) with a murine IgG2a Fc backbone, which best recapitulates hlgG1-hFcγRI/HFcγRIII interactions. Consistent with the lack of expression of hCD20 in the mouse early B cell progenitor population, only CD20+ immature and recirculating mature B cells in the bone marrow were depleted (Fig. 2C).

In addition to the depletion of circulating B cells, treatment also resulted in depletion of B220+ cells from lymph nodes and peritoneal cavity of hCD20 Tg+ mice (Fig. 2A, middle and bottom row). Intriguingly, the kinetics of depletion differed among these three compartments. Although >90% of circulating B cells were depleted within 3 h following i.v. administration of anti-hCD20 mAbs, lymph node B cells were depleted within 2 days, and peritoneal B cells required ~21 days for >90% depletion despite i.p. administration of the anti-hCD20 mAb. Because peritoneal B cells recirculate more slowly than lymph node B cells, the distinct kinetics of depletion paralleled the kinetics of lymphocyte circulation (11).

Although circulating mature B cells were completely depleted, ~33% of B220+ splenocytes remained following anti-hCD20 mAb treatment (Fig. 3A). Analysis of splenic B cell subsets revealed that FO B cells were significantly depleted (>90% depletion). Additional analysis of 2-wk-old mice, in which immature B cells are more abundantly represented, demonstrated a similar >90% depletion of both T1 and T2 immature B cell subsets following anti-hCD20 mAb treatment (data not shown). In contrast, the CD21highCD21low MZ B cell compartment exhibited greater apparent resistance to anti-hCD20 mAb depletion. Approximately 50% of these cells remained following anti-hCD20 mAb therapy (Fig. 3B). Resistance was not due to the lack of hCD20 expression in MZ B cells as hCD20 was expressed at a higher level in MZ as compared with FO B cells (Fig. 1A), nor due to the lack of accessibility of the therapeutic mAb as CD20 on resistant splenic B cells were nearly saturated with the in vivo administered anti-hCD20 mAb (Fig. 3C). Neither administration of anti-hCD20 mAb up to 10 mg/mouse (~15-fold greater than the clinical dose of rituximab for treatment of NHL) nor continued treatment of mice every other week for 4 mo with anti-hCD20 mAb resulted in any greater depletion of the MZ B cell compartment (data not shown).

In contrast to the relative resistance of the MZ compartment, germlinal center (GC) B cells resident within Peyer’s patches demonstrated greatest resistance to anti-hCD20 mAb treatment. Although mature B220+CD38high B cells were readily depleted, the B220+CD38low GC B cells were resistant to anti-hCD20 mAb therapy (Fig. 3D). To extend our observations on Peyer’s patch resident GC B cells, we tested whether splenic GC B cells, generated through immunization with SRBC, were similarly resistant. As GCs are maximally formed by day 8 following immunization, mice were treated on day 8 with anti-hCD20 or control IgG mAbs. Although unimmunized mice did not develop B220+ PNA+ GC B cells, SRBC immunized mice developed PNA+ GC B cells that were resistant to anti-hCD20 mAb killing (Fig. 3E). Resistance was independent of: 1) hCD20 expression, as both Peyer’s patch resident or splenic GC B cells expressed higher levels of hCD20 than the sensitive mature circulating B cells (Fig. 1A and data not shown); 2) binding of mAb to GC cells, as in vivo recovered GC B cells were saturated with the administered mAb; 3) treatment dose; and 4) duration of treatment (data not shown). Hence, a hierarchy of sensitivity to anti-hCD20 mAb treatment exists for B cells within different splenic microenvironments with FO (most sensitive) > MZ > GC (most resistant) compartments.

The residual B cells in treated mice were functional as anti-hCD20 mAb-treated mice were capable of mounting substantial, albeit reduced, immune responses to immunogens and bacteria (data not shown). Conversely, baseline serum IgM levels were not decreased following 4 wk of anti-hCD20 mAb treatment (Fig. 2D). Because the serum half-life of IgG is longer than IgM, we assessed
the effects of chronic administration of anti-hCD20 mAb (>12 mo of anti-hCD20 mAb every other week) on serum IgG levels. Serum IgG1, IgG2b, and IgG3 were not altered with long-term chronic anti-hCD20 mAb treatment (Fig. 2E). Interpretation of the serum IgG2a levels was not possible because the administered anti-hCD20 IgG2a mAb interfered with measurement of the serum IgG2a levels. These data are consistent with the differential effects observed on self- and non-self-reactive Abs in patients treated with rituximab (reviewed in Ref. 12).

