Therapeutic Effects of Anti–Bone Morphogenetic Protein and Activin Membrane-Bound Inhibitor Treatment in Psoriasis and Arthritis

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Objective. The transforming growth factor β (TGFβ) inhibitor BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) has been shown to control differentiation of CD4+ T lymphocytes into either tolerogenic Treg cells or pathogenic Th17 cells, through the regulation of TGFβ and interleukin-2 (IL-2) signaling strength. The present study was undertaken to explore the potential beneficial effects of this strategy of pharmacologic inhibition using novel anti-BAMBI monoclonal antibodies (mAb) in different experimental murine models of chronic skin and joint inflammatory/autoimmune disease.

Methods. Development of Saccharomyces cerevisiae mannan-induced psoriatic arthritis (MIP) (n = 18–30 mice per group), imiquimod-induced skin psoriasis (n = 20–30 mice per group), or type II collagen–induced arthritis (CIA) (n = 13–16 mice per group) was analyzed in a total of 2–5 different experiments with either wild-type (WT) or BAMBI-deficient B10.RIII mice that were left untreated or treated with mAb B101.37 (mouse IgG1 anti-BAMBI), a mouse IgG1 anti-TNP isotype control, anti-CD25, or anti-TGFβ mAb.

Results. Treatment of normal mice with IgG1 anti-BAMBI mAb clone B101.37 led to expansion of Treg cells in vivo, and had both preventive and therapeutic effects in mice with MIP (each P < 0.05 versus controls). The conferred protection against disease progression was found to be mediated by Treg cells, which controlled the activation and expansion of pathogenic IL-17–producing cells, and was dependent on the level of TGFβ activity. Furthermore, treatment with B101.37 mAb blocked both the development of skin psoriasis induced by imiquimod and the development of CIA in mice (each P < 0.05 versus controls). Finally, pharmacologic inhibition of BAMBI with the IgM anti-BAMBI mAb B143.14 also potentiated the suppressive activity of Treg cells in vitro (P < 0.001 versus controls).

Conclusion. These results in murine models identify BAMBI as a promising new therapeutic target for chronic inflammatory diseases and other pathologic conditions modulated by Treg cells.

INTRODUCTION

Regulatory CD4+ T cells (Treg cells) exhibit suppressive properties, and their absence and/or abnormal function promote autoimmune diseases (1–5). Treg cells also prevent allograft rejection (6). Therefore, therapeutic strategies aimed at increasing the numbers and/or activity of Treg cells are currently under investigation in different clinical trials (7,8). Treg cells can be subdivided into thymus-derived Treg cells, which differentiate within the thymus from immature T cells, and peripheral Treg cells, which are derived from Cantabria, CSIC-Universidad de Cantabria-SODERCAN, Santander, Spain. Drs. J. Merino and R. Merino share senior authorship. Drs. Augustin and Tamayo contributed equally to this work. Drs. J. Merino and R. Merino own stock or stock options in Inhibitec-Anticuerpos S. L., which possesses the license for the commercialization and exploitation of a patent application entitled “Monoclonal antibodies against BAMBI and use for the treatment of inflammatory diseases.” No other disclosures relevant to this article were reported. Address correspondence to Ramón Merino, MD, PhD, Instituto de Biomedicina y Biotecnología de Cantabria, Calle Albert Einstein 22, PCTCAN, 39011 Santander, Spain. Email: merinor@unican.es. Submitted for publication March 25, 2019; accepted in revised form March 24, 2020.
from CD4+ T cells that are activated in secondary lymphoid organs in the presence of transforming growth factor β (TGFβ) and interleukin-2 (IL-2) (1,2,9).

TGFβ also participates in the conversion of CD4+ T lymphocytes into Th17 cells (10–12), a subpopulation of effector cells that are known to be involved in the defense against extracellular bacteria and fungi (13), but also known to be involved in many inflammatory diseases (14). The capacity of TGFβ to promote the differentiation of Th17 cells depends on the co-presence of proinflammatory cytokines such as IL-6, IL-23, IL-1β, and/or IL-21 (10–12,15,16). In addition, we have recently reported that BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) regulates the activity of TGFβ during differentiation of Th17 and Treg cells (17).

