

Sage et al., BAFF-R and atherosclerosis

BAFF (B Cell Activating Factor) Receptor Deficiency Reduces the Development of Atherosclerosis in Mice

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Word count: 2633.

Abstract

Objective— To assess the role of BAFF (B cell activating factor) receptor in B cell regulation of atherosclerosis.

Methods and Results— Male low-density lipoprotein 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 LDL. 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.

Word count: 161

Key words: lymphocytes, immunity, atherosclerosis.

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 proteins¹⁻⁴. 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 athero-protective role of the B lymphocyte subset^{5,6}. 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 pro-atherogenic and have pinpointed T cell regulatory subsets with potent anti-atherogenic properties⁷⁻⁹. More recently, we reconsidered the role of B lymphocytes in atherosclerosis. We clearly showed that contrary to expectations based on previous studies^{5,6}, depletion of mature B cells in mice reduces lesion development, indicating the presence of a pro-atherogenic B cell subset¹⁰. These results have been confirmed by another group of investigators¹¹ 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 pro-atherogenic effect is mainly driven by the common B2 subset, which responds to T cell-dependent antigens and is part of the adaptive immune response^{10,11}. In contrast, the athero-protective 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^{12,13}. This protective property may largely depend on the capacity of natural IgM to recognize oxidation-specific epitopes on oxidized LDL and cellular debris¹⁴. However, a recent study has shown that B cell homing to atherosclerotic lesions might be important for the athero-

protective effects of B cells, independently of IgM levels¹⁵. 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 BAFF (B cell activating factor), a member of the tumor necrosis factor family¹⁶. 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 trials¹⁶. The survival role of BAFF on the B2 cell subset is mediated through BAFF receptor (BAFF-R), mostly expressed on mature B cells¹⁶. 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^{16,17}. We therefore hypothesized that inhibition of BAFF-R signaling would reduce B2 pro-atherogenic activity while preserving B1 athero-protective 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 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 Supplemental Methods). The mice were put on a high fat diet for 6 weeks to address the role of BAFF-R on immune responses and lesion development in the context of atherosclerosis. Body weights (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 (Supplemental Fig. I). 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 Supplemental Fig. II). 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 (Supplemental Fig. III). However, plasma levels of IgG1 and IgG2c antibodies to malondialdehyde (MDA)-modified LDL were significantly reduced in mice reconstituted with *BAFF-R*^{-/-} bone marrow compared with controls (Figure 1C). Interestingly, serum levels of total or anti-MDA-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 athero-protective^{12, 18}. In a previous study from our group¹⁰, 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 (Supplemental Fig. IV). BAFF-R deficiency was also associated with a marked reduction of T cell activation (Figure 1 D) 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 towards decreased ratio of MOMA2 staining to total lesion area (0.59 ± 0.29 vs 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 vs 8.50 ± 1.03 g/L, $P=0.82$). Selective BAFF-R deficiency on B cells (see Supplemental Methods) did not alter T cell count

(Supplemental Fig. I) but led to selective depletion of B2 cells, associated with a reduction of T cell activation despite no change in DC activation (Supplemental Fig. V). Spleen T cells from these mice (20% BAFF-R^{-/-} T cells) showed the same reduction in proliferation *in vitro* compared to controls as mice with 100% BAFF-R^{-/-} T cells (data not shown), indicating the affect 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 three 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 MDA-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*^{-/-} mice¹⁹, which is in agreement with the present results. Our studies on the mixed μ MT/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.

Acknowledgements: None.

Source of Funding: British Heart Foundation, Fondation Leducq.

Disclosures: The authors have no conflicting financial interests to disclose.

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FIGURE LEGENDS

Figure 1. 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 Supplemental Methods) in bone marrow (BM), blood (Bl), spleen (Sp) 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 Supplemental Methods) in the same groups of mice. C) Plasma antibody (IgG1, IgG2c and IgM) levels against MDA-modified LDL in the same groups of mice. D) Quantitative analysis of CD69⁺ and CD44^{high} expression using flow cytometry on spleen-derived CD4⁺ cells in the 2 groups of mice after 8 weeks 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 ± S.D. are represented (± s.e.m. for T cell proliferation). *P<0.05.

Figure 2. BAFF-R deficiency in bone marrow cells reduces the development of atherosclerosis. A-C) *Ldlr*^{-/-} mice were lethally irradiated and reconstituted with either a wild type or *BAFF-R*^{-/-} bone marrow, or a mixture of 80% bone marrow from μ MT mice and 20% bone marrow from either WT (μ MT/WT) or *BAFF-R*^{-/-} mice (μ MT/BR^{-/-}). After 4 weeks of recovery, they were fed a high fat diet for 6 or 8 weeks. A) Quantitative analysis of intimal lesion area at the aortic root on Oil red O-stained sections. B)

Quantitative analysis of macrophage area at the aortic root on MOMA2-stained sections.

C) Quantitative analysis of T cell numbers at the aortic root on CD3-stained sections.

*P<0.05.