B Cell Depletion Inhibits Spontaneous Autoimmune Thyroiditis in NOD.H-2h4 Mice

Shiguang Yu, Robert Dunn, Marilyn R. Kehry and Helen Braley-Mullen

*J Immunol* 2008; 180:7706-7713; doi: 10.4049/jimmunol.180.11.7706

http://www.jimmunol.org/content/180/11/7706

References
This article cites 64 articles, 30 of which you can access for free at:
http://www.jimmunol.org/content/180/11/7706.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
B Cell Depletion Inhibits Spontaneous Autoimmune Thyroiditis in NOD.H-2h4 Mice

Shiguang Yu,*† Robert Dunn,§ Marilyn R. Kehry,§ and Helen Braley-Mullen2*‡

B cells are important for the development of most autoimmune diseases. B cell depletion immunotherapy has emerged as an effective treatment for several human autoimmune diseases, although it is unclear whether B cells are necessary for disease induction, autoantibody production, or disease progression. To address the role of B cells in a murine model of spontaneous autoimmune thyroiditis (SAT), B cells were depleted from adult NOD.H-2h4 mice using anti-mouse CD20 mAb. Anti-CD20 depleted most B cells in peripheral blood and cervical lymph nodes and 50–80% of splenic B cells. Flow cytometry analysis showed that marginal zone B cells in the spleen were relatively resistant to depletion by anti-CD20, whereas most follicular and transitional (T2) B cells were depleted after anti-CD20 treatment. When anti-CD20 was administered before development of SAT, development of SAT and anti-mouse thyroglobulin autoantibody responses were reduced. Anti-CD20 also reduced SAT severity and inhibited further increases in anti-mouse thyroglobulin autoantibodies when administered to mice that already had autoantibodies and thyroid inflammation. The results suggest that B cells are necessary for initiation as well as progression or maintenance of SAT in NOD.H-2h4 mice. The Journal of Immunology, 2008, 180: 7706–7713.

S

pontaneous autoimmune thyroiditis (SAT) is in NOD.H-2h4 mice, a chronic organ-specific autoimmune disease characterized by infiltration of the thyroid by CD4+ and CD8+ T cells, B cells, and plasma cells and destruction of thyroid follicles by infiltrating inflammatory cells. All NOD.H-2h4 mice develop SAT when they are given 0.05% NaI in their drinking water (1–6). All mice produce anti-mouse thyroglobulin (MTg)-specific autoantibodies, and autoantibody levels generally correlate with SAT severity scores (1, 2). CD4+ T cells are the primary pathogenic effector cells for SAT and are required for both initiation and maintenance of SAT (1–3). B cells are required for development of SAT, because B cell-deficient NOD.H-2h4 mice do not develop SAT (7). Our previous studies suggested that B cells were required for activation of pathogenic CD4+ T cells, possibly functioning as important APCs (7).

B cells are important for development of most spontaneous autoimmune diseases including SAT, diabetes in NOD mice, systemic lupus erythematosus (SLE) in MRL/Mp-lpr/lpr mice, scleroderma in tight-skin mice, and arthritis in K/BxN mice (7–12). B cells produce autoantibodies that contribute to the pathogenesis of autoimmune diseases by multiple mechanisms (13). However, B cells, including B cells from transgenic mice unable to secrete Ab, can also function in disease pathogenesis, e.g., as important APCs for development of proinflammatory T cell responses (7–9, 14–18).

Rituximab is a chimeric mAb that recognizes human CD20, a marker expressed on essentially all mature B cells (13, 19, 20). Rituximab depletes B cells, and it has been used for treatment of lymphoma, rheumatoid arthritis, and SLE in humans (13, 20–22). Although rituximab has been effective for treating several autoimmune diseases and lymphomas, the basis for its efficacy is not entirely clear because the extent of B cell depletion in tissues other than the blood is unknown, and many patients who have benefited from rituximab (anti-CD20) therapy do not have remarkable decreases in autoantibody levels (13, 20–22). These results suggest that the therapeutic benefits of rituximab do not result simply from decreased autoantibodies and indicate a need for further mechanistic studies.