BAMBI is a transmembrane protein homologous to TGFβ type I receptors (TGFβRI) that antagonizes TGFβ superfamily signals by preventing the formation of active receptor complexes upon ligand binding (18). We have previously demonstrated that BAMBI fixes the intensity of TGFβ signaling and regulates CD25 expression and IL-2 signaling strength in CD4+ T lymphocytes. Consequently, in a state of BAMBI deficiency, in vitro Treg cell differentiation is increased and in vivo Th17 cell differentiation is decreased (17). These results point to this pseudoreceptor as a potential new therapeutic target in chronic inflammatory diseases. In the present study, we explored this possibility by evaluating the preventive and therapeutic effects of treatment with a novel anti-BAMBI monoclonal antibody (mAb) in several experimental murine models of chronic inflammatory skin and joint disease.

MATERIALS AND METHODS

Mice and mAb. Wild-type (WT) C57BL/6 (B6.WT) and WT B10.RIII (B10.RIII.WT) mice were obtained from Charles River. The generation of B6 and B10.RIII mice deficient in BAMBI (B6.BAMBI-KO and B10.RIII.BAMBI-KO, respectively) has been described previously (17). All experimental disease models were performed in 2–3-month-old male B10.RIII mice, and B6.WT and B10.RIII. WT littermates were used as controls in the experiments with BAMBI-KO mice. All mice were maintained in a conventional animal room at the University of Cantabria animal facilities.

Genotyping of mice was performed using polymerase chain reaction (PCR) to analyze the genomic tail DNA of each mouse. For mouse bone marrow (BM) chimeras, 2-month-old male B10. RIII.WT and B10.RIII.BAMBI-KO mice were irradiated uniformly at 950 cGy, with a dose rate of 130 cGy/minute, and reconstituted with 10^5 BM cells (BMCs) from either B10.RIII.WT or B10. RIII.BAMBI-KO mice. Two months later, the level of reconstitution of hematopoietic cells was controlled via flow cytometry. All animal care and experimental procedures were approved by the University of Cantabria Institutional Laboratory Animal Care and Use Committee (approval reference no. 2017/05).

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In all experimental models, the clinical severity and histologic scores of disease were determined by 2 blinded observers (PA and OA). For generation of Saccharomyces cerevisiae mannan (SCM)-induced psoriatic arthritis (MIP), IgG1-C– or B101.37-treated WT BAMBI-KO and WT BM-chimeric mice were injected intraperitoneally with 10 mg of SCM (Sigma-Aldrich) dissolved in 200 μl of phosphate buffered saline (PBS), on day 0 only or for multiple exposures on day 0 followed by days 7 and 14, as described previously (20). The severity of skin psoriasis was evaluated daily by measuring ear thickening macroscopically using a digital caliper (Somet C2), at the end of the experiment and then again after histologic analysis of paraffin-embedded skin tissue sections stained with hematoxylin and eosin (H&E).

Induction and assessment of chronic inflammatory diseases and treatments. In all experimental models, the clinical severity and histologic scores of disease were determined by 2 blinded observers (PA and OA). For generation of Saccharomyces cerevisiae mannan (SCM)-induced psoriatic arthritis (MIP), IgG1-C– or B101.37-treated WT BAMBI-KO and WT BM-chimeric mice were injected intraperitoneally with 10 mg of SCM (Sigma-Aldrich) dissolved in 200 μl of phosphate buffered saline (PBS), on day 0 only or for multiple exposures on day 0 followed by days 7 and 14, as described previously (20). The severity of skin psoriasis was evaluated daily by measuring ear thickening macroscopically using a digital caliper (Somet C2), at the end of the experiment and then again after histologic analysis of paraffin-embedded skin tissue sections stained with hematoxylin and eosin (H&E).

Paw swelling was scored daily in a manner as described previously (17,21). At the end of the experiment, the hind paws were fixed in 10% phosphate buffered formaldehyde solution and decalcified in Parengy’s decalcification solution overnight. Thereafter, paraffin-embedded paw tissue sections (4 μm) were stained with H&E.

Expression levels of messenger RNA (mRNA) for the IL-1β, tumor necrosis factor (TNF), IL-6, IL-17A, and IL-23 cytokines in the ears and hind paws were explored by quantitative real-time reverse transcription–PCR 6 days after injection of PBS or SCM, as described previously (21). Results (analyzed in triplicate experiments) were normalized to the expression levels of GAPDH and measured in parallel in each sample.