Intravascular access as a major determinant of susceptibility

To explore factors that contribute to the relative resistance of the MZ B cell compartment, we first evaluated the intrinsic sensitivity of the MZ B cells to anti-hCD20 mAb-mediated depletion. MZ B cells were mobilized into the vasculature with the coadministration of anti-α4 and anti-α6 integrin mAbs (Fig. 4A; panels 1–3) (13). Mobilization of CD21highCD23low MZ B cells rendered them sensitive to anti-CD20 mAb-mediated depletion (compare Fig. 4A, panel 2 vs panel 5 and panel 3 vs panel 6, and Fig. 4B). Histologic analysis of the spleen confirmed the preferential depletion of MZ B cells outside of the MOMA-1 staining marginal sinus with the combined treatment of anti-integrins and anti-hCD20 mAbs as compared with anti-hCD20 mAb alone (Fig. 4D, panel 2 and panel 3). In contrast, mobilization of cells from the MZ into the follicle with administration of LPS did not result in depletion of the CD21highCD23low MZ splenic B cells (Fig. 4C, and D, panel 4) (14). Together, these data indicate that MZ B cells are not intrinsically resistant to anti-hCD20 mAb-mediated killing and that trafficking of MZ B cells into the vasculature can render them susceptible to depletion.

Conversely, we tested whether retaining mature B cells within the lymph node would interfere with B cell depletion. Mice, treated with vehicle control or a sphingosine 1-phosphate receptor ligand (compound SEW2871), were treated with anti-hCD20

FIGURE 1. Generation of a mouse model of hCD20 Tg expression. A. Surface expression of hCD20 during B cell ontogeny. B cell progenitors and subsets in the bone marrow (top row), spleen (middle row), and other lymphoid organs (bottom row) were analyzed for hCD20 expression. The thick red lines represent staining for hCD20 from a hCD20 Tg+ mice and the thin blue lines represent hCD20 staining from a control hCD20 Tg+ littermate. Mean fluorescence intensity MN is represented numerically in each plot. These data are representative of over 20 independent analyses. B. Expression of hCD20 in circulating lymphocytes. Peripheral lymphocytes were analyzed for surface expression of B220 and CD3 (upper left panel), B220+ (upper right), CD3+ (lower right), and CD3+ B220+ (lower left) cells were analyzed for hCD20 expression. Expression of hCD20 was not detectable on Tg+ littermates (shaded). C. Immunohistochemical analysis of hCD20 expression in Tg+ mice. Spleens from Tg+ (left column) or Tg- (right column) mice were stained for IgM (green), hCD20 (red), or CD5 (blue). Single staining for IgM (top panels) and hCD20 (middle panels) is shown. The bottom panel represents merging of all three markers.
mAbs (15). Consistent with the inhibitory effects of sphingosine 1-phosphate receptor ligands on lymphocyte egress from lymph node to circulation (16), both B and T cells were significantly decreased in the blood of mice treated with compound SEW2871 (Fig. 4F). Although lymph node B cells were readily depleted by anti-hCD20 mAbs in vehicle-treated mice, they were minimally affected by anti-hCD20 mAbs in the presence of compound SEW2871 (Fig. 4E, left panel). The effect was specific to B cells as no effects were observed on T cells (Fig. 4E, right panel). Together, these data support the requirement for B cells to access the circulation for efficient depletion.

**Contributions of microenvironment to B cell susceptibility**

Although access to the intravascular compartment was important for efficient B cell killing, we further analyzed the roles of the microenvironment to anti-hCD20 mAb-mediated depletion. We first used cellular competition experiments to evaluate the importance of the microenvironment. Chimeric mice were generated from 100% hCD20+/H11001 or a mixture of hCD20+/H11001:hCD20+/H11002 bone marrow-derived cells. Similar to the hCD20 Tg+ mice (Fig. 3A), treatment of chimeric mice generated from 100% hCD20+ bone marrow resulted in 50% depletion of the MZ B cell compartment (Fig. 5A, top panels). In contrast, treatment of chimeric mice derived from the mixture of hCD20+/H11001:hCD20+/H11002 bone marrow with anti-hCD20 mAbs resulted in >90% depletion of the hCD20+ MZ B cell compartment (Fig. 5A, bottom panels). Conversely, >99% of FO B cells were depleted from both groups of chimeric mice (data not shown). Hence, cellular competition, potentially reflected by B cell survival factors within the MZ microenvironment, can alter the threshold of anti-hCD20 mAb-mediated killing.

