BAFF Receptor Deficiency Reduces the Development of Atherosclerosis in Mice—Brief Report

Andrew P. Sage, Dimitrios Tsiantoulas, Lauren Baker, James Harrison, Leanne Masters, Deirdre Murphy, Celine Loinard, Christoph J. Binder, Ziad Mallat

Objective—The goal of this study was to assess the role of B-cell activating factor (BAFF) receptor in B-cell regulation of atherosclerosis.

Methods and Results—Male LDL receptor-deficient mice (Ldlr−/−) were lethally irradiated and reconstituted with either wild type or BAFF receptor (BAFF-R)–deficient bone marrow. After 4 weeks of recovery, mice were put on a high-fat diet for 6 or 8 weeks. BAFF-R deficiency in bone marrow cells led to a marked reduction of conventional mature B2 cells but did not affect the B1a cell subtype. This was associated with a significant reduction of dendritic cell activation and T-cell proliferation along with a reduction of IgG antibodies against malondialdehyde-modified low-density lipoprotein. In contrast, serum IgM type antibodies were preserved. Interestingly, BAFF-R deficiency was associated with a significant reduction in atherosclerotic lesion development and reduced numbers of plaque T cells. Selective BAFF-R deficiency on B cells led to a similar reduction in lesion size and T-cell infiltration but in contrast did not affect dendritic cell activation.

Conclusion—BAFF-R deficiency in mice selectively alters mature B2 cell-dependent cellular and humoral immune responses and limits the development of atherosclerosis.

Key Words: atherosclerosis □ cytokines □ immune system □ leukocytes □ transgenic models

Atherosclerosis is an inflammatory disease of the arterial wall driven by innate and adaptive immune responses to a variety of endogenous agents; the most studied ones being lipoproteins and heat shock proteins. Several inflammatory cell subsets have been shown to promote atherosclerosis, including monocytes/macrophages, neutrophils, mast cells, and T lymphocytes. A notable exception was the proposed atheroprotective role of the B lymphocyte subset. However, these concepts have been refined during the last few years. For example, we and others have clearly shown that not all T lymphocytes are proatherogenic and have pinpointed T-cell regulatory subsets with potent antiatherogenic properties. More recently, we reconsidered the role of B lymphocytes in atherosclerosis. We clearly showed that contrary to expectations based on previous studies, depletion of mature B cells in mice reduces lesion development, indicating the presence of a proatherogenic B-cell subset. These results have been confirmed by another group of investigators and have important consequences for our understanding of the immune response of atherosclerosis and the identification of suitable targets for disease modulation. A brief review of the previous and recent work on the role of B cells in atherosclerosis indicates that the proatherogenic effect is mainly driven by the common B2 subset, which responds to T-cell–dependent antigens and is part of the adaptive immune response. In contrast, the atheroprotective effect is mostly attributed to the minor innate B1a subset, which responds to T-cell–independent antigens and is responsible for the production of natural IgM antibodies. This protective property may largely depend on the capacity of natural IgM to recognize oxidation-specific epitopes on oxidized low-density lipoprotein (LDL) and cellular debris. However, a recent study has shown that B cell homing to atherosclerotic lesions might be important for the atheroprotective effects of B cells, independently of IgM levels. Thus, more work is still needed to fully capture the multiple roles of B-cell subtypes in atherosclerosis.

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Interestingly, the development, survival, proliferation, and functions of the various B-cell subsets are driven by distinct factors. One important factor is B-cell activating factor (BAFF), a member of the tumor necrosis factor family. BAFF is produced both by hematopoietic and non-hematopoietic cells, and signals through 3 different receptors. BAFF is required for B cell maturation beyond the transitional T1 stage and supports the survival of low-affinity self-reactive B cells. As such, BAFF is involved in a variety of autoimmune-mediated diseases, and therapeutic strategies that interrupt its signaling pathways are currently approved or being tested in several clinical trials. The survival role...
of BAFF on the B2-cell subset is mediated through BAFF receptor (BAFF-R), mostly expressed on mature B cells. Mice deficient for BAFF-R show impaired B-cell maturation beyond the transitional T1 stage and therefore marked depletion of mature B2 cells. However, the B1 cell subset does not require BAFF-R signaling for its survival and is preserved in BAFF-R-deficient mice. We therefore hypothesized that inhibition of BAFF-R signaling would reduce B2 proatherogenic activity while preserving B1 atheroprotective potential, thereby limiting lesion development.

