B-Lymphocyte Deficiency Increases Atherosclerosis in LDL Receptor–Null Mice

Amy S. Major, Sergio Fazio, MacRae F. Linton

Objective—Atherosclerosis is an inflammatory disease characterized by innate and adaptive immune responses. We investigated the role of B cells and antibodies in the development of atherosclerosis in low density lipoprotein (LDL) receptor–deficient (LDLR−/−) mice.

Methods and Results—Using wild-type and B cell–deficient mice as bone marrow donors, we were able to generate LDLR−/− mice that possessed <1.0% of their normal B cell population. B cell–deficient LDLR−/− mice on a Western diet showed marked decreases in total serum antibody and anti–oxidized LDL antibody. B cell deficiency was associated with a 30% to 40% increase in the lesion area in the proximal and distal aortas. Real-time reverse transcription–polymerase chain reaction and enzyme-linked immunospot analyses showed a decrease in proatherogenic (interferon-γ) and antiatherogenic (interleukin-10 transforming growth factor-β) cytokine mRNA and a decrease in interleukin-4– and interferon-γ–producing cells. Additionally, we observed a decrease in splenocyte proliferation to oxidized LDL in the B cell–deficient LDLR−/− mice, suggesting that B lymphocytes may play a role in the presentation of lipid antigen.

Conclusions—Collectively, these data demonstrate that B cells and/or antibodies are protective against atherosclerosis and that this protection may be conferred by B cell–mediated immune regulation. (Arterioscler Thromb Vasc Biol. 2002; 22:1892-1898.)

Key Words: B-lymphocyte deficiency • B cells • atherosclerosis • LDL receptors

It is well established that atherosclerosis is an inflammatory disease involving the innate and acquired immune systems. Much of the focus in studying immune-associated mediators of atherosclerosis has been on macrophages. Recently, however, increasing attention has been given to determining the role of T and B lymphocytes in the development of atherosclerosis. Early atherosclerotic lesions in humans and mice have been shown to contain significant numbers of T cells and some B cells.1–2 Although these lymphocytes are present in the artery wall at most stages of disease, their relative contribution to the development of atherosclerosis is not yet well understood.

Many experiments show a decrease in lesion development in lymphocyte-deficient mice compared with control mice,3–5 suggesting that T cells may be proatherogenic. When the role of subsets of T cells is examined, it appears that CD4+ T cells may contribute to the atherosclerotic process.6 Studies in apoE-deficient (apoE−/−) mice and LDL receptor–deficient (LDLR−/−) mice null for both T and B cells have demonstrated decreased lesion formation, supporting proatherogenic effects of these lymphocytes.3–5 There is also sufficient evidence that cytokines associated with a proinflammatory T helper-1 response (interferon [IFN]-γ and interleukin [IL]-12) are important in atherogenesis and that T helper-2–associated cytokines (IL-10) are protective against lesion development.7–9 Collectively, these reports strongly suggest a role for acquired immunity in the development of atherosclerosis.

Although T cells and their associated cytokines can be proatherogenic or antiatherogenic, much less is known regarding the role of B cells in atherosclerosis. B cells produce antibodies that are thought to be involved in atherosclerosis. These antibodies are specific for self-antigens, such as heat shock protein 60 and β2-glycoprotein, “modified-self” antigens, such as oxidized LDL (oxLDL) and its phospholipid components, and bacterial antigens, such as Streptococcus and Chlamydia.10–13 Some of these antibodies have been characterized as “natural” antibodies and are thought to be derived from a specific subset of B cells, B-1 B cells.10 Autoantibodies against many of these antigens have been found in humans with coronary heart disease (CHD) and in animal models of atherosclerosis, and titers of anti–oxidized LDL (anti-oxLDL) antibodies are directly correlated with the severity of disease.14 Studies in animals have demonstrated that humoral immunity leading to the production of autoantibodies against modified lipoproteins provides protection.
against atherosclerosis, usually in the absence of significantly large changes in lipoprotein levels or distribution. Immunization of atherosclerosis-prone rabbits and mice with oxidative forms of LDL has resulted in the production of large amounts of anti-oxLDL autoantibody and in protection from atherosclerosis.\(^{15,16}\) Recently, it has been shown that splenectomized apoE\(^{-/-}\) mice have increased atherosclerotic lesion areas compared with sham-operated mice, even in the presence of extreme hyperlipidemia.\(^{17}\) Adoptive transfer of B cells from atherosclerotic apoE\(^{-/-}\) mice has been found to be protective against this increased atherosclerosis. Collectively, these data offer indirect evidence that B cells and antibodies can be beneficial in the prevention of atherosclerosis.