Imiquimod-induced skin psoriasis was induced after topical application of a cream containing 5% imiquimod (Aldara; 3M Pharmaceuticals) for 5 consecutive days (12.5 mg imiquimod/day) on the right ears of IgG1-C– or B101.37-treated male WT or BAMBI-KO mice. Left ears were kept untreated as a control. The severity of psoriasis was evaluated daily by analyzing the extent of erythema and desquamation in the skin according to a grading scale of 0–3, as follows: 0 = normal skin, 1 = small changes, 2 = moderate changes, and 3 = big changes. In addition, ear thickening was evaluated using a digital caliper to measure the thickness of imiquimod-treated ears in comparison to their respective untreated control ears, which was graded on a scale of 0–4, as follows: 0 = no thickening, 1 = 1–10% increase in thickening, 2 = 11–25% increase in thickening, 3 = 26–50% increase
in thickening, and 4 = >50% increase in thickening. The scores of these individual aspects of dermatitis were added to obtain the cumulative score of psoriasis severity.

Imiquimod-treated and control ears were analyzed by histology at the end of the experiment. Sections (4 μm) of paraffin-embedded ear tissue were stained with H&E. For quantification of epidermal and dermal thickening, images from 3 different sections in each ear were captured and quantified with an Axio Scan Z1 equipped with ZEN 2012 software (blue edition; Zeiss Iberica). Thickening was evaluated in 3 different ear regions at each side of the cartilage (total of 6 measurements per section per mouse). Total values represent the mean of the 18 individual measurements of thickness per ear.

For induction of CIA, bovine type II collagen was emulsified with Freund's complete adjuvant (CII-CFA) containing 4 mg/ml of Mycobacterium tuberculosis (MD Bioproducts). The induction of CIA in IgG1-C– or B101.37-treated male WT and BAMBI-KO mice and the clinical and radiologic evaluations of arthritis severity were performed as described previously (17,21). Sections of paraffin-embedded hind paws were stained with H&E. The severity levels of synovial inflammation, bone erosion, cartilage damage, and leukocyte infiltration were each assessed in at least 4 different sections using a scale of 0–4, as follows: 0 = normal, 1 = mild, 2 = moderate, 3 = marked, and 4 = severe. The histologic score of CIA was calculated by summing the scores from each of these scales.

B6.BAMBI-KO and B6.WT mice treated with IgG1-C or B101.37 mAb were injected with JES6-1A12 containing IL-2–anti–IL-2 immune complexes (ICs) as described previously (22). The effects of these treatments on Treg cells in the lymph nodes and spleen were evaluated 5 days later by flow cytometry.

For in vivo inhibition of BAMBI, mice were treated intraperitoneally with 2 mg/week of B101.37. Control mice were treated with 2 mg/week of IgG1-C mAb. For CD4+CD25+ T cell depletion, WT and BAMBI-KO mice were treated intraperitoneally with 1 mg of anti-CD25 mAb at 1 week before injection of SCM. For in vivo inhibition of TGFβ or IL-17, WT and BAMBI-KO mice were injected with 1 mg of the respective mAb at 1 day before SCM injection.

**Cell cultures.** Naïve CD4+ T cells and Treg cells from B6.WT mice were purified by cell sorting on a FACS aria (BD Biosciences). Antigen-presenting cells (APCs) were obtained from irradiated spleen cells. For the assessment of Treg cell activity, 5 × 10⁴ CD4+CD25– cells were cultured in triplicate over 3 days in complete RPMI medium and stimulated with 0.5 μg/ml of anti-CD3 mAb in the presence of 5 × 10⁴ APCs, decreasing ratios of Treg cells, and 20 μg/ml of IgM anti-BAMBI B143.14 mAb or 20 μg/ml of murine IgM (Sigma). Cultures were pulsed with 1 μCi of ℎ-ethyl-thymidine for the final 6 hours of culture, and thereafter cells were harvested and counted.