**Methods**

We used male Ldr−/− and BAFF-R−/− mice on C57Bl/6 background. Details of Methods can be found in the online-only Data Supplement.

**Results**

In order to test this hypothesis, we subjected male Ldr−/− mice to lethal total body irradiation followed by reconstitution with either wild type or BAFF-R−/− bone marrow (see Methods in the online-only Data Supplement). The mice were put on a high fat diet for 6 weeks to address the role of BAFF-R in immune responses and lesion development in the context of atherosclerosis. Body weights (wild type [WT]: 32.6±2.5 g, BAFF-R−/−: 33.2±3.4 g) and plasma cholesterol levels (WT: 5.96±0.73 g/L, BAFF-R−/−: 6.5±0.74 g/L) did not differ between the 2 groups of mice. Mice reconstituted with BAFF-R−/− bone marrow showed selected and marked depletion of B cells in bone marrow, blood, spleen, and lymph nodes (Figure 1A) compared with control mice. Monocyte numbers (in spleen, blood, bone marrow, and peritoneum) were similar between the groups (data not shown). Numbers of T lymphocytes (CD4+ and CD8+ cells, determined in spleen and lymph nodes) were also similar between the groups, except for a significant reduction of CD4+ cells in spleen (Figure I in the online-only Data Supplement). When we looked in more detail at the B-cell subsets as exemplified by analysis of the peritoneal B-cell population, we found a profound depletion of B2 cells (86% reduction) in face of a smaller reduction of B1b cells (48% reduction) and a preservation of innate and athero-protective B1a cells (Figure 1B and Figure II in the online-only Data Supplement). We then addressed the impact of BAFF-R deficiency on both the humoral and cellular immune response. Total plasma Ig levels did not differ between the groups, except for IgG2c (Figure III in the online-only Data Supplement). However, plasma levels of IgG1 and IgG2c antibodies to malondialdehyde-modified LDL were significantly reduced in mice reconstituted with BAFF-R−/− bone marrow compared with controls (Figure 1C). Interestingly, serum levels of total or antimalondialdehyde-LDL IgM antibodies were not altered by BAFF-R deficiency (Figure 1C and data not shown), a finding consistent with the preservation of B1a cells that are responsible for serum levels of natural IgM antibodies, shown to be atheroprotective. In a previous study from our group, we reported an important impact of mature B-cell depletion on dendritic cell (DC) activation and T-cell proliferation. In the present study, we also found a significant reduction of DC activation in mice reconstituted with BAFF-R−/− bone marrow, as exemplified by the reduction of CD40 and MHC-II expression on CD11c+DCs (Figure IV in the online-only Data Supplement). BAFF-R deficiency was also associated with a marked reduction of T-cell activation (Figure 1D) and T-effector cell proliferation (Figure 1E) in the absence of any change in the percentage or suppressive function of CD4+CD25+ regulatory T cells (data not shown). This finding suggests an important role for B2 cells in the maintenance of effector T-cell responses in the context of atherosclerosis.

Finally, we addressed the effect of BAFF-R deficiency on the development of atherosclerotic lesions. As shown in Figure 2A, quantification of intimal area in the aortic root after Oil red O staining at 6 weeks of high-fat diet revealed a significant...
reduction of lesion size in mice reconstituted with BAFF-R−/− bone marrow compared with controls. Interestingly, BAFF-R deficiency was also associated with a reduction of lesion inflammation, as revealed by the significant decrease of total macrophage accumulation (MOMA2 staining) in the aortic root (Figure 2B) and a trend toward decreased ratio of MOMA2 staining to total lesion area (0.59±0.29 versus 0.84±0.35, P=0.11). The reduction of lesion size in mice reconstituted with BAFF-R−/− bone marrow was also significant after 8 weeks on high-fat diet (Figure 2A) despite similar serum cholesterol levels between the 2 groups of mice (8.65±1.36 versus 8.50±1.03 g/L, P=0.82). Selective BAFF-R deficiency on B cells (see Methods in the online-only Data Supplement) did not alter T-cell count (Figure I in the online-only Data Supplement) but led to selective depletion of B2 cells, associated with a reduction of T-cell activation despite no change in DC activation (Figure V in the online-only Data Supplement). Spleen T cells from these mice (20% BAFF-R−/− T cells) showed the same reduction in proliferation in vitro compared to mice with 100% BAFF-R−/− T cells (data not shown), indicating the effect is due to the lack of B cells rather than BAFF-R on T cells. Indeed, we did not detect expression of BAFF-R on freshly isolated spleen T cells by flow cytometry (data not shown). Interestingly, selective BAFF-R deficiency on B cells also led to a significant reduction of lesion development (Figure 2A) despite no change in serum cholesterol levels (9.84±1.45 vs 9.84±1.82 g/L, P=1.00). Additionally, BAFF-R deficiency, whether in all cells or selectively in B cells, led to decreased numbers of T cells present in atherosclerotic plaque (Figure 2C).