Because studies in humans are generally limited to assessment of B cell depletion in peripheral blood, and peripheral blood B cells represent <2% of all B cells (23), mechanistic studies in animal models are needed to determine the mechanisms by which anti-CD20 antibody therapy inhibits autoimmune diseases. Until recently, effective reagents for depleting B cells in adult mice were not available. Abs directed against murine CD20 are now available and have shown substantial in vivo depletion of B cells in normal mice (19, 24–30), as have mAb to human CD20 (hCD20) in transgenic mice that express hCD20 on B cells (25, 29, 31). These reagents provide new tools to study in detail the role of B cells in autoimmune diseases.

In this study, mAb directed against murine CD20 were used to determine whether B cell depletion would inhibit development of SAT in NOD.H-2h4 mice. The results indicate that even though most splenic marginal zone (MZ) B cells were resistant to depletion, anti-CD20 markedly inhibited development of SAT and suppressed further development of thyroid lesions and increases in autoantibody production in mice that already had mild SAT when treatment was initiated.

*Research Service, Harry S. Truman Memorial Veterans Affairs Hospital, Columbia, MO 65201; †Department of Internal Medicine and ‡Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65212; and §Biogen Idec, San Diego, CA 92122

Received for publication January 22, 2008. Accepted for publication April 2, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by a Merit Review Grant from the Department of Veterans Affairs, the Arthritis National Research Foundation, Children’s Miracle Network, and the Lottie Caroline Hardy Trust.

2 Address correspondence and reprint requests to Dr. Helen Braley-Mullen, Department of Internal Medicine, Division of Immunology and Rheumatology, University of Missouri, M307 Health Science Center, One Hospital Drive, Columbia, MO 65212. E-mail address: mullenh@health.missouri.edu

3 Abbreviations used in this paper: SAT, spontaneous autoimmune thyroiditis; MTg, mouse thyroglobulin; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; MZ, marginal zone; FO, follicular; CLN, cervical lymph node; hCD20, human CD20.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

www.jimmunol.org
Materials and Methods

**Mice**

NOD.H-2h4 mice express H-2K^k^, I-A^k^, and D^b^ on the NOD background (1, 32). They were maintained in the animal facility at the University of Missouri as previously described (2, 6, 7). Both male and female mice were used, but all mice in an individual experiment were of the same sex. All animal protocols were approved by the University of Missouri Animal Care and Use Committee.

**Anti-CD20 and experimental design**

IgG1 anti-CD20 mAb 18B12 was generated as previously described (29). The IgG2a 18B12 mAb was made by combining the VH and VL regions of the IgG1 hybridoma with mouse y2a and k constant regions, respectively, and expressing the two polypeptide chains in Chinese hamster ovary cells (33). The IgG1 mouse anti-human 2B8 CD20 mAb with no cross-reactivity to mouse CD20 was used as the isotype control for experiments in mice given anti-CD20 IgG1, and the IgG2a form of the same mAb was used as the isotype control for experiments using anti-CD20 IgG2a. The IgG1 and IgG2a 2B8 lgs were expressed in Chinese hamster ovary cells. All isotype control and anti-CD20 mAbs were purified on protein A columns as previously described (29). Anti-CD20 and isotype control mAbs (250–300 μg) were initially injected i.v. with subsequent injections given i.p. Results obtained with either isotype control mAb were comparable and did not differ from those in mice given no treatment (data not shown). As previously described (2, 6, 7), 7- to 8-wk-old mice were given 0.05% NaI in the drinking water to induce SAT, and thyroids were removed 7–9 wk later.