In the present study, we directly examined the role of B cells in atherosclerosis. We used B cell-deficient (\(\mu\)MT) mice and C57BL/6 mice as bone marrow donors for transplantation into LDLR\(^{-/-}\) mice. \(\mu\)MT mice were generated by disrupting the gene encoding the membrane \(\mu\)-chain of the B cell receptor.\(^{18}\) Using this transplantation approach, we were able to generate LDLR\(^{-/-}\) mice that completely lacked B cells and, subsequently, circulating antibodies. We show that atherosclerosis is increased in B cell-deficient LDLR\(^{-/-}\) mice and that the effect of B cells and/or autoantibody is apparent in early and late atherosclerotic lesions. This increase in atherosclerosis was not due to an obvious increase in inflammatory responses in the absence of B cells but might be due to autoantibody production or immune regulation via antigen presentation.

Methods

Animals

LDLR\(^{-/-}\) (B6, 129S-Ldl\(^{em162\text{r}}\)) and \(\mu\)MT (B6, 129S-Igh-6\(^{pm1\text{cm}}\)) mice, both on the C57BL/6 background, were purchased from The Jackson Laboratory (Bar Harbor, Me). Animals were kept in microisolator cages and, unless otherwise stated, were given normal chow and acidified water (pH 2.5). Donor female C57BL/6 mice were obtained from our mouse colony. All experiments involving animals were conducted under the approval of Vanderbilt University’s Institutional Animal Care and Use Committee.

Bone Marrow Transplantation

Bone marrow transplantation (BMT) was conducted as previously described.\(^{19}\) Four weeks after BMT, the mice were started on a Western diet containing 21% fat and 0.15% cholesterol.

Cell Preparation and Flow Cytometry Analysis

Five weeks after BMT, peripheral blood mononuclear cells (PBMCs) were collected from both groups of mice. PBMCs were isolated by Ficoll gradient centrifugation at 800g for 30 minutes. Cells were washed and stained for specific cell surface markers. Splenocytes from recipients were isolated at various times after BMT and washed in either PBS or RPMI-1640 containing 10% FBS, L-glutamine, 2\(\beta\)-mercaptoethanol, and antibiotics (referred to hereafter as TCM). Cells were counted and resuspended to the necessary concentration in either flow cytometry medium (1% FBS/PBS and 0.02% sodium azide) or TCM.

All antibodies used for flow cytometry were purchased from Pharmingen. Cell surface markers were stained by incubation of PBMCs with antibodies against Thy1.1, B220, and Mac-1. The percentage of B cells in spleens was determined by staining with biotin–anti-CD19 and avidin-conjugated PerCP. Percent CD4\(^{+}\) and CD8\(^{+}\) T cells were determined by FITC–anti-CD4 and PE–anti-CD8, respectively. Macrophages were visualized by staining with PE–anti-Mac1. Staining was analyzed by using a FacsCalibur flow cytometer and Cell Quest software (Becton Dickinson).