**Discussion**

We have presented 3 different experiments showing that BAFF-R deficiency in bone marrow-derived cells reduces atherosclerosis. BAFF-R deficiency is associated with a reduction of T cell activation, reduced T-cell numbers in plaques, and attenuation of adaptive humoral responses against malondialdehyde-LDL. This contrasts with the preservation of the B1a cell subtype and the production of natural IgM antibodies. The result is a significant attenuation of macrophage accumulation within the vessel wall and thus a reduction of lesion development in Ldlr−/− mice. During the review process, Kyaw et al reported reduction of lesion development in Apoe−/−/BAFF-R−/− compared with Apoe−/− mice,19 which is in agreement with the present results. Our studies on the mixed μMT/BAFF-R chimeras also show that BAFF-R expression on B cells is sufficient to drive T-cell activation and atherogenesis, which is a unique and novel aspect. In addition, and in contrast to BAFF-R deficiency in total bone marrow-derived cells, DC activation is not altered in mice with selective BAFF-R deficiency in B cells. These results indicate that BAFF-R signaling in B cells directly drives T-cell activation/proliferation and atherosclerosis independently of DC activation. Finally, our results clearly suggest that blockade of BAFF-R signaling may constitute an interesting therapeutic strategy to limit the development of atherosclerosis, particularly in patients with associated BAFF-dependent immune-mediated diseases.

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**Disclosures**

None.

**References**


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BAFFR and Atherosclerosis

Supplemental Material
SUPPLEMENTAL METHODS

Mice

Ldlr−/−, µMT and BAFF−R− mice were purchased originally from Jackson labs and were on a C57Bl/6 background. Male 6 week old Ldlr−/− mice were lethally irradiated (9.5 Gy) then injected i.v. with 1x10^7 bone marrow cells from male BAFF-R^+/+ (WT) or BAFF-R−/− mice. After 4 weeks recovery, mice were fed a high fat diet (21% Fat, 0.15% Cholesterol) for 6 or 8 weeks. In order to assess the effects of selective deficiency of BAFF-R on B cells, lethally irradiated Ldlr−/− mice reconstituted with a mixture of 80% bone marrow from µMT mice (which do not develop B cells due to disruption of the membrane exon of the mu heavy chain gene) and 20% bone marrow from either WT (control) or BAFF-R−/− mice, as previously described 1. In this case, the B cell precursors of WT or BAFF-R−/− bone marrow were able to sufficiently repopulate the spleen B cell compartment of the recipient mice (Supplemental Fig. VI). Thus, reconstitution with 80% µMT/20% BAFF-R−/− results in bone marrow chimeras with only BAFF-R−/− B cells. However, 80% of antigen presenting cells and T cells still express BAFF-R. After 4 weeks recovery, mice were fed a high fat diet for 8 weeks.

Flow cytometry

Single cell suspensions of bone marrow, spleen, lymph node, blood and peritoneum were stained with fluorophore-conjugated antibodies to B220 (Clone RA3-6B2), IgM (II/41), CD11c (N418), MHC Class II (M5/114.15.2), CD40 (1C10) (eBioscience), CD5 (53-7.2) and CD11b (M1/70) (BD) and analyzed using a CyAN ADP analyzer (Beckman)
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Coulter). Data was analysed using FlowJo software (TreeStar, OR, USA). B2 cells were defined as B220^{hi} \text{IgM}^{+} in the lymphocyte gate. For peritoneal analysis, cells were gated on B220^{+} \text{IgM}^{+} and B2 cells were defined as CD5^{-}CD11b^{-}, B1a cells as CD5^{-}CD11b^{+} and B1b cells as CD5^{-}CD11b^{+}.