**Flow cytometry.** Frequencies of Treg cells and Th17 cells in the spleen, lymph nodes, and ears of IgG1-C– or B101.37–treated WT and BAMBI-KO mice were explored by flow cytometry using commercially labeled antibodies (from BioLegend and eBioscience) introduced into the cultures at 6 days after injection of PBS or SCM. Intracellular cytokine staining was performed using an intracellular staining kit (BD Biosciences) as described previously (17,21). For ear cell suspensions, samples were pooled from 3–5 ears per experimental group. Skin sheets from the naïve and diseased mouse ears were separated from cartilage and processed as described previously (23). Cells were analyzed in a FACSCanto II flow cytometer using FACS Diva software (BD Biosciences).

**Statistical analysis.** Statistically significant differences between the groups were analyzed by one-way and two-way analyses of variance, with Tukey’s or Dunnett’s post hoc test for multiple comparisons, and by Student’s t-test, using GraphPad Prism version 6.0 software. P values less than 0.05 were considered significant.

**RESULTS**

Enhancement of Treg cell expansion in vivo and increase in suppressive activity of Treg cells in vitro following pharmacologic inhibition of BAMBI with anti-BAMBI mAb. We recently described the generation of 2 novel anti-mouse BAMBI mAbs, clones B101.37 (mouse IgG1) and B143.14 (mouse IgM) (17). Herein, we explored the in vivo inhibitory activity of the B101.37 mAb. Treatment of B6.WT mice with B101.37 caused a slight but significant increase in the percentage of Treg cells in the lymph nodes and spleen, when compared to that in B6.WT mice receiving IgG1-C control treatment (Figure 1A). As previously reported (17), the injection of IL-2 ICs containing the JES6-1A12 mAb, which selectively targets T cells expressing CD25 (22), promoted a greater expansion of Treg cells in the lymph nodes and spleen of B6.BAMBI-KO mice compared to B6.WT mice (Figure 1A). Interestingly, the increase in Treg cells following injection of IL-2 ICs in B6.WT mice was also higher after B101.37 mAb treatment than after IgG1-C injection (Figure 1A). Further information on these findings is available from the corresponding author upon request.

Compared to B6.WT mice, Treg cells from B6.BAMBI-KO mice exhibited higher expression of CD25 (17) (Figure 1B). After treatment with B101.37 mAb, Treg cell expression of CD25 in B6.WT mice increased to the levels found in untreated B6.BAMBI-KO mice and was higher than the levels observed in IgG1-C–treated WT mice, regardless of whether or not the mice had received an injection of IL-2 ICs (Figure 1B).

Our previous findings showed that BAMBI deficiency also enhances the in vitro suppressive activity of Treg cells (17). Accordingly, the suppressive activity of B6.WT mouse Treg cells...
was increased after the addition of B143.14 mAb to the cultures, which inhibited BAMBI in vitro (17), whereas the IgM isotype control antibody had no effect (Figure 1C). Taken together, these results indicate that BAMBI inhibition with the B101.37 mAb and the B143.14 mAb caused the expansion of Treg cells in vivo and increased their suppressive potential in vitro, respectively.

Effect of BAMBI deficiency or its pharmacologic inhibition with B101.37 mAb in MIP. Since treatment with B101.37 mAb inhibited BAMBI and expanded Treg cells in vivo, we explored its potential therapeutic effect in a recently described experimental murine model of psoriatic arthritis, MIP (20). Following a single intraperitoneal injection of 10 mg of SCM, susceptible B10.RIII.WT mice treated with IgG1 anti-TNP isotype control mAb (IgG1-C) or B101.37 mAb were injected intraperitoneally with phosphate buffered saline (PBS) or 6 μg JES6-1A12–containing IL-2 ICs during 3 consecutive days. Percentages of Treg cells in the lymph nodes and spleen of individual mice were evaluated by flow cytometry 5 days later. B, Expression levels of CD25 were determined in Treg cells from individual mice in each group following injection of PBS or JES6-1A12–containing IL-2 ICs. Values are the mean ± SD uptake of 3H-methyl-thymidine in triplicate cultures. Cumulative results of 3 independent experiments are shown. Statistical differences were analyzed by Student’s t-test (A and B) or two-way analysis of variance with Sidak’s post hoc test for multiple comparisons (C). * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