Serum Cholesterol and Triglycerides

Serum cholesterol and triglyceride levels were determined as previously described.\(^{20}\)

ELISA for Total and OxLDL Antibody

Normal human serum was collected, and LDL was isolated by density centrifugation and copper-oxidized by using 10 \(\mu\)mol/L CuO\(_2\), as previously described.\(^{21}\) Ninety-six–well plates were coated with 50 \(\mu\)L oxLDL (10 \(\mu\)g/mL) in 0.1 mol/L NaHCO\(_3\) overnight at 4°C. The plates were washed twice with PBS containing 0.5% Tween 20 (PBS-T) and blocked with 5% BSA/PBS for 2 hours at room temperature. Plates were washed, and serum samples were added, followed by incubation overnight at 4°C. Bound antibody was detected with biotin-conjugated anti-mouse immunoglobulin secondary antibody (Southern Biotech), followed by incubation with avidin-conjugated peroxidase (Sigma Chemical Co). Color was developed with the addition of the substrate 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma). Optical density was determined at 405 nm.

Total serum antibody titers were determined by coating 96-well plates with 50 \(\mu\)L of anti-mouse immunoglobulin (1 \(\mu\)g/mL). The remaining ELISA procedure was conducted as described above for oxLDL. Serum was diluted 1:300 and 1:10\(^3\) for oxLDL and total antibody ELISAs, respectively.

Quantification of Atherosclerosis in the Proximal Aorta

At 4 and 12 weeks after initiation of the Western diet, mice were euthanized and perfused with 20 mL PBS through the right ventricle. Hearts and aortas were removed, and the hearts were embedded in OCT and frozen in liquid nitrogen. Atherosclerosis was analyzed according to the method of Paigen et al.\(^{22}\) Lesion area was measured by using Imaging System KS300 2.0.

Quantification of Atherosclerosis of Distal Aorta by En Face Preparations

Mice were perfused as described above. Aortas, from the aortic valve to the iliac bifurcation, were collected, fixed in 4% paraformaldehyde, cleaned of any surrounding tissue, opened longitudinally, pinned flat, and stained with Sudan IV, as previously described.\(^{23}\) Lesion area was measured by use of Imaging System KS300 2.0 and expressed as a percentage of total aorta area.

Histology

Masson’s trichrome staining and macrophage staining using anti–MOMA-2 were performed as previously described.\(^{20,23}\) B cell staining was performed by fixing 5-\(\mu\)m cryosections with cold acetone and incubating them with rat anti-CD19 (clone 1D3, Pharmingen) at a 1:100 dilution. Slides were washed twice in PBS-T and incubated with goat anti-rat biotin-conjugated antibody (1:100 dilution, Pharmingen). Positive cells were detected by using FastRed (Sigma). Positive and negative controls for CD19 staining included staining of spleen sections from C57BL/6 and \(\mu\)MT mice (data not shown).

Real-Time RT-PCR

After the mice were fed a Western diet for 4 weeks, spleens were removed from 4 mice per group, and total spleen RNA was isolated by using TRIZol reagent (Invitrogen). mRNA levels for IL-4, IL-10, IFN-\(\gamma\), and transforming growth factor (TGF)–\(\beta\) were measured by using the One-Step Reverse Transcription–Polymerase Chain Reaction (RT-PCR) Master Mix Reagents as per the manufacturer’s instructions (Applied Biosystems) and the Sequence Detection System (ABI Prism 7700 Sequence Detection System and software, Applied Biosystems). Primer and probes were designed according to Xia et al.\(^{25}\) Cycling conditions for IL-4, IFN-\(\gamma\), and IL-10 were as
ELISPOT Assay