**Cell culture**

Whole spleen cells were isolated by passing through a 40 µm sieve. Total CD4^{+}CD25^{-} T cells and CD11c^{+} antigen presenting cells were isolated using magnetic beads and an AutoMACS SeparatorPRO (Miltenyi). Proliferation of whole spleen or effector T cells in the presence of CD11c^{+} cells was induced with anti-CD3\text{ε} antibody (1 µg/ml; BD) and quantified by ^{3}H-thymidine incorporation as previously described.

**Serum parameters**

Serum antibody levels including antibodies to malondialdehyde modified (MDA)-LDL were quantified as previously described. Total cholesterol was quantified using a Cholesterol RTU kit (Biomerieux).

**Analysis of atherosclerotic lesions**

Aortic root atherosclerotic lesions were analysed by Oil Red O, MOMA-2 and CD3 staining as previously described. Images were captured and analysed using a Leica DM6000B microscope and accompanying software.

**Statistics**
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Results were analyzed in GraphPad Prism (LaJolla, CA, USA) using unpaired t-test or two-way analysis of variance and presented as mean ± S.D. A P value of <0.05 was considered significant.

References


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Supplemental Figures I to VI
Supplemental Figure I. Effect of BAFF-R deficiency in bone marrow cells (see Supplemental Methods) on CD4 lymphocyte count in spleen. A) Numbers of CD4+ lymphocytes in spleen of Ldlr⁻/⁻ mice reconstituted with either a wild type (WT, n=11) or BAFF-R⁻/⁻ (n=10) bone marrow and put on a high fat diet for 6 weeks. B) Numbers of CD4+ lymphocytes in spleen of Ldlr⁻/⁻ mice reconstituted with either a 80% μMT/20% WT (μMT/WT, n=10) or 80% μMT/20% BAFF-R⁻/⁻ (μMT/BAFF-R⁻/⁻, n=10) bone marrow and put on a high fat diet for 8 weeks. Mean values ± S.D. are represented. *P<0.05.
Supplemental Figure II. Examples of flow cytometry gateings (A) to distinguish the different B cell subsets in the peritoneal cavity of Ldlr−/− mice reconstituted with either a wild type (WT) or BAFF-R−/− bone marrow (B) and put on a high fat diet for 6 weeks.
Supplemental Figure III. Effect of BAFF-R deficiency in bone marrow cells on total Ig levels. Plasma levels of total antibody IgG1, IgG2c and IgM antibodies in Ldlr−/− mice reconstituted with either a wild type (WT, n=11) or BAFF-R−/− (n=10) bone marrow and put on a high fat diet for 6 weeks. Mean values ± S.D. are represented. *P<0.05.
Supplemental Figure IV. BAFF-R deficiency in bone marrow cells impairs DC activation. Quantitative analysis of MHC-II and CD40 expression using flow cytometry on spleen-derived CD11c+ DCs in Ldlr−/− mice reconstituted with either a wild type (WT, n=11) or BAFF-R−/− (n=10) bone marrow and put on a high fat diet for 6 weeks. MFI indicates mean fluorescence intensity. Mean values ± S.D. are represented. *P<0.05.
Figure V

A

Bar chart showing the percentage of B cells in different subsets. The subsets are labeled as T1, T2, and B2. The data points are represented by bars with error bars, and the significance is indicated by asterisks.

B

Two histograms showing the MFI (Mean Fluorescence Intensity) for different subsets. The subsets are represented by the labels 'WT' and 'BR'. The bars are colored red and black, and the significance is indicated by asterisks.

C

A scatter plot showing the CPM (Counts Per Minute) for different conditions. The data points are represented by markers, and the significance is indicated by asterisks.