Figure 1. Effects of BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) monoclonal antibody (mAb) treatment on interleukin-2 immune complex (IL-2 IC)–induced expansion of Treg cells in vivo and regulatory activity of Treg cells in vitro. A, Two-month-old B6 mice deficient in BAMBI (B6.BAMBI-KO) and B6 wild-type (B6.WT) mice treated with murine IgG1 anti-TNP isotype control mAb (IgG1-C) or B101.37 mAb were injected intraperitoneally with phosphate buffered saline (PBS) or 6 μg JES6-1A12–containing IL-2 ICs during 3 consecutive days. Percentages of Treg cells in the lymph nodes and spleen of individual mice were evaluated by flow cytometry 5 days later. B, Expression levels of CD25 were determined in Treg cells from individual mice in each group following injection of PBS or JES6-1A12–containing IL-2 ICs. Values are the mean ± SD uptake of 3H-methyl-thymidine in triplicate cultures. Cumulative results of 3 independent experiments are shown. Statistical differences were analyzed by Student’s t-test (A and B) or two-way analysis of variance with Sidak’s post hoc test for multiple comparisons (C). * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
was observed both in B10.RIII.BAMBI-KO mice and in B10.
RIII.WT mice treated with B101.37 mAb from the time of SCM
injection (Figures 2A–C). Further information on these findings is
available from the corresponding author upon request.

BAMBI-KO mice exhibited a system-wide BAMBI defi-
ciency (24), and it was hypothesized that administration of
B101.37 mAb might also cause a widespread inhibition of
this pseudoreceptor in WT mice. Therefore, the reduced

Figure 2. Development of Saccharomyces cerevisiae mannan (SCM)–induced psoriatic arthritis in B6.BAMBI-KO mice or in B6.WT mice after pharmacologic inhibition with B101.37 mAb. A, Following injection with 10 mg of SCM, mice in each group were examined for the kinetics of skin psoriasis (increase in ear thickening) and paw swelling over 6 days. Results are the mean ± SD of 5–7 mice per group. B and C, Representative images show macroscopic ear skin lesions (upper panels) and histologic ear skin lesions (lower panels; original magnification × 10) (B) and macroscopic paw swelling (C).

D, Cytokine expression in the ears and hind paw footpads of mice was determined by quantitative real-time reverse transcription–polymerase chain reaction 6 days after SCM injection. PBS was used as a control. Cumulative results are the mean ± SD fold change in expression of each cytokine relative to that of GAPDH (n = 7–9 mice per group). E, Percentages of Treg cells and Th17 cells in the lymph nodes and spleen of individual mice were determined by flow cytometry. F, Dot plots show the mean percentages of CD4+FoxP3+ cells and CD4+IL-17+ cells in a representative pool of ear cells from each group of mice. G, Percentages of Treg cells and Th17 cells were determined in individual ear cell pools. Symbols in E and G represent individual mice; horizontal lines show the mean. Statistical differences were analyzed by two-way analysis of variance (ANOVA) with Sidak’s post hoc test for multiple comparisons (A), Student’s t-test (D), or one-way ANOVA with Tukey’s post hoc test for multiple comparisons (E). * = P < 0.05; ** = P < 0.01; *** = P < 0.001. TNF = tumor necrosis factor; NT = not treated (see Figure 1 for other definitions).
disease severity that was observed in both BAMBI-KO and B101.37 mAb–treated mice could possibly be attributed to the absence of BAMBI or inhibition of BAMBI in immune cell populations directly involved in the pathogenesis of this process and/or in other cell types controlling the activity of these relevant immune cells. To discriminate between these 2 possible mechanisms of disease attenuation, lethally irradiated B10.RIII.BAMBI-KO and B10.RIII.WT mice were reconstituted with 10⁷ BMCs from either B10.RIII.BAMBI-KO or B10.RIII.WT mice (Figure 3A). Two months later, the development of MIP was studied in the different groups of mouse BM chimeras. Similar to the observations in nonchimeric mice (Figure 2A), the WT→WT and BAMBI-KO→BAMBI-KO chimeric mice developed MIP that was either severe (in the former) or mild (in the latter) (Figure 3B).