Enzyme-linked immunospot (ELISPOT) assay for the quantification of numbers of cytokine-producing splenocytes was performed as previously described. Briefly, 96-well nitrocellulose plates (Millipore) were coated overnight at 4°C with 100 μL of either anti–IL-4 or anti–IFN-γ (both from Pharmingen) diluted in PBS. Plates were blocked with TCM for 2 hours at room temperature, and pooled splenocytes from 4 mice per group were added to the wells in the presence or absence of 10 μg/mL oxLDL. Plates were incubated 24 hours at 37°C and 5% CO₂, and then the cells were lysed, and the plates were washed with PBS-T. The plates were incubated for 2 hours at room temperature with 100 μL of either biotin-conjugated anti–IL-4 or IFN-γ (Pharmingen). After another wash cycle, the plates were blocked with TCM for 1 hour at room temperature with 100 μL avidin–biotin-peroxidase followed by development with substrate solution containing 0.15 mg/mL 5-bromo-4-chloro-3-indolylphosphate. Spot development occurred for ~30 minutes and was stopped by adding dH₂O. Cytokine spot-forming cells were counted by use of a dissecting scope.

Splenocyte Proliferation

After 4 weeks of the Western diet, spleen cells from 4 mice per group were isolated as described above and pooled. Cells were plated at 3x10⁵ cells per well in a 96-well plate in TCM and incubated in 1894 Arterioscler Thromb Vasc Biol. November 2002

Results

Mice were lethally irradiated and reconstituted with either C57BL/6 or μMT bone marrow. Flow cytometric analyses of PBMCs confirmed the reconstitution of irradiated LDLR⁻/⁻ mice, demonstrating that at 5 weeks after BMT, ~5% of the circulating monocytes were B cells in C57BL/6→LDLR⁻/⁻ mice, whereas <1.0% of the circulating monocytes in μMT→LDLR⁻/⁻ mice were B cells (data not shown). Analysis of splenocytes 12 weeks after BMT showed that the B cell compartment was reconstituted in C57BL/6→LDLR⁻/⁻ mice (Figure 1A) but was completely absent in the μMT→LDLR⁻/⁻ mice (Figure 1B). The percentages of CD4⁺ and CD8⁺ cells and Mac-1⁺ cells were increased in the μMT recipients (Table 1). However, this did not result in an increase in the absolute numbers of these cells because the μMT→LDLR⁻/⁻ group had significantly lower numbers of total spleen cells compared with the control group.

Concentrations of total serum antibody were similar for all recipient mice before BMT. However, starting at 4 weeks on the diet (8 weeks after BMT), the μMT→LDLR⁻/⁻ mice compared with C57BL/6 recipients had a significantly lower

<table>
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<tr>
<th>TABLE 1. Splenocyte Number and Cell Surface Phenotype in Bone Marrow–Transplanted LDLR⁻/⁻ Mice</th>
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<td>Donor Strain</td>
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<tr>
<td>C57BL/6</td>
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<tr>
<td>μMT</td>
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Data are represented as cell No. or percent (±SEM) of three animals in each group.

Splenocytes were isolated and analyzed at 12 weeks after BMT.

*Statistical significance (P<0.01) compared to the C57BL/6 group as determined by Student’s t test.
total serum antibody concentration (Figure 2A). In a similar manner, antibodies against oxLDL were also dramatically reduced in the \( \mu MT \rightarrow LDLR^{−/−} \) group compared with the C57BL/6→LDLR\(^{−/−} \) control group (Figure 2B). These data show that transplantation of B cell-deficient marrow results in LDLR\(^{−/−} \) mice that lack B cells and antibody without change in the absolute numbers of other immune cells, such as T cells and macrophages.

**B Cell Deficiency Does Not Result in Significant Change in Serum Cholesterol or Triglycerides**

There were no significant differences in fasting serum cholesterol or triglyceride levels, at any time during the study, in LDLR\(^{−/−} \) mice that were transplanted with either \( \mu MT \) or C57BL/6 bone marrow (Table 2). Before the initiation of the Western diet, mice in both groups had cholesterol and triglyceride levels that ranged between 200 and 400 mg/dL and between 100 and 200 mg/dL, respectively. After the initiation of the Western diet, the levels of serum cholesterol and triglycerides dramatically increased in both groups, as expected. In addition, there were no significant differences in body weight between the 2 groups at any time point measured.