D

Three histograms showing the percentage of CD69+ and CD44hi CD4+ T cells for different conditions. The conditions are represented by the labels 'WT' and 'BR/BR-/-'. The bars are colored red and black, and the significance is indicated by asterisks.
Supplemental Figure V. BAFF-R deficiency in B cells markedly depletes B2 cells, impairs T cell activation and proliferation but does not alter DC activation. A) Percentages of B2 and transitional T1 and T2 B lymphocytes (see Supplemental Methods) in spleen of Ldlr−/− mice reconstituted with 100% WT bone marrow, 100% BAFF-R−/− bone marrow (BR−/-), a mixture of 80% bone marrow from μMT mice (which do not develop B cells) and 20% bone marrow from either WT (μMT/WT) or BAFF-R−/− mice (μMT/BR−/-). After 4 weeks recovery, mice were fed a high fat diet for 8 weeks. B) Quantitative analysis of MHC-II and CD40 expression using flow cytometry on spleen-derived CD11c+ DCs in mice reconstituted with 80% μMT/20% wild type (μMT/WT) or 80% μMT/20% BAFF-R−/− (μMT/BR−/-) bone marrow and put on a high fat diet for 8 weeks. MFI indicates mean fluorescence intensity. C) Anti-CD3 antibody-stimulated splenocyte proliferation using tritiated thymidine (see Methods). D) Quantitative analysis of CD69+ and CD44high expression using flow cytometry on spleen-derived CD4+ cells. Mean values ± S.D. are represented. *P<0.05.
Supplemental Figure VI. Repopulation of the spleen and peritoneal B cell compartments in lethally irradiated Ldlr-/- mice reconstituted with 100% WT bone marrow, 100% BAFF-R/- bone marrow (BR-/-), a mixture of 80% bone marrow from μMT mice (which do not develop B cells) and 20% bone marrow from either WT (μMT/WT) or BAFF-R/- mice (μMT/BR-/-). After 4 weeks recovery, mice were fed a high fat diet for 8 weeks. The B cell precursors of WT or BAFF-R/- bone marrow were able to repopulate the spleen (A) and peritoneal (B) B cell compartments of the recipient mice. BAFF-R deficiency profoundly reduced the numbers/percentages of mature B2 cells. #P<0.05 vs WT. *P<0.01 vs WT or vs μMT/WT.
B-cell activating factor (BAFF) 수용체 결핍은 죽상경화를 저해시킨다.

한 기 훈 교수
서울아산병원 심장내과

Summary

배경
본 연구는 B 임파구에 존재하는 B-cell activating factor (BAFF) 수용체가 죽상경화의 발전에 미치는 영향을 평가하고자 하였다.

방법 및 결과
LDL 수용체가 결핍된 수컷 생쥐(Ldlr⁻/⁻)들을 방사선 조사한 후 골수를 BAFF 수용체 결핍(BAFF-R) 골수로 대치하였다. 4주 후 생쥐들을 대상으로 고지방식이를 시작하여 6~8주간 시행하였다. BAFF-R 결핍골수 생쥐들은 B2 임파구의 결핍을 초래하였지만 B1a 임파구에는 영향이 없었다. 이는 수지상세포 (dendritic cell)의 감소, T 임파구 증식의 감소와 malondialdehyde-modified low-density lipoprotein에 반응한 IgG 항체의 감소를 가져왔다. 그러나, 기존 죽상경화를 저해한다고 알려진 IgM형 항체의 생산에는 영향이 없었다. 흥미롭게도 B 임파구의 BAFF-R 수용체의 결핍은 죽상경화반 내 T 임파구의 감소와 함께 죽상경화반의 크기 감소를 야기하였지만 수지상세포의 활성에는 영향이 없었다.

결론
생쥐의 BAFF-R 결핍은 B2 세포 의존성 세포 및 체액성 면역반응을 선택적으로 억제함으로써 죽상경화의 발전을 저해한다.
Commentary

죽상경화 혈관 안에 염증세포들이 존재한다는 것은 잘 알려져 있다. 그들의 역할에 대해서는 아직도 명확하지 않은 부분이 있는 것이 사실이다. 이중 B 임파구에 대해서는 더욱 알려진 것들이 없다. 2002년 B 임파구의 역할에 대한 주요한 결과가 보고되었는데, oxidized LDL을 주사한 후의 생쥐에서 분리된 B 임파구에서는 T 임파구로부터의 소위 Th2 사이토카린 분비에 의하여 oxidized LDL을 인지하는 IgM이 분비되어 대식세포로의 oxidized LDL의 함입이 차단되고 죽상경화의 발전이 저해된다는 사실이 알려졌다.


