A novel modality of BAFF-specific inhibitor AMG623 peptibody reduces B-cell number and improves outcomes in murine models of autoimmune disease

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Abstract

Objectives

AMG623, also known as A-623, is an antagonist of B-cell activating factor (BAFF). The present study was to evaluate the effects of AMG623 on murine models of autoimmune diseases.

Methods

AMG623 was generated through phage library. Inhibitory activities of AMG623 against human and murine BAFF were measured by biacore binding and BAFF-mediated B-cell proliferation assay. Pharmacological effects of AMG623 were studied in BALB/c mice, collagen-induced arthritis model (CIA) and in the NZBxNZW F1 lupus model.

Results

AMG623 binds to both soluble and cell surface BAFF. AMG623 blocks both human murine BAFF binding to the receptors. Treatment of AMG623 resulted in B-cell number reduction, and improvement of arthritis and lupus development in mice.

Conclusion

AMG623 is a novel modality of BAFF antagonist. AMG623 is a potential therapeutic agent for the treatment of SLE, rheumatoid arthritis, and other B-cell-mediated autoimmune diseases.

Key words

AMG623, BAFF, B-cell, SLE, CIA, autoimmune disease

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Introduction

BAFF is a cytokine of the Tumour Necrosis Factor (TNF) ligand family essential for B-cell survival (1). Its expression is mainly restricted to monocytes, macrophages, dendritic cells, and neutrophil. Like most other type II transmembrane proteins, BAFF may exert its effects either as the full-length membrane-bound form, or as a soluble protein arising from proteolytic cleavage. BAFF is able to bind to 3 receptors expressed on B-cells: BAFFR, TACI and BCMA. BCMA and TACI are able to bind APRIL, another TNF family member.

The essential role of BAFF in B-cell homeostasis suggests that deregulation of BAFF may lead to autoimmune diseases. Indeed, high levels of soluble BAFF have been associated with several B-cell-mediated autoimmune diseases, including lupus (2), rheumatoid arthritis (3), or Sjögren's syndrome (4). Abnormal expression of membrane bound BAFF has also been documented in B-cells from patients with active SLE (5). Several BAFF antagonists are under clinical development. Belimumab, an anti-BAFF monoclonal antibody, has been recently approved for SLE treatment (6). Belimumab cannot be tested in murine models of autoimmune disease because of its lack of cross-reactivity with murine BAFF (7). LY2127300, another BAFF antibody currently being developed for autoimmune disease, also does not bind murine BAFF (8). Atacicept, a TACI-Fc fusion protein that binds both BAFF and APRIL, is in ongoing phase II/III SLE trials (9).

Here we report development and preclinical studies of a novel therapeutic modality, AMG623 peptibody which binds both soluble and membrane BAFF and specifically blocks both human and murine BAFF. AMG623 is currently being developed for the treatment of SLE and other B-cell-mediated autoimmune diseases under the name A-623 by Anthera Pharmaceuticals, Hayward, California.

Materials and methods

For the biacore binding assay, biacore sensor chip surface was immobilised with soluble receptor proteins according to manufacturer's instructions (BIACore, Inc., Piscataway, NJ). 1 nM of human or murine BAFF, or 2nM of murine APRIL described previously (10-11), was pre-incubated with AMG623 before injection over the biacore chip for 25 minutes at 10 μl/min. For the B-cell proliferation assay, 10^5 purified mouse spleen B-cells were cultured in 96-well plate in MEM media plus 10% FBS, 10 ng/ml BAFF protein, 2 μg/ml Goat $F(ab')_2$ anti-IgM, and a serially diluted amount of AMG623 for 48 hours. Proliferation was measured by (^{3}H) thymidine update.

To study the effect of AMG623 treatment on B-cells, female BALB/c mice (n=6/group, 8 weeks of age) were dosed intravenously three times weekly for two weeks with PBS, human Fc, or AMG623. B-cells in peripheral blood were determined by B220 staining in FACS analyses.

For the CIA model, ten-week-old male DBA/1 mice were injected intradermally at the base of the tail with 100 μg of bovine type II collagen emulsified in CFA on day -21. Three weeks later, mice were given a booster injection with 100 μg of CII emulsified in IFA (Difco, Franklin Lakes, NJ) on day 0. Starting from the booster day, mice (n=10) were treated with PBS, human Fc (5 mg/kg), or AMG623 (5 mg/kg), intraperitoneally 3 times/week for 4 weeks.