**B Cell Deficiency Increases Atherosclerotic Lesions in Proximal and Distal Aortas in LDLR\(^{−/−} \) Mice**

To determine the effect of B-cell deficiency on early and late atherosclerotic lesions, transplanted LDLR\(^{−/−} \) mice were euthanized at 4 and 12 weeks after the initiation of the Western diet. Oil red O analyses of the proximal aorta revealed a 2-fold increase in early fatty streak lesions after 4 weeks on the Western diet (8 weeks after BMT) in the \( \mu MT \rightarrow LDLR^{−/−} \) female mice (32 000±4000 \( \mu m^2 \) per section) compared with the C57BL/6→LDLR\(^{−/−} \) female control mice (17 000±6000 \( \mu m^2 \) per section; Figure 3A, left). This difference was still apparent after 12 weeks on the diet (16 weeks after BMT), when lesions are more complicated (Figure 3A, right). Male \( \mu MT \rightarrow LDLR^{−/−} \) mice (n=8) also showed a significant increase in atherosclerotic lesion area (\( P=0.046 \)) compared with male C57BL/6→LDLR\(^{−/−} \) control mice (n=7) after 4 weeks on the diet (10 221±2528 versus 2552±603 \( \mu m^2 \) per section, respectively). This increase in atherosclerosis after 4 weeks on the diet in B cell-deficient LDLR\(^{−/−} \) male mice also occurred without any differences in serum cholesterol (data not shown).

En face analyses of atherosclerosis in the distal aorta showed a 2-fold increase in lesion area after 12 weeks on the diet in the \( \mu MT \rightarrow LDLR^{−/−} \) mice (0.46±0.11%) compared with the control mice (0.22±0.04%, Figure 3B). However, this difference did not quite reach statistical significance (\( P=0.052 \)).

Masson’s trichrome staining demonstrated that the lesions in both groups of animals after 12 weeks on the diet had significant but similar collagen deposition (blue) that was deposited adjacent to, but not within, the macrophage–foam cell area of the lesion (Figure 4A through 4D). In addition, immunostaining for CD19 showed no presence of B lymphocytes in atherosclerotic lesions of the proximal aorta in either the \( \mu MT \rightarrow LDLR^{−/−} \) or the C57BL/6→LDLR\(^{−/−} \) mice (Figure 4E and 4F).

**Levels of Cytokine mRNA and Cell Proliferation Are Reduced in Spleens of B Cell-Deficient LDLR\(^{−/−} \) Mice**

To test the hypothesis that the absence of B cells in peripheral secondary lymphoid organs affects T helper-1/2 responses during atherogenesis, mRNA levels for IL-10, IL-4, TGF-\( \beta \), and IFN-\( \gamma \) were measured in total spleen RNA after 4 weeks on the diet by quantitative real-time RT-PCR. There was a 30% to 50% decrease in all cytokine mRNA levels in the spleens of \( \mu MT \rightarrow LDLR^{−/−} \) mice compared with control

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**TABLE 2. Body Weight and Serum Cholesterol and Triglyceride in BMT LDLR\(^{−/−} \) Mice**

<table>
<thead>
<tr>
<th>Donor Strain</th>
<th>Weight</th>
<th>Chol</th>
<th>Trig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Weeks</td>
<td>4 Weeks</td>
<td>12 Weeks</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>14.3 (0.3)</td>
<td>298.2 (5.5)</td>
<td>107.4 (4.0)</td>
</tr>
<tr>
<td>( \mu MT )</td>
<td>13.1 (0.5)</td>
<td>309.3 (23.9)</td>
<td>103.2 (2.3)</td>
</tr>
</tbody>
</table>

Body weight is expressed as g (±SEM); serum cholesterol (Chol) and triglyceride (Trig) are expressed as mg/dL (±SEM).