본 논문에서 사용한 B cell-activating factor receptor (BAFFR)는 성숙한 B2 임파구의 유지에 중 요한 역할을 하는 수용체이다. 논문 저자는 bone marrow cell의 BAFFR를 선택적으로 제거하였으며 이로 인하여 B2 세포를 제거하였고 이러한 상황에서 죽상경화가 저해되는 현상을 관찰하였다.

또한 이러한 상황에서 죽상경화가 죽상경화반 내의 CD4 T 임파구가 줄어드는 것을 보고하여 B2 임파구의 제거는 죽상경화를 촉진시키는 CD4 T 임파구의 증식을 저해함을 시사하였다.

이러한 사실은 B 임파구의 역할에 따라 죽상경화의 진행에 영향을 미치는 역할이 존재한다는 것을 의미한다. 특히, 최소한 B2 임파구는 죽상경화를 촉진하는 역할을 할 가능성이 높다. 그러나 아직도 명확히

REFERENCE
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Objective—The goal of this study was to assess the role of B-cell activating factor (BAFF) receptor in B-cell regulation of atherosclerosis.

Methods and Results—Male LDL receptor-deficient mice (Ldlr<sup>−/−</sup>) were lethally irradiated and reconstituted with either wild type or BAFF receptor (BAFF-R)–deficient bone marrow. After 4 weeks of recovery, mice were put on a high-fat diet for 6 or 8 weeks. BAFF-R deficiency in bone marrow cells led to a marked reduction of conventional mature B2 cells but did not affect the B1a cell subtype. This was associated with a significant reduction of dendritic cell activation and T-cell proliferation along with a reduction of IgG antibodies against malondialdehyde-modified low-density lipoprotein. In contrast, serum IgM type antibodies were preserved. Interestingly, BAFF-R deficiency was associated with a significant reduction in atherosclerotic lesion development and reduced numbers of plaque T cells. Selective BAFF-R deficiency on B cells led to a similar reduction in lesion size and T-cell infiltration but in contrast did not affect dendritic cell activation. Conclusion—BAFF-R deficiency in mice selectively alters mature B2 cell-dependent cellular and humoral immune responses and limits the development of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2012;32:1573–1576.)

Key Words: atherosclerosis ■ cytokines ■ immune system ■ leukocytes ■ transgenic models

Atherosclerosis is an inflammatory disease of the arterial wall driven by innate and adaptive immune responses to a variety of endogenous agents; the most studied ones being lipoproteins and heat shock proteins. Several inflammatory cell subsets have been shown to promote atherosclerosis, including monocytes/macrophages, neutrophils, mast cells, and T lymphocytes. A notable exception was the proposed atheroprotective role of the B lymphocyte subset. However, these concepts have been refined during the last few years. For example, we and others have clearly shown that not all T lymphocytes are proatherogenic and have pinpointed T-cell regulatory subsets with potent antiatherogenic properties. More recently, we reconsidered the role of B lymphocytes in atherosclerosis. We clearly showed that contrary to expectations based on previous studies, depletion of mature B cells in mice reduces lesion development, indicating the presence of a proatherogenic B-cell subset. These results have been confirmed by another group of investigators and have important consequences to our understanding of the immune response of atherosclerosis and the identification of suitable targets for disease modulation. A brief review of the previous and recent work on the role of B cells in atherosclerosis indicates that the proatherogenic effect is mostly attributed to the minor innate B1a subset, which responds to T-cell–independent antigens and is responsible for the production of natural IgM antibodies. This protective property may largely depend on the capacity of natural IgM to recognize oxidation-specific epitopes on oxidized low-density lipoprotein (LDL) and cellular debris. However, a recent study has shown that B cell homing to atherosclerotic lesions might be important for the atheroprotective effects of B cells, independently of IgM levels. Thus, more work is still needed to fully capture the multiple roles of B-cell subtypes in atherosclerosis.