**Evaluation of SAT severity**

After 7–9 wk on NaI water, thyroids were removed, and one thyroid lobe was fixed in formalin, sectioned, and stained with H&E as previously described (2, 6, 7). The other thyroid lobe was snap frozen in liquid nitrogen, and stored at −70°C for use in immunohistochemical staining. All slides were scored by two individuals, one of whom had no knowledge of the experimental groups. Thyroid histopathology scores were based on the percentage of thyroid follicles replaced by infiltrating lymphocytes as previously described in detail (2, 6, 7). Briefly, a score of 0 indicates a normal thyroid, and 0+ indicates a few inflammatory cells infiltrating the thyroids and/or mild follicular changes. A 1+ severity score is defined as an infiltrate of at least 125 inflammatory cells in one or several foci; a 2+ score represents 10–20 foci of cellular infiltration, each the size of several follicles, with replacement or destruction of up to one-fourth of the gland; a 3+ score indicates that one-fourth to one-half of the thyroid follicles are destroyed or replaced by infiltrating inflammatory cells; and 4+ indicates that greater than one-half of the gland is replaced or destroyed.

**Autoantibody determination**

MTg-specific autoantibodies were determined by ELISA using serum from individual mice as previously described (2, 7).

**Immunohistochemical staining**

Frozen thyroids were sectioned and stained as previously described (6) using anti-CD4 (GK1.5; American Type Culture Collection) and anti-B220 (Caltag Laboratories). Biotinylated goat anti-rat-IgG (Caltag Laboratories) was used as secondary Ab, and 0.3% hydrogen peroxide was used to block endogenous peroxidase. Sections were incubated with Vectastain Elite avidin-biotin complex (Vector Laboratories), and peroxidase activity was visualized using Nova Red substrate (Vector). Negative controls used IgG isotype controls as the primary Ab, with the remaining steps performed as described previously. These controls were always negative.

**Flow cytometry**

Peripheral blood, spleen, or cervical lymph node (CLN) cells from experimental mice were analyzed for expression of CD4, CD19, B220, CD21, and CD23 by flow cytometry (FACScan and FACSCalibur) as described previously (7). Abs were obtained from eBioscience or BD Pharmingen.

**Statistical analysis**

A two-tailed Student’s t test was used to determine the significance of differences in SAT severity between different groups. The p values are given in the footnotes to the tables, and p values of <0.05 were considered significant.

**Results**

**Differential susceptibility of B cells in spleen, lymph node, and blood to depletion by anti-CD20**

To determine whether anti-CD20 could effectively deplete B cells in NOD.H-2h4 mice, 7- to 8-wk-old mice were given a single injection of 250 μg of anti-CD20 mAb 18B12 (mouse IgG1 or mouse IgG2a) or the corresponding isotype control i.v. The percentages of total B cells in peripheral blood, CLN, and spleens were determined 4 or 7, 14, and 21 days later. After 4 or 7 days, 80–90% of B cells in peripheral blood (Fig. 1 and Table I) and CLNs (data not shown) were depleted using either of the anti-CD20 mAbs. However, only ~50% of splenic CD19^+^ B cells were depleted after 7–14 days in most mice given a single injection of anti-CD20 IgG1 mAb, whereas depletion of splenic B cells was usually greater (60–70%) after 7 or 14 days in most mice given anti-CD20 IgG2a mAb (Fig. 1 and Table I). Increasing the amount of anti-CD20 to 500–1000 μg did not result in increased depletion of splenic B cells, and a lower dose of 100 μg was less effective (data not shown). B cell depletion in blood, CLNs, and spleen was usually comparable 1 and 2 wk after a single injection of 250–300 μg of either anti-CD20 mAb (Table I). After 3 wk, B cells remained at very low levels in blood, but splenic B cell numbers remained at 50–70% of control levels in some experiments.