Body weight, serum cholesterol, and triglyceride were measured at 0, 4, and 12 weeks after the initiation of Western diet. There were no significant differences between groups in body wt or serum cholesterol and triglyceride at any time point as determined by Student’s t-test.

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![Figure 3](https://example.com/figure3.png)

**Figure 3.** Atherosclerotic lesion area was determined in the proximal aorta by oil red O staining (A) and in the distal aorta by en face analysis (B) 12 weeks after initiation of the Western diet as described in Methods. Bars represent the mean±SE of the C57BL/6→LDLR\(^{−/−} \) (open bars) and \( \mu MT \rightarrow LDLR^{−/−} \) (closed bars) groups. *\( P<0.05 \) and **\( P=0.052 \) by Student t test. Numbers of mice per group are labeled on bars.
mice (Figure 5A). ELISPOT analyses on splenocytes from mice on the diet for 12 weeks showed that the frequency of IL-4–producing cells in the μMT→LDLR−/− group was 270 per 10⁷ cells compared with 507 per 10⁷ cells in the C57BL/6→LDLR−/− group. Likewise, IFN-γ–producing cells were reduced in the μMT→LDLR−/− splenocytes compared with control splenocytes (6 per 10⁷ cells versus 22 per 10⁷ cells, respectively). In addition to a decrease in cytokine production in the spleen ex vivo, we also observed a significant decrease in spleen cell proliferation after restimulation with oxLDL (Figure 5B).

Discussion

The present study is the first to examine the effect of a specific and total B cell deficiency on atherogenesis. We describe a protective role for B cells and/or antibody during atherosclerotic lesion development in LDLR−/− mice on a Western diet. This protection was observed without any obvious difference in serum cholesterol or triglyceride levels between experimental and control animals. B cell deficiency was deleterious for early fatty streak lesions (after 4 weeks on the diet) as well as for more advanced lesions (after 12 weeks on the diet) in the proximal aorta. Increased atherosclerosis was also evident in the distal aorta after 12 weeks on the Western diet. The increased atherosclerosis in μMT→LDLR−/− mice did not appear to be due to increased inflammatory responses in these mice because we observed similar decreases in “antiatherogenic” (IL-10 and TGF-β) and “proatherogenic” (IFN-γ) cytokine mRNA levels as well as cytokine-producing cells in the spleen. We also demonstrate a decrease in splenocyte proliferation in response to oxLDL in the absence of available B cells as antigen-presenting cells.

Recently, Caligiuri et al. showed that, compared with a sham operation, removal of the spleen (which serves as a large reservoir for T and B lymphocytes) from apoE−/− mice increased atherosclerosis. This difference was observed in a severe hyperlipidemic environment, inasmuch as the animals were placed on a Western diet for 12 weeks. The authors were able to show reduction of atherosclerosis when splenic B cells from atherosclerotic apoE−/− mice were adoptively transferred to splenectomized as well as sham-operated animals. These data suggest that during atherosclerosis development, protective effector and/or memory B cells are generated in the spleen. However, because only splenic B cells were removed in splenectomized mice, the importance of other B cell pools in sites such as lymph nodes, bone marrow, and the peritoneal cavity (as well as nonimmune functions of the spleen) in protection against atherosclerosis was not addressed.

In the present study, we were able to assess the modulation of atherosclerosis after complete, not partial, B cell and antibody deficiency. Furthermore, we demonstrated possible antiatherogenic properties of B cells beyond antibody production. The overall reduction in cytokine production and cell proliferation in spleens of μMT→LDLR−/− mice compared
with control mice (Figure 4A and 4B) indicates that B cells may regulate immunity during the development of atherosclerosis, perhaps via antigen presentation of lipid antigen to T cells.