Interestingly, the development, survival, proliferation, and functions of the various B-cell subsets are driven by distinct factors. One important factor is B-cell activating factor (BAFF), a member of the tumor necrosis factor family. BAFF is produced both by hematopoietic and non-hematopoietic cells, and signals through 3 different receptors. BAFF is required for B cell maturation beyond the transitional T1 stage and supports the survival of low-affinity self-reactive B cells. As such, BAFF is involved in a variety of autoimmune-mediated diseases, and therapeutic strategies that interrupt its signaling pathways are currently approved or being tested in several clinical trials. The survival role

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of BAFF on the B2-cell subset is mediated through BAFF receptor (BAFF-R), mostly expressed on mature B cells. Mice deficient for BAFF-R show impaired B-cell maturation beyond the transitional T1 stage and therefore marked depletion of mature B2 cells. However, the B1 cell subset does not require BAFF-R signaling for its survival and is preserved in BAFF-R–deficient mice. We therefore hypothesized that inhibition of BAFF-R signaling would reduce B2 proatherogenic activity while preserving B1 atheroprotective potential, thereby limiting lesion development.

**Methods**

We used male Ldlr−/− and BAFF-R−/− mice on C57Bl/6 background. Details of Methods can be found in the online-only Data Supplement.

**Results**

In order to test this hypothesis, we subjected male Ldlr−/− mice to lethal total body irradiation followed by reconstitution with either wild type or BAFF-R−/− bone marrow (see Methods in the online-only Data Supplement). The mice were put on a high fat diet for 6 weeks to address the role of BAFF-R in immune responses and lesion development in the context of atherosclerosis. Body weights (wild type [WT]; 32.6±2.5 g, BAFF-R−/−: 33.2±3.4 g) and plasma cholesterol levels (WT: 5.96±0.73 g/L, BAFF-R−/−: 6.5±0.74 g/L) did not differ between the 2 groups of mice. Mice reconstituted with BAFF-R−/− bone marrow showed selected and marked depletion of B cells in bone marrow, blood, spleen, and lymph nodes (Figure 1A) compared with control mice. Monocyte numbers (in spleen, blood, bone marrow, and peritoneum) were similar between the groups (data not shown). Numbers of T lymphocytes (CD4+ and CD8+ cells, determined in spleen and lymph nodes) were also similar between the groups, except for a significant reduction of CD4+ cells in spleen (Figure I in the online-only Data Supplement). When we looked in more detail at the B-cell subsets as exemplified by analysis of the peritoneal B-cell population, we found a profound depletion of B2 cells (86% reduction) in face of a smaller reduction of B1b cells (48% reduction) and a preservation of innate and atheroprotective B1a cells (Figure 1B and Figure II in the online-only Data Supplement). We then addressed the impact of BAFF-R deficiency on both the humoral and cellular immune response. Total plasma Ig levels did not differ between the groups, except for IgG2c (Figure III in the online-only Data Supplement). However, plasma levels of IgG1 and IgG2c antibodies to malondialdehyde-modified LDL were significantly reduced in mice reconstituted with BAFF-R−/− bone marrow compared with controls (Figure 1C). Interestingly, serum levels of total or malondialdehyde-LDL IgM antibodies were not altered by BAFF-R deficiency (Figure 1C and data not shown), a finding consistent with the preservation of B1a cells that are responsible for serum levels of natural IgM antibodies, shown to be atheroprotective. In a previous study from our group, we reported an important impact of mature B-cell depletion on dendritic cell (DC) activation and T-cell proliferation. In the present study, we also found a significant reduction of DC activation in mice reconstituted with BAFF-R−/− bone marrow, as exemplified by the reduction of CD40 and MHC-II expression on CD11c+DCs (Figure IV in the online-only Data Supplement). BAFF-R deficiency was also associated with a marked reduction of T-cell activation (Figure 1D) and T-effector cell proliferation (Figure 1E) in the absence of any change in the percentage or suppressive function of CD4+CD25+ regulatory T cells (data not shown). This finding suggests an important role for B2 cells in the maintenance of effector T-cell responses in the context of atherosclerosis.