**Figure 3.** Treg cell– and transforming growth factor β (TGFβ)–dependent protection against *Saccharomyces cerevisiae* mannan (SCM)–induced psoriatic arthritis (MIP) in B10.RIII.BAMBI-KO mice or in B10.RIII.WT mice after pharmacologic inhibition with B101.37 mAb. A, Schematic diagram showing the generation of mouse bone marrow cell (BMC) chimeras. B, Kinetics of skin psoriasis and paw swelling in MIP mouse chimeras in 2 independent experiments (Exp.). Results are the mean ± SD of 3–6 mice per group. C and D, Two-three-month-old B10.RIII.BAMBI-KO mice (C) and IgG1-C– or B101.37 mAb–treated WT mice (D) were depleted of CD4+CD25+ Treg cells using an anti–CD25 antibody or treated with anti-TGFβ mAb after receiving a single injection of 10 mg SCM. The kinetics of skin psoriasis and paw swelling were evaluated over 6 days. Results are the mean ± SD of 4–6 mice per group. E, IgG1-C– or B101.37–treated B10.RIII.WT and BAMBI-KO mice were depleted of CD4+CD25+ Treg cells and treated with anti–IL-17A mAb, or left untreated, after receiving a single injection of 10 mg SCM. The severity of skin psoriasis and paw swelling was assessed in individual mice at 6 days after SCM injection. Symbols represent individual mice; horizontal lines show the mean. Statistical differences were analyzed by two-way analysis of variance with Tukey’s post hoc test (B) or Dunnnett’s post hoc test (C and D) for multiple comparisons, or by Student’s *t*-test (E). * = *P < 0.05; ** = *P < 0.01; *** = *P < 0.001. See Figure 1 for other definitions.
Interestingly, BAMBI-KO→WT chimeric mice also failed to develop an aggressive disease. In contrast, a similar level of disease severity was observed in WT→BAMBI-KO and WT→WT chimeric mice (Figure 3B), indicating that the development of MIP or protection against MIP in the different groups of chimeric mice was correlated with the phenotype of the donor BMCs.

To further explore the mechanism of disease protection in B10.RIII.BAMBI-KO and B101.37-treated B10.RIII.WT mice, the expression pattern of mRNAs encoding for different proinflammatory cytokines was explored in the ears and hind paws of these animals. A reduced expression of mRNAs for IL-6, TNF, IL-17A, and IL-23 in the ears and mRNAs for IL-1β, IL-6, and IL-17A in the hind paws was observed in both of these groups of disease-protected animals in comparison to IgG1-C–treated B10.RIII.WT controls (Figure 2D). TNF mRNA expression was also reduced in the hind paws of B10.RIII.BAMBI mice, but not in B101.37-treated B10.RIII.WT mice (Figure 2D).

The pattern of cytokine mRNA expression described above was suggestive of an impaired Th17 cell activity in mice protected from disease progression. To analyze this hypothesis and the potential role of Treg cells in this process, changes in the distribution of Treg cells and Th17 cells were evaluated in mice after MIP induction. Enhanced expansions of Treg cells and reduced expansions of Th17 cells were observed in the lymph nodes, spleen, and ears of B10.RIII.BAMBI-KO and B101.37-treated B10.RIII.WT mice after SCM injection as compared to IgG1-C–treated B10.RIII.WT controls (Figures 2E–G).

Furthermore, the depletion of CD4+CD25+ Treg cells with a cytokotoxic anti-CD25 mAb prior to disease induction promoted the development of MIP in otherwise-protected B10.RIII.BAMBI-KO and B101.37-treated B10.RIII.WT mice (Figures 3C and D). Interestingly, in vivo blockade of IL-17A with an anti–IL-17A mAb (19) restored the protection against MIP in CD4+CD25+ Treg cell–depleted B10.RIII.BAMBI-KO and B101.37-treated B10.RIII.WT mice (Figure 3E), thus stressing the involvement of IL-17A–producing cells in disease development in Treg cell–depleted mice. Further information on these findings is available from the corresponding author upon request.