As an example of such survival factors, we analyzed the contribution of the B cell-activating factor belonging to the TNF family (BAFF)/BLyS/TALL-1 member of the TNF superfamily (17). BAFF/BLyS/TALL-1 plays an important role in the survival and maturation of immature T2, FO, and MZ B cells and enhances
competitive survival of autoreactive B cells (18–20). Overexpression of a soluble form of BAFF/BLyS/TALL-1 in mice results in B cell hyperplasia, hypergammaglobulinemia, and autoimmune lupus-like syndrome (21). Conversely, treatment of lupus-prone mice with a BAFFR/BR3-Fc fusion protein, which neutralizes BAFF/BLyS, results in improved autoimmune serologies, renal pathology, and mortality (22). As expected, treatment of hCD20+ mice with BAFFR/BR3-Fc for ~2 wk resulted in a marked decrease in MZ and T2/FO B cells (Fig. 5B, panel 3). Combined treatment of BAFFR/BR3-Fc and anti-hCD20 mAb, surprisingly, resulted in the depletion of all splenic B cell subsets (Fig. 5B, panel 4). To further explore the potential synergy of BAFF neutralization and anti-hCD20 mAb, we quantified the extent of B cell loss 4 days following treatment with single doses of anti-hCD20 mAb and BAFFR/BR3-Fc. Although treatment with single doses of anti-hCD20 mAb or BAFFR/BR3-Fc resulted in ~40–50% loss of MZ B cells and ~33–70% loss of FO B cells, the combination anti-hCD20 mAb and BAFFR/BR3-Fc resulted in >90% loss of MZ and FO B cells (Fig. 5C). Hence, survival factors also play an important role in determining susceptibility to anti-hCD20 mAb-mediated B cell depletion.

**Effector mechanisms required for depletion**

To analyze the effector mechanisms important for in vivo depletion, we assessed the contributions of complement and Fc receptor effector function. In vivo depletion of C3 by administration of CVF (23) did not affect the ability of wild-type anti-hCD20 mAbs to deplete circulating, lymph node, or splenic FO B cells (Fig. 6A, panels 1, 2, and 5). In contrast, the absence of C3 abrogated the ability of anti-CD20 mAbs to deplete MZ B cells (Fig. 6A, panel 4). A similar requirement of C3 for MZ B cell depletion was also observed in hCD20+ C3−/− mice (data not shown). Hence, the MZ B cell compartment exhibits a selective dependence on complement for anti-hCD20 mAb killing.

As Fc receptor binding has been demonstrated to be required for the efficacy of rituximab in the elimination of CD20+B cell xenografts (24), we assessed the contributions of Fc effector function through the use of an anti-hCD20 mAb with two mutations within the Fc effector domain (D265A, N297A). This mutant exhibited no binding to FcγR (RI, RII, and RIII) but also a partial reduction in complement activation (data not shown). Treatment of mice with the anti-hCD20 (D265A, N297A) mutant resulted in the loss of ability of the mutant anti-CD20 mAb to deplete circulating, lymph node, and splenic FO B cells (Fig. 6B, panels 1, 2, and 5). This was not due to altered pharmacodynamics as recovery of these B cells demonstrated saturation of surface CD20 with the in vivo administered mutant anti-hCD20 mAb (data not shown). In contrast, MZ B cells were still depleted by the mutant anti-hCD20 mAb though to a lesser degree than wild type anti-CD20 mAb (Fig. 6B, panel 4). The intermediate depletion of MZ B cells observed with the D265A, N297A mutant was likely due to the partial loss of complement activation. Consistent with this idea, administration of CVF reversed the partial depletion by the mutant anti-CD20 mAb (Fig. 6C). Hence, different mechanisms appear important for depletion of distinct splenic B cell compartments with complement dependent mechanisms playing a dominant role in the MZ B cell compartment and Fc receptor-mediated mechanisms in the elimination of circulating, lymph node, and splenic FO B cells.

We next assessed the cellular components (neutrophils, macrophages, and NK cells) required for depletion of circulating, lymph
node, and FO B cells. Depletion of macrophages using clodronate-loaded liposomes resulted in loss of B cell killing (Fig. 7A, panel 3 and panel 4) (25). Greater than 90% of B cells remained in circulation despite anti-hCD20 mAb treatment and these cells were saturated with the in vivo administered mAb (Fig. 7A, panel 3 and data not shown). In contrast, depletion of NK cells and neutrophils did not affect B cell killing (data not shown). Hence, macrophages play important roles as effectors in anti-hCD20 mAb-mediated B cell depletion.

Because macrophages within the RES represent a major modality for clearance of apoptotic cells and immune complexes (26), we examined the contributions of the spleen and liver to B cell depletion. Surprisingly, splenectomy mice did not compromise the ability of anti-hCD20 mAbs to deplete B cells, but actually resulted in accelerated B cell depletion (Fig. 7B), an effect that was likely secondary to reduced B cell numbers in splenectomized mice. Compromise of blood flow to the liver through ligation of both portal vein and celiac artery resulted in a significant loss in the depleting ability of anti-hCD20 mAbs (Fig. 7C). Histological examination of livers 15 min following anti-hCD20 mAb administration demonstrated colocalization of B220+ staining B cells within F4/80+ staining macrophages in anti-hCD20 mAb-treated, but not control IgG-treated mice.
Hence, consistent with the function of the RES, the liver and, to a lesser degree, the spleen represents the major site of depletion of circulating B cells (data not shown).