Finally, we addressed the effect of BAFF-R deficiency on the development of atherosclerotic lesions. As shown in Figure 2A, quantification of intimal area in the aortic root after Oil red O staining at 6 weeks of high-fat diet revealed a significant

![Figure 1. B-cell activating factor receptor (BAFF-R) deficiency in bone marrow cells markedly depletes B2 cells, alters the production of anti-MDA-LDL antibodies and impairs T cell activation and proliferation. A, Percentages of B lymphocytes (see Methods in the online-only Data Supplement) in bone marrow (BM), spleen (Sp), blood (Bl) and lymph nodes (LN) of Ldlr−/− mice reconstituted with either a wild type (WT; n=11) or BAFF-R−/− (n=10) bone marrow and fed a high fat diet for 6 weeks. B, Percentages of peritoneal B-cell subsets (see Methods in the online-only Data Supplement) in the same groups of mice. C, Plasma antibody (IgG1, IgG2c, and IgM) levels against malondialdehyde-modified LDL in the same groups of mice. D, Quantitative analysis of CD69− and CD44+ expression using flow cytometry on spleen-derived CD4+ cells in the 2 groups of mice after 8 wk of high-fat diet. E, T-effector cell proliferation using tritiated thymidine after stimulation of purified spleen-derived CD4+CD25− with anti-CD3 antibody (see Methods). Mean values±SD are represented (±SEM for T-cell proliferation). *P<0.05.](https://example.com/figure1.png)
reduction of lesion size in mice reconstituted with BAFF-R−/− bone marrow compared with controls. Interestingly, BAFF-R deficiency was also associated with a reduction of lesion inflammation, as revealed by the significant decrease of total macrophage accumulation (MOMA2 staining) in the aortic root (Figure 2B) and a trend toward decreased ratio of MOMA2 staining to total lesion area (0.59±0.29 versus 0.84±0.35, P=0.11). The reduction of lesion size in mice reconstituted with BAFF-R−/− bone marrow was also significant after 8 weeks on high-fat diet (Figure 2A) despite similar serum cholesterol levels between the 2 groups of mice (8.65±1.36 versus 8.50±1.03 g/L, P=0.82). Selective BAFF-R deficiency on B cells (see Methods in the online-only Data Supplement) did not alter T-cell count (Figure I in the online-only Data Supplement) but led to selective depletion of B2 cells, associated with a reduction of T-cell activation despite no change in DC activation (Figure V in the online-only Data Supplement). Spleen T cells from these mice (20% BAFF-R−/− T cells) showed the same reduction in proliferation in vitro compared to mice with 100% BAFF-R−/− T cells (data not shown), indicating the effect is due to the lack of B cells rather than BAFF-R on T cells. Indeed, we did not detect expression of BAFF-R on freshly isolated spleen T cells by flow cytometry (data not shown). Interestingly, selective BAFF-R deficiency on B cells also led to a significant reduction of lesion development (Figure 2A) despite no change in serum cholesterol levels (9.84±1.45 vs 9.84±1.82 g/L, P=1.00). Additionally, BAFF-R deficiency, whether in all cells or selectively in B cells, led to decreased numbers of T cells present in atherosclerotic plaque (Figure 2C).

**Discussion**

We have presented 3 different experiments showing that BAFF-R deficiency in bone marrow-derived cells reduces atherosclerosis. BAFF-R deficiency is associated with a reduction of T cell activation, reduced T-cell numbers in plaques, and attenuation of adaptive humoral responses against malondialdehyde-LDL. This contrasts with the preservation of the B1a cell subtype and the production of natural IgM antibodies. The result is a significant attenuation of macrophage accumulation within the vessel wall and thus a reduction of lesion development in Ldlr−/− mice. During the review process, Kyaw et al reported reduced formation of atherosclerotic plaque in Apoe−/−/BAFF−/− mice compared with Apoe−/− mice, which is in agreement with the present results. Our studies on the mixed µMT/BAFF-R chimeras also show that BAFF-R expression on B cells is sufficient to drive T-cell activation and atherogenesis, which is a unique and novel aspect. In addition, and in contrast to BAFF-R deficiency in total bone marrow-derived cells, DC activation is not altered in mice with selective BAFF-R deficiency in B cells. These results indicate that BAFF-R signaling in B cells directly drives T-cell activation/proliferation and atherosclerosis independently of DC activation. Finally, our results clearly suggest that blockade of BAFF-R signaling may constitute an interesting therapeutic strategy to limit the development of atherosclerosis, particularly in patients with associated BAFF-dependent immune-mediated diseases.

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**Disclosures**

None.

**References**