![FIGURE 1. B cell depletion by anti-CD20 in peripheral blood and spleen. NOD.H-2h4 mice were given 250 μg of anti-CD20 IgG1 or IgG2a or isotype control i.v. B cell depletion was monitored by flow cytometry in peripheral blood (top) after 4 days and in spleen (bottom) after 7 days. The percentages of CD19^+^ cells are indicated in each panel. Results are representative of five to six individual mice per group.](http://www.jimmunol.org/content/jimmunol/191/8/7707/F1.large.jpg)

<table>
<thead>
<tr>
<th>Isotype control</th>
<th>IgG1 anti-CD20</th>
<th>IgG2a anti-CD20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Spleen</td>
<td>Spleen</td>
</tr>
<tr>
<td>4 days</td>
<td>2 wk</td>
<td>1 wk</td>
</tr>
<tr>
<td>Isotype control</td>
<td>25.5 ± 3.6</td>
<td>19.6 ± 4.9</td>
</tr>
<tr>
<td>IgG1 anti-CD20</td>
<td>3.6 ± 1.2</td>
<td>2.6 ± 1.5</td>
</tr>
<tr>
<td>IgG2a anti-CD20</td>
<td>0.5 ± 0.3</td>
<td>2.6 ± 0.3</td>
</tr>
</tbody>
</table>

* Mice were given a single injection of 300 μg of anti-CD20 IgG1 or IgG2a or the IgG2a isotype control i.v. CD19^+^ B cells in blood and spleen were determined by flow cytometry at different times after injection as indicated. Results represent mean percentages of CD19^+^ B cells in total lymphocytes from 3–6 individual mice. Similar results were obtained in another experiment.
increased to 60–70% of those in mice given either of the isotype control mAbs (Table I and data not shown). In general, B cells in blood began to increase ∼4 wk after a single injection of 250–300 μg of anti-CD20 but did not return to levels seen in mice given isotype control or no mAb for 7–8 wk (data not shown). Although the extent of splenic B cell depletion was variable in the many individual mice examined in these experiments, the overall conclusion is that both IgG1 and IgG2a anti-CD20 mAbs effectively deplete B cells in blood and CLNs of NOD.H-2h4 mice, but depletion of splenic B cells is incomplete.

**MZ B cells in NOD.H-2h4 mice are resistant to depletion by anti-CD20**

Because some splenic B cells were resistant to depletion by anti-CD20, it was of interest to determine whether a particular subpopulation of splenic B cells might be resistant to depletion by anti-CD20. To address this issue, three-color flow cytometry was used to determine the phenotypes of residual splenic B cells in mice given anti-CD20 7 days previously (Fig. 2). B220<sup>+</sup>CD21<sup>high</sup>CD23<sup>low</sup> cells represent MZ B cells, B220<sup>+</sup>CD21<sup>int</sup>CD23<sup>high</sup> cells represent follicular (FO) B cells and B220<sup>+</sup>CD21<sup>high</sup>CD23<sup>high</sup> represent transitional 2 (T2) MZ precursor B cells (34–36). Most FO and T2 B cells were depleted in spleens of anti-CD20 treated NOD.H-2h4 mice. Most residual splenic B cells in mice given anti-CD20 were MZ B cells, although some FO and T2 B cells were also resistant to depletion by both isotypes of anti-CD20 (Fig. 2). As mentioned above, both anti-CD20 mAbs effectively depleted 80–90% of B cells in CLNs and blood, and this is consistent with the fact that MZ B cells are non-circulating; i.e., B cells in blood and LN are primarily FO B cells (Ref. 34 and data not shown).

**Effect of anti-CD20 on development of SAT**

To determine whether anti-CD20 could inhibit development of SAT, 7-wk-old NOD.H-2h4 mice were given 300 μg of IgG1
Effect of anti-CD20 given to mice with SAT

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>SAT Severityb</th>
<th>Anti-MTg IgG(OD)</th>
<th>Splenic B Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotype control</td>
<td>0 1+ 2+ 3+</td>
<td>0.567 ± 0.042</td>
<td>45 ± 3.5</td>
</tr>
<tr>
<td>IgG2a anti-CD20</td>
<td>2 1 0</td>
<td>0.342 ± 0.046</td>
<td>16 ± 3.9</td>
</tr>
<tr>
<td>Isotype control</td>
<td>1 0 6 0</td>
<td>0.626 ± 0.200</td>
<td>41 ± 2.5</td>
</tr>
<tr>
<td>IgG2a anti-CD20</td>
<td>5 5 0</td>
<td>0.124 ± 0.049</td>
<td>21 ± 5.1</td>
</tr>
<tr>
<td>Isotype control</td>
<td>0 1 4 3</td>
<td>0.398 ± 0.05</td>
<td>39.3 ± 0.9</td>
</tr>
<tr>
<td>IgG2a anti-CD20</td>
<td>2 4 2</td>
<td>0.030 ± 0.01</td>
<td>5.7 ± 0.3</td>
</tr>
</tbody>
</table>