In addition to TGFβ, BAMBI negatively regulates the activities of activin and bone morphogenetic protein (BMP) (18) and potentiates Wnt signaling (25). However, the administration of an anti-TGFβ mAb, which selectively inhibits TGFβ without affecting activin, BMP, or Wnt/β-catenin signaling pathways, induced the development of MIP in B10.RIII.BAMBI-KO and B101.37-treated B10.RIII.WT mice (Figures 3C and D). Further information on these findings is available from the corresponding author upon request.
Preventive and therapeutic effects of B101.37 mAb treatment in chronic MIP. It has been previously demonstrated that the disease induced in mice after a single injection of SCM is self-limited, whereas multiple injections will cause a sustained disease (20). We used this chronic disease variant of the MIP model to evaluate whether the B101.37 mAb treatment possesses not only preventive effects, but also therapeutic effects in this process. In addition, we explored whether a single injection of B101.37 mAb at the time of disease induction would promote persistent tolerance and block long-term disease development. As previously described (20), a weekly intraperitoneal injection of SCM into IgG1−C−treated B10.RIII.WT mice resulted in development of a chronic skin psoriasis and paw swelling (Figure 4). The severity of these manifestations was significantly reduced in B10.RIII.WT mice treated with B101.37 from the time of the first SCM injection up to the end of disease surveillance (preventive regimen) (Figure 4). Although a single injection of B101.37 at the time of disease induction significantly delayed the development of MIP, the long-term evolution of the disease was similar to that in IgG1−C−treated B10.RIII.WT mice (Figure 4). Treatment with B101.37 mAb that was initiated at 3 days after the first SCM injection, when both skin inflammation and paw swelling were already evident, and then maintained through the end of disease surveillance (therapeutic regimen) controlled the evolution of the disease (Figure 5).
disease to a similar extent as that in B10.RIII.WT mice receiving a preventive regimen of B101.37 (Figure 4).

Prevention of imiquimod-induced skin psoriasis and CIA development with B101.37 mAb treatment. Our previous results clearly showed that BAMBI plays an active role in modulating Treg cell and Th17 cell differentiation during the development of MIP; and that treatment with the inhibitory B101.37 mAb exhibited both preventive and therapeutic effects in this chronic inflammatory disease. To further support these findings, and since none of the existing experimental models of psoriasis have reproduced all of the characteristics of the disease observed in humans (26), we explored the effects of BAMBI deficiency or its pharmacologic inhibition with B101.37 in a second experimental model of skin psoriasis, the imiquimod-induced psoriasis model (27). Topical application of a cream containing 5% imiquimod for 5 consecutive days on the right ears of IgG1-C–treated B10.RIII.WT mice induced an inflammatory skin disease (Figure 5A) with histologic features of psoriasis (Figure 5B). The absence of BAMBI in mutant mice or the treatment of WT mice with B101.37 significantly reduced the clinical and histopathologic signs of the disease in these animals (Figure 5C). Further information on these findings is available from the corresponding author upon request.

Figure 6. Effects of BAMBI deficiency or treatment with B101.37 mAb in B10RIII mice with type II collagen (Col II)–induced arthritis (CIA). Two–three-month-old IgG1-C– or B101.37 mAb–treated B10RIII.WT mice and B10RIII.BAMBI-KO mice were immunized with Col II–Freund’s complete adjuvant. A, The kinetics of arthritis development (clinical score) and arthritis severity at 8 weeks after immunization in individual mice were assessed. Symbols represent individual mice; horizontal lines and bars show mean ± SD. B, Top, Representative radiologic images of the paws show the extent of CIA at 8 weeks after immunization with Col II. Bottom, Individual radiologic signs at 8 weeks after immunization were scored in each group of mice. Results are the mean ± SD of 7–8 mice per group. C, Representative histologic images show the presence of cartilage and bone destruction, synovitis, and pannus formation in the joints of each group of mice with CIA (left), and histologic scores were determined in individual mice (right). Statistical differences were analyzed by two-way analysis of variance with Dunnett’s post hoc test for multiple comparisons (A) or Student’s t-test (B and C). * = P < 0.05; ** = P < 0.01; *** = P < 0.001. NI = non-immunized (see Figure 1 for other definitions).
Furthermore, treatment of B10.RIII.WT mice with B101.37 during the first 4 weeks after immunization with CII-CFA inhibited the development of CIA to the same extent as that in B10.RIII. BAMBI-KO mice (Figure 6A). In contrast, B10.RIII.WT mice treated with IgG1-C developed severe CIA (Figure 6A). The attenuated disease observed in B10.RIII.BAMBI-KO and B101.37-treated B10.RIII.WT mice was further confirmed by radiologic analysis of different signs associated with aggressive arthritis, and by histology showing the presence of cartilage and bone destruction, synovitis, and pannus formation in the joints of B10.RIII.WT mice, but not in the joints of B101.37-treated B10.RIII.WT and B10.RIII. BAMBI-KO mice (Figures 6B and C). Further information on these findings is available from the corresponding author upon request.