**Discussion**

These data identify two in vivo mechanisms by which anti-hCD20 mAbs eliminate B cells. Upon administration of anti-hCD20 mAbs, the mAb rapidly binds CD20<sup>+</sup> B cells and circulating B cells are cleared through the RES. B cells residing within lymphoid tissues, including splenic FO B cells, Peyer's patch mature B cells, lymph node B cells, and peritoneal cavity B cells require access to the vasculature to deliver the targeted B cells to effector cells within the RES. This mechanistic model accounts for the longer durations required for depletion of slower recirculating peritoneal and lymph node B cells as compared with the circulating B cells. Accordingly, compromise of RES function, as has been described in some patients with systemic lupus erythematosus, may contribute to observations of a lower efficiency of peripheral B cell clearance by rituxan in systemic lupus erythematosus patients as compared with patients with rheumatoid arthritis (5, 27, 28).
In contrast to the mechanism used for circulatory B cells, B cells within the MZ compartment, with reduced circulatory capacity, exhibit a greater dependence on complement-dependent, rather than Fc receptor, mediated mechanisms for depletion. This notwithstanding, not all B cells within the MZ compartment are depleted even with longer duration of anti-hCD20 mAb treatment and the extent of depletion of the MZ compartment varies among different mouse genetic backgrounds (data not shown). Hence, other factors, including cellular competition, microenvironment, and differential expression of inhibitory proteins likely contribute to additional mechanistic determinants of sensitivity. As an example of the latter, both CD55 and CD59 complement regulatory proteins are highly regulated during B cell differentiation and activation that, in turn, may alter the thresholds for complement mediated lysis (data not shown). Expression of CD59 on leukemic cells and NHL-derived cell lines has been reported to be associated with resistance; conversely, neutralization by an anti-CD59 blocking mAb restores sensitivity to anti-CD20 mAb-mediated killing (29, 30). Our studies also illustrate the importance of the BAFF/BLyS survival factor in defining the threshold for anti-hCD20 mAb-mediated killing. As this survival factor has been recently demonstrated to promote NHL B cell survival (31), elucidation of factors contributing to resistance of MZ (e.g., BAFF/BLyS) and GC (e.g., CD40/CD40L) B cell compartments may parallel those used by lymphomas that are not responsive or relapse following rituximab treatment (32, 33).
Similar to the study of the in vivo importance of the FcRI and FcγRIII pathways in B cell depletion by anti-mouse CD20 has been recently recognized (8). However, in contrast to our study, the effect of anti-mouse CD20 mAb depletion in the splenic compartment was more dramatic. Depletion of B220⁺ lymphocytes (~90%) was achieved with a panel of IgG2a anti-mouse CD20 mAbs. In contrast, our studies demonstrate only ~70% depletion of B220⁺ B cells. These differences in the sensitivity of the MZ B cell compartment may reflect differences between anti-CD20 mAbs directed against mouse and human CD20 Ags; the epitopes within hCD20 reactive with rituximab or 2H7 are not conserved in mouse CD20. Additionally, strain differences in the genetic backgrounds used in these studies may also contribute to the apparent differences. Finally, the composition of the residual B cell compartment following B cell depletion and sensitivity of GC B cells to anti-mouse CD20 was not examined in this recent report (8). Differential sensitivity of GC and MZ B cells to anti-hCD20 mAbs has also been described in cynomolgous monkeys, though these differences appear more prominent in the lymph node than the spleen (34, 35). Hence, species- and strain-specific differences in lymphocyte circulation, complement activation, environmental factors (e.g., infections) that alter circulatory dynamics and survival factors together modulate the hierarchy of B cell sensitivities in rituximun immunotherapy.

The contributions of microenvironment and the distinct in vivo effector mechanisms may also be reflected in the recently identified genetic factors associated with a more favorable prognosis in rituxan immunotherapy. Genetic polymorphisms in HLA class II genes and the HLA-DRB1*15:01, HLA-DRB1*15:02, and HLA-DRB1*15:03 alleles have been associated with a more favorable outcome in patients with NHL (90%). However, in contrast to our study, the effect of HLA DRB1*15:02 genotype on degree of B cell depletion by rituximab in the treatment of systemic lupus erythematosus. Arthritis Rheum. 48:453.

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