a Seven-week-old NOD.H-2h4 mice were given 300 μg of IgG2a anti-CD20 i.v. (lines 2, 4, and 6) or the IgG2a isotype control (lines 1, 3, and 5), and injections were repeated i.p. 3 and 6 wk later. Each isotype vs anti-CD20 pair represents a separate experiment. All mice were given 0.05% NaI in their drinking water at 8 wk of age, and thyroids were removed 2 wk after the last injection.

b Numbers of mice with various degrees of severity of SAT 8 wk after NaI water (p < 0.01; line 1 vs line 2). Anti-MTg IgG (1/100 serum dilutions) expressed as OD410 ± SEM (p < 0.05, line 1 vs line 2).

c Percentages of splenic CD19+ B cells at termination of the experiments (mean ± SEM of all mice in the group) (p < 0.01; line 1 vs line 2).

d Numbers of mice with various degrees of severity of SAT before and after administration of anti-CD20 or IgG2a isotype control. p < 0.05 (line 2 vs line 3 and line 5 vs line 6).

Effect of anti-CD20 on ongoing SAT

Although anti-CD20 is effective for treating established autoimmune diseases in humans, it had little effect when given after disease onset in several murine models (26–28). Therefore, it was important to determine whether SAT would be inhibited by the 18B12 anti-CD20 IgG2a mAb if injections began after mice had begun to develop thyroid lesions and anti-MTg autoantibodies. To address this question, groups of mice were given IgG2a anti-CD20 or isotype control beginning 5 (Table IV, lines 1–3) or 8 (Table IV,
lines 4–6) weeks after NaI water. Thyroids and serum were obtained from groups of five mice in each experiment to confirm the extent of inflammation and levels of autoantibodies present when anti-CD20 injections began (Table IV). Thyroid lesions and anti-MTg autoantibodies were present in most mice 5 (Table IV, line 1) or 8 (Table IV, line 4) weeks after NaI water when anti-CD20 injections began. Other groups of mice in the same experiments were given three injections of IgG2a anti-CD20 or the IgG2a isotype control at 3-wk intervals, and experiments were terminated 2 wk after the last injection. SAT severity was significantly reduced in both experiments in mice given IgG2a anti-CD20 after inflammation had begun to develop (Table IV, line 2 vs line 3 and line 5 vs line 6). When B cell depletion was initiated in mice that had begun to develop SAT and produce autoantibody, circulating anti-MTg autoantibodies in anti-CD20-treated mice were only slightly higher than those present before anti-CD20 treatment (Table IV, line 1 vs line 3 and line 4 vs line 6), whereas anti-MTg autoantibodies in mice given isotype control mAb continued to increase (Table IV). When thyroids were removed for evaluation of thyroid histopathology, 40–60% of splenic B cells were depleted in anti-CD20-treated mice (Table IV), and as mentioned above, there was no correlation between the percentage of splenic CD19+ B cells present at the time of thyroid removal with the SAT severity scores. This suggests that depletion of circulating B cells, which was always essentially complete throughout the experiment, was the most important predictor of disease suppression in anti-CD20-treated mice. Depletion of B cells in blood and spleens of mice given anti-CD20 IgG2a after disease had begun to develop was comparable with that shown for naive mice in Table I (data not shown).