For the lupus model, female 6-month-old NZBWF1 mice (Jackson Labs, Bar Harbor, ME) were screened for low levels of protein in the urine using the Albustix strips (Bayer Diagnostics, Pittsburg, PA). Animals with less than 100 mg/dl protein in urine were included in the study. Mice were injected intraperitonially with PBS, 4mg/kg human Fc or 4mg/kg AMG623, three times per week for 5 months.

Results

To select for BAFF-binding peptides, a 12-mer constrained phage library was screened by repeated panning with recombinant Fc-BAFF protein immobilised on protein A magnetic beads. The BAFF-binding peptides were fused in tandem copies in-frame to the N-terminus of the Fc region of human IgG1 (named as peptibody) and expressed

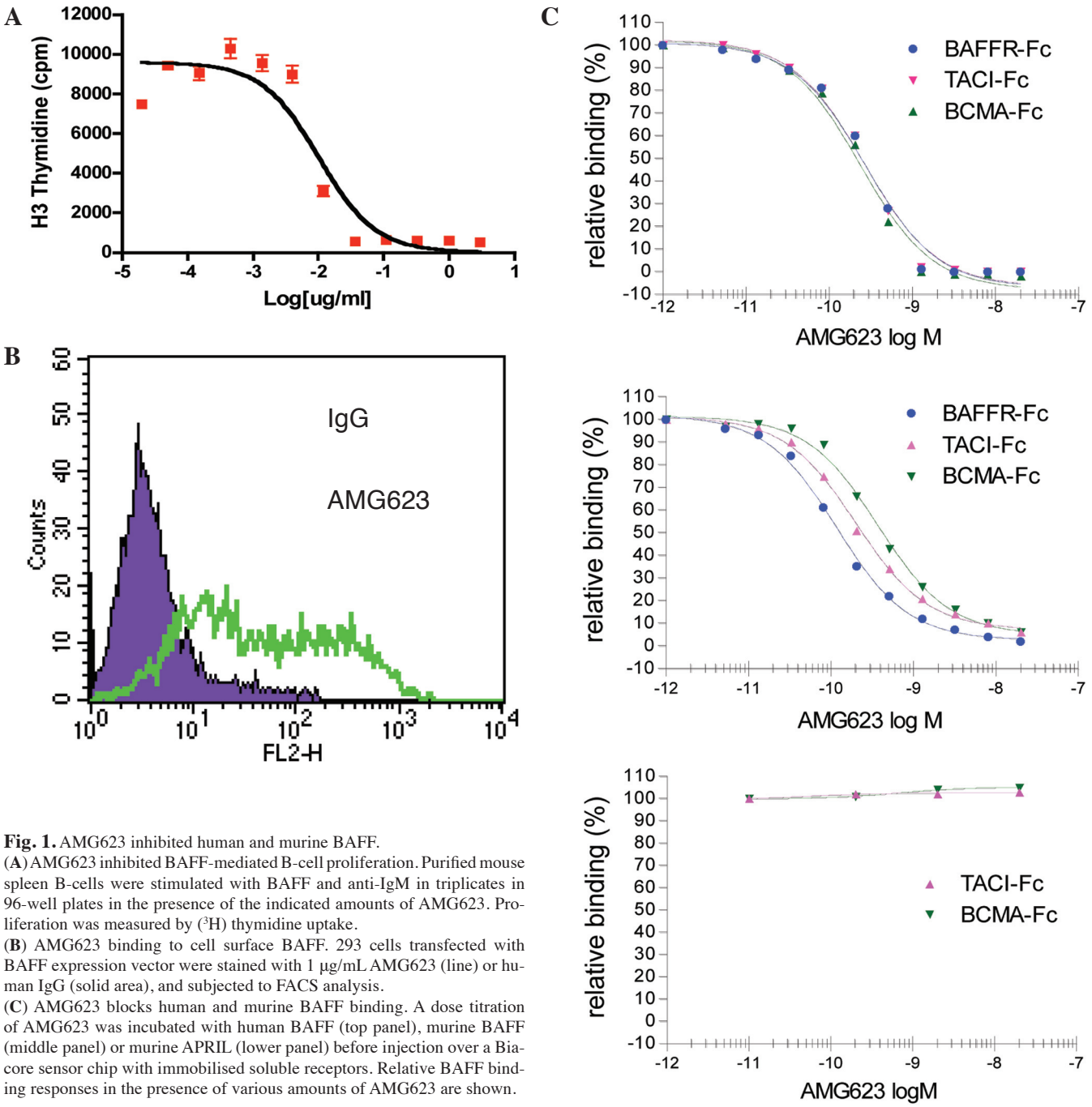

Fig. 1. AMG623 inhibited human and murine BAFF.
(**A**) AMG623 inhibited BAFF-mediated B-cell proliferation. Purified mouse spleen B-cells were stimulated with BAFF and anti-IgM in triplicates in 96-well plates in the presence of the indicated amounts of AMG623. Proliferation was measured by (^{3}H) thymidine uptake.
(**B**) AMG623 binding to cell surface BAFF. 293 cells transfected with BAFF expression vector were stained with 1 μg/mL AMG623 (line) or human IgG (solid area), and subjected to FACS analysis.
(**C**) AMG623 blocks human and murine BAFF binding. A dose titration of AMG623 was incubated with human BAFF (top panel), murine BAFF (middle panel) or murine APRIL (lower panel) before injection over a Biacore sensor chip with immobilised soluble receptors. Relative BAFF binding responses in the presence of various amounts of AMG623 are shown.