The exact role of B cells or autoantibodies in atherosclerosis is not well defined. We focus mainly on the production of oxLDL antibodies because this most likely would be one of the major “autoantigens” present in LDLR−/− mice on an atherogenic diet. Several studies have demonstrated the beneficial effects of anti-oxLDL antibody on the progression of atherosclerosis.30,31,32,33 Shaw et al33 showed that a human-derived anti-oxLDL antibody could block the uptake of oxLDL and apoptotic cells by macrophages, 2 mechanisms that would be considered antiatherogenic. This could most likely occur without affecting total cholesterol levels because our data show increased atherosclerosis in the absence of antibody, with no differences in total serum cholesterol (Table 2). In addition to blocking oxLDL uptake, antibody-antigen complexes have been shown to regulate macrophage and other immune cell responses via Fcγ receptors.28 Much evidence has accumulated to implicate a role for Fcγ receptor signaling in atherosclerosis. Several classes of Fcγ receptors have been detected in human atherosclerotic lesions,29 and patients with advanced atherosclerosis have reduced levels of CD32 (Fcγ receptor IIA) monocyte, a molecule known to downregulate cell activation.30 These studies, in addition to the present study, support a protective role of antibody-mediated effects on atherosclerosis.

The effects of antibody may be proatherogenic or antiatherogenic, depending on the target antigen studied. Immunization of animals with heat shock protein 65 or β2-glycoprotein has been shown to lead to specific humoral immunity against these antigens with increased lesion development.31–33 However, whether the increase in antibody production is a cause or effect of atherosclerosis is not known. It would be interesting to determine whether immunization of B cell–deficient mice with proatherogenic antigens would lead to further increases in atherosclerosis. Indirect in vitro evidence that antibody function could be proatherogenic demonstrated that oxLDL immune complexes, via Fcγ receptor binding, activated a human monocyte/macrophage cell line.34 In addition, antibodies are also important activators of the complement system, which has been shown to be associated with C-reactive protein in atherosclerotic lesions.35 Therefore, it appears that the role of autoantibody in atherosclerosis is complicated and may be system and antigen dependent.

B cells can regulate immune responses, independent of antibody production, in other inflammatory situations, such as ulcerative colitis and type-1 diabetes.36,37 Adoptive transfer of activated B cells mediated protection in type-I diabetes by the induction of apoptosis of diabetic T cells via the Fas/FasL signaling pathway.37 In addition, B cells are important for the development of memory CD4+ T cells in the spleen and peripheral populations of a specific subset of regulatory T cells (CD4+CD25+) that serve to decrease inflammatory responses.38,39 These data are consistent with our observations that T cell–associated immune responses to oxLDL are decreased in the spleen in the absence of B cells (Figure 5A and 5B). The observation of lower numbers of cytokine-producing cells and less proliferation in response to oxLDL indicates that B cells may act as a major lipid antigen–presenting cell to other immune cells, such as T cells. Our present data and the studies of others demonstrate that CD19+ B cells are not present in the aortic lesion (Figure 4E and 4F and REF3). Because CD19 is not expressed on plasma cells, it is possible that fully differentiated plasma cells are present in the lesions of LDLR−/− mice after transplantation. At any rate, our data suggest that B cells may not have a direct local effect in the artery wall. One may hypothesize that in the absence of B cells, a subset of regulatory cells that serve to decrease inflammatory responses in the growing lesion or that inhibit lymphocyte migration to atherosclerotic plaques is not activated in the periphery. This hypothesis is supported by data showing that splenectomized apoE−/− mice had increased numbers of CD4+ T cells in arterial lesions, which decreased to control levels after splenic B cell transfer.37 Additional studies examining B cells as antigen-presenting cells for oxLDL and as regulators of specific immune responses are necessary to make further conclusions.

In summary, we have definitively shown that in LDLR−/− mice, B cells are protective against the early and late stages of atherosclerosis. This protection is likely related to antibody production but may also involve the regulation of cellular immunity against antigens such as oxLDL. Therefore, by increasing our understanding of the mechanisms by which B cells and antibodies confer protection against atherosclerosis, it may be possible to develop future immune-modulating therapies for individuals suffering from CHD.

Acknowledgments

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References


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