**Reduction of both T and B cells in thyroids after anti-CD20 treatment**

IgG2a anti-CD20 markedly inhibited SAT development, and inflammatory cell infiltration of the thyroid was therefore reduced (Fig. 3). When anti-CD20 injections were initiated after SAT had begun to develop, T and B cells had already migrated to the thyroid. Infiltration of both B and T cells was reduced in mice given anti-CD20, and clustering of T and B lymphocytes which was evident in isotype controls was minimal (Fig. 3). These results suggest that B cells may be required for maintenance of inflammation in SAT, and when circulating B cells (including those that migrate to the thyroid) are depleted, effector CD4+ T cells are not retained in the inflammatory site.

**Discussion**

The results of this study indicate that development of SAT and anti-MTg autoantibody responses were reduced in NOD.H-2b4
mice given anti-CD20 IgG2a mAb. SAT severity scores were also reduced in mice given anti-CD20 IgG2a if treatment was initiated after disease had begun to develop (Table IV). Because plasma cells reportedly do not express CD20 (21, 39), anti-MTG autoantibody responses in anti-CD20-treated mice were comparable with those present when treatment began, whereas Ab responses continued to increase in isotype controls. In these experiments (Table IV), SAT severity scores were relatively mild (2+/ in most mice) when treatment was initiated. It is not known whether anti-CD20 would be effective in mice with more severe SAT.

In other studies, anti-CD20 given before disease onset prevented development of collagen induced arthritis, fibrosis in tight-skin mice, Sjögren’s syndrome, and diabetes in mice but had little effect in mice with ongoing disease (26–28, 38). However, the same anti-CD20 IgG2a Ab used here effectively inhibited proteoglycan-induced arthritis in mice that had already begun to develop arthritis and autoantibodies (30). The IgG1 18B12 mAb inhibited ongoing SLE in MRL/lpr mice (29), and anti-hCD20 effectively suppressed some clinical signs of SLE and diabetes in transgenic MRL/lpr (29) or NOD (31) mice expressing hCD20 on B cells. The reason(s) for the differences in effectiveness of anti-CD20 in suppressing ongoing disease in different animal models are not known but may be due to differences in requirements for B cells in disease initiation vs their role in maintaining disease in different models. Previous studies have established an important role for B cells in initiation of many autoimmune diseases, where they presumably function as important APCs (7–9, 14–18) to promote activation of pathogenic CD4+ T cells (29–31, 40).

Experiments using B cell-deficient mice and mice depleted of B cells beginning at birth indicate that B cells are required for development of many autoimmune diseases including SAT (7–12, 14–18). In those studies, B cell depletion was essentially complete, and in B cell-deficient mice, B cells are completely absent throughout development; this may result in abnormalities that extend beyond simple B cell depletion (40). The results presented here indicate that anti-CD20 given to adult NOD.H-2h4 mice reduced development of SAT and autoantibody responses even though most splenic MZ B cells were resistant to depletion. These results are consistent with those of others (29, 31, 38), indicating that B cells in autoimmunity-prone mouse strains are relatively resistant to depletion by anti-CD20 compared with those in non-autoimmunity-prone strains. B cells in autoimmunity-prone mice could be more resistant to depletion because autoreactive B cells are presumably activated before development of autoimmune diseases, and activated B cells could be more resistant to depletion by anti-CD20 (29). Alternatively, the relatively greater resistance of B cells from autoimmunity-prone mice, particularly those of NOD mice, to depletion by anti-CD20 could be due to deficient FcγRI binding of IgG2a anti-CD20 and/or decreased numbers of splenic monocytes in NOD mice compared with C57BL/6 mice (38). Because NOD.H-2h4 mice differ from NOD mice only at the MHC locus (2, 32), the defects in FcγR effector functions in NOD mice (38, 41, 42) are presumably also present in NOD.H-2h4 mice. The relative resistance of some splenic B cells to depletion by anti-CD20 in our studies might also be explained by the fact that some NOD.H-2h4 mice given anti-CD20 IgG2a generated an immune response against the variable domain of the IgG2a anti-CD20 Ab, because circulating anti-idiotypic Abs were detected in some mice (data not shown). This explanation, however, is unlikely to account for the relative resistance to depletion of splenic B cells in most mice because only a very small percentage of sera had detectable anti-idiotypic Abs, these Abs were never detected in mice given IgG1 anti-CD20, and MZ B cells were resistant to depletion in all mice.