in *E. coli*. Purified peptibodies were analysed in the BAFF-mediated B-cell proliferation assay for antagonist activity. AMG623, a potent peptibody obtained through this exercise, inhibited BAFF-mediated B-cell proliferation with IC50 6 ng/ml (Fig. 1A). AMG623 was able to bind cell surface BAFF expressed on the BAFF transfected 293 cells (Fig. 1B). AMG623 binds to human BAFF with K_D of 1 pM analysed by KinExA method (data not shown). AMG623 blocked both human and murine BAFF binding to BAFFR, TACI, and BCMA (Fig. 1B), but did not affect APRIL binding (Fig. 1C).
Since AMG623 blocks murine BAFF binding to its receptors, we examined its impact on the mouse B-cell compartment. Treatment of AMG623 in BALB/c mice resulted in significant reduction of B-cell numbers in peripheral blood and spleen (Fig. 2A). T-cell numbers were not changed in all dosage groups (data not shown). In the collagen induced arthritis model, significantly reduced arthritis scores were observed after AMG623 treatment (Fig.2B). In the lupus prone NZB/NZW F1 mice, AMG623 treatment delayed proteinuria onset and prolonged the survival (Fig. 2C).

Discussion

Comparison of BAFF antagonist in preclinical and clinical studies will be very informative to the scientific community in the comparison of animal disease models with human disease devel-

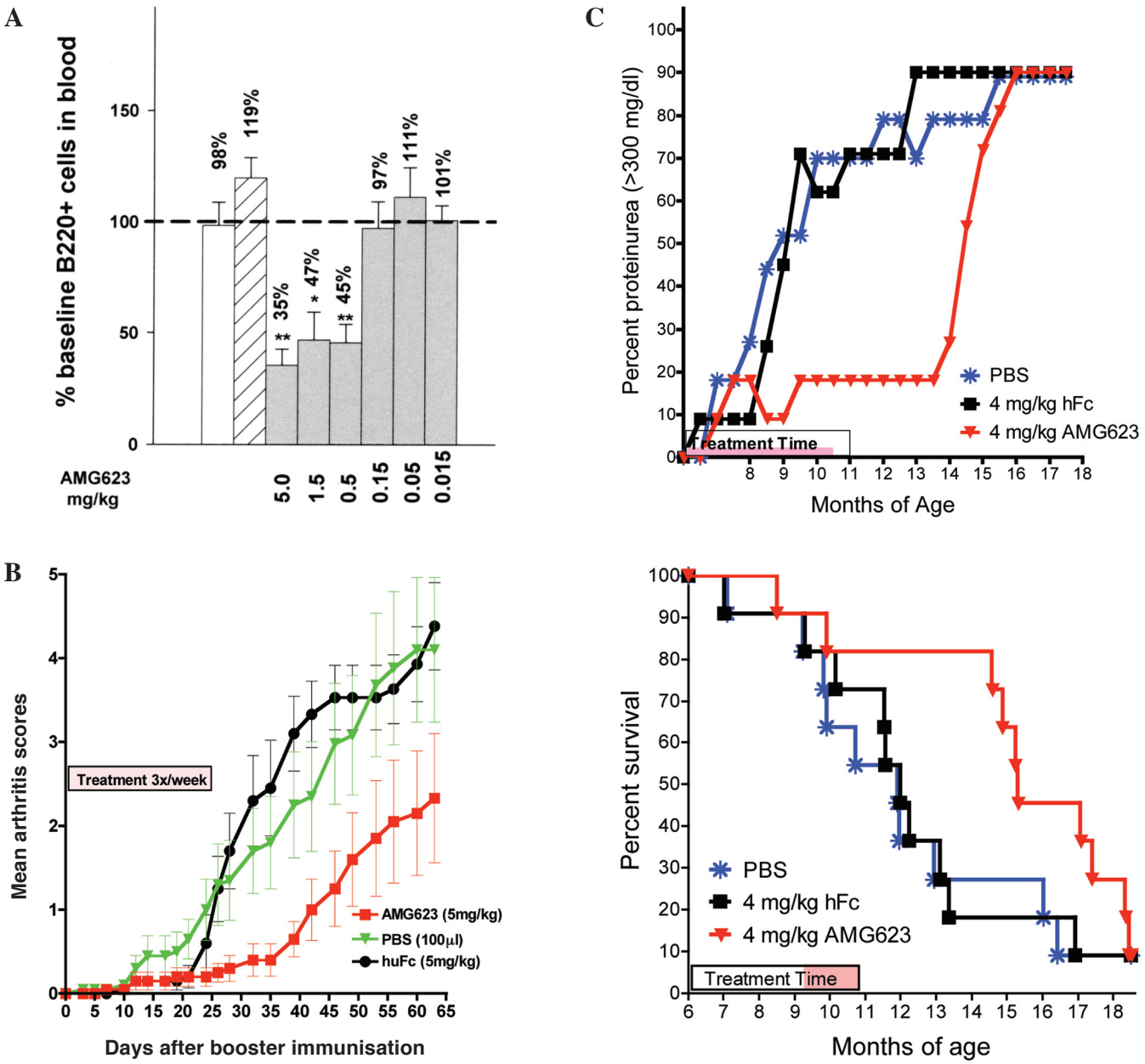

Fig. 2. Characterisation of AMG623 in murine autoimmune disease models.
(**A**) AMG623 reduced B-cells in mouse. BALB/c female mice were treated with indicated amount of AMG623 or control human Fc protein intravenous (IV) 3 times weekly for 2 weeks (Day 0 to Day14). Blood was collected on Day 0 prior to the treatment and on Day 14 for FACS analysis. *p<0.05 compared to PBS treatment using the paired student's t-test.
(**B**) AMG623 reduced disease severity in mouse CIA model. DBA/1 mice (n=10) were treated with PBS, 5mg/kg of AMG623 or human intraperitoneally 3x per week starting the collagen for 4 weeks. Significant reduction of arthritis scores (p<0.05) was observed for AMG623 treated mice compared with human Fc analysed by Dunnett's test.
(**C**) AMG623 reduced disase severity in mouse lupus model. Six-month-old NZBxNZB F1 female mice (n=11) were treated with PBS, AMG623 or human IgG Fc intraperitoneally 3x per week for 5 months. Percent of mice developed proteinuria >300mg/dL was shown on the upper panel. Percent of survival throughout the study was shown on the lower panel. p=0.04 compared to the human Fc treatment group using the Tarone modified log-rank statistics.

opment. Several BAFF antagonists are currently under clinical development for autoimmune diseases. However, the lack of murine cross-reactivity of belimumab and LY2127300 prohibited their studies in rodent models. Atacicept, a fusion protein containing extracellular domain of TACI fused with Fc region, binds both BAFF and APRIL, limiting its value in the understanding of BAFF specific biology. AMG623, on the other hand, blocks both human and murine BAFF specifically. We showed here AMG623 treatment in mice delayed disease onset in both lupus and arthritis models. The efficacy waned 3 months after the last treatment in the lupus model, suggesting continuous BAFF inhibition is required for disease control.

Like TNF, BAFF is a type II transmembrane protein which exerts its biological effects either as the full-length membrane-bound form or as a soluble protein arising from proteolytic cleav-

age. Belimumab was reported to only bind soluble BAFF but not cell surface BAFF (7). AMG623 binds to both cell surface and soluble BAFF, therefore, it may have more profound impact on BAFF mediated B-cell survival. It remains to be tested if AMG623 has same or different efficacy than belimumab in human.

Overall, AMG623 is a new class of BAFF inhibiting biologics, currently in clinical development by Anthera Pharmaceuticals under the name A-623. We believe the preclinical studies described here along with the ongoing clinical studies may provide insights in the translation of mouse disease model to human disease development. AMG623.

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