Previous studies by others indicated that circulating mature B cells were almost completely depleted by anti-hCD20 in transgenic mice expressing hCD20 on B cells after injection of anti-hCD20, but most MZ B cells were resistant to depletion (25). The results presented here indicate that the CD21(high)CD23(low) splenic MZ B cell compartment in NOD.H-2h4 mice was almost completely resistant to depletion by anti-CD20, whereas most splenic FO and transitional T2 B cells were depleted (Fig. 2). The resistance of MZ B cells to depletion by anti-CD20 is due to lack of CD20 expression by MZ B cells or to lack of binding of the mAb to MZ B cells (25, 38). In some models, MZ, T2, and FO B cells were depleted to a similar extent by anti-CD20 (26–30, 38). To our knowledge, our studies are the first to show that splenic MZ B cells are relatively resistant to depletion by anti-CD20 compared with other splenic B cell subsets. This suggests that B cell subsets in different mouse strains might differ in their susceptibility to depletion by anti-CD20. However, other factors are apparently also important because a different anti-CD20 mAb effectively depleted MZ B cells in NOD mice (38), which are closely related to the NOD.H-2h4 mice used here. Importantly, the splenic B cells (primarily MZ B cells) that were not depleted in NOD.H-2h4 mice given IgG2a anti-CD20 were apparently insufficient and/or were not required to initiate development of SAT in NOD.H-2h4 mice.

The finding that anti-CD20 was relatively effective when anti-CD20 was given to mice that had already begun to develop SAT (Table IV) suggests that B cells play a role both in initiation of SAT and in maintaining chronic inflammation in the thyroid. Several studies support an active role for B cells in the diversification of autoreactive T cell responses, and T cell–B cell interactions constitute a positive feedback loop that enables diversification and continuous amplification of autoimmune responses (43–47). B cells also contribute to inflammation by functioning as APCs for activation of autoreactive T cells (8, 14–18) and by producing autoantibodies and proinflammatory cytokines (28, 47–50). In the current study, infiltration of both T and B cells in thyroids was reduced when anti-CD20 was given to mice that had already developed SAT (Fig. 3). This is consistent with a recent report indicating that rituximab depleted intrathyroidal B cells in a patient with Graves’ disease (51). B cells that migrate to the thyroid may promote infiltration and proliferation of autoreactive CD4+ T cells to form tertiary lymphoid organs found in many autoimmune disease target tissues, including thyroids of NOD.H-2h4 mice with SAT (6, 48–50, 52, 53). B cells can be important for the formation and maintenance of these structures (49, 50), and B cell depletion could lead to dissolution of these organized lymphoid infiltrates as suggested by our finding that thyroid-infiltrating cells in anti-CD20-treated mice tended to be scattered, whereas those in mice given isotype control form clusters (Fig. 3), shown previously to be comprised of CD4+ T cells and B220+ B cells (6). Because infiltration of both CD4+ T cells and B cells decline after treatment with anti-CD20, T cells apparently die or are not retained in the thyroid after B cells are depleted.

In addition to their role in promoting autoimmune diseases, B cells can function as regulatory cells to suppress progression and/or promote recovery in several murine models of chronic inflammation (54), including inflammatory bowel diseases (54–56), rheumatoid arthritis (36, 57–59), and experimental autoimmune encephalomyelitis (60). In some reports, B cells were shown to negatively regulate inflammatory responses by producing IL-10 (36, 54, 57–60). In some of these studies, IL-10-producing regulatory B cells were MZ-like B cells, characterized by expression of high levels of CD1d and responsiveness to LPS (57), whereas in others, regulatory B cells were reported to be mesenteric (56) or transitional type 2 cells (36, 57, 59) that include precursors of MZ