**DISCUSSION**

BAMBI has been identified as part of a rheostat-like machinery involved in the control of Treg cell and Th17 cell differentiation. Its deficiency inhibits the development of CIA, indicating that this molecule could be a novel therapeutic target in chronic inflammatory/autoimmune diseases (17). In the present study, we describe the in vivo inhibitory activity of a mouse IgG1 anti-BAMBI mAb, clone B101.37. Treatment with this mAb expanded Treg cells and reduced Th17 cells, showing both preventive and therapeutic effects in different experimental murine models of psoriasis and arthritis involving Treg cell- and TGFβ-dependent mechanisms.

Multiple clinical trials have been performed or are currently in progress to explore the benefits of Treg cell-based therapies in humans (7,8). However, the protocols employed to purify and expand these cells in vitro in sufficient numbers must deal with important technical challenges that have not yet been sufficiently resolved. One promising approach consists of the in vivo expansion of this cell population using IL-2 IC-targeting activity of a mouse IgG1 anti-BAMBI mAb, such as Treg cells (22). This approach has already been employed successfully in the clinic for different diseases (28–30). Herein, we show that the combined administration of IL-2 ICs with BAMBI inhibitors such as B101.37 mAb improves this type of Treg cell-based therapy. Furthermore, our results demonstrate that the blockade of BAMBI with specific mAb potentiates the inhibitory activity of murine Treg cells, at least in vitro. Taken together, these findings encourage the possibility of using BAMBI inhibitors for the in vivo or in vitro Treg cell expansion protocols in humans.

Using 2 different murine models of psoriasis (either associated with or not associated with paw inflammation) and a murine model of CIA, we demonstrate herein that treatment with B101.37 mAb conveyed beneficial effects on these chronic inflammatory/autoimmune diseases. Since B101.37 mAb is specifically an IgG1 mAb, these therapeutic effects are unrelated to a complement-dependent depletion of cells expressing BAMBI (31). In addition, the expansion of Treg cells observed after B101.37 treatment and the fact that BAMBI expression is induced after the activation of CD4+ T cells (17) argues against the possibility that the effects of this mAb could be attributed to an antibody-dependent mechanism of cellular cytotoxicity. Instead, we postulate that the observed effects of the B101.37 mAb are directly associated with the functional inactivation of BAMBI in those cells in which BAMBI is expressed. In this regard, the extracellular part of BAMBI, the region recognized by B101.37, influences the capacity of the TGFβRI receptor to form homodimers, a process that is required for TGFβ signaling (18).

The cellular mechanisms accounting for the in vivo therapeutic effects of B101.37 have been explored in detail in the murine model of MIP. Our studies with mouse BM chimeras clearly show that the therapeutic effects of B101.37 mAb are mediated by BM-derived cells and not by other cell populations, such as keratinocytes, that are potentially involved in disease development (32–34). Furthermore, we demonstrate herein that Treg cells mediated the protection against the development of MIP in both BAMBI-KO and B101.37-treated WT mice, a finding that is consistent with the inhibition of CIA development that was observed in BAMBI-KO mice (17). Recently, Treg cells have been shown to control the severity of imiquimod-induced psoriasis by inhibiting the production of type I interferon by mononuclear phagocytes and by suppressing the infiltration of granulocyte-macrophage colony-stimulating factor–producing CD4+ T cells into the lesioned skin (35,36). Our present results show that, in correlation with the reduction in disease severity, the absence or pharmacologic inhibition of BAMBI promoted a higher increase in the number of Treg cells found in different locations, including the skin, than was observed in untreated WT mice.

These Treg cells control the activity of Th17 cells and/or other components of the Th17 immune axis. In this regard, disease development observed in BAMBI-KO and B101.37-treated WT mice after Treg cell depletion with anti-CD25 mAb was blocked after inhibition of IL-17A. The reduced numbers of Th17 cells in the spleen, lymph nodes, and skin of BAMBI-KO and B101.37-treated WT mice suggest that these cells may be crucial targets of Treg cell activity. However, MIP development has been shown to be independent of αβ T cells (20). Thus, the protective effect of Treg cells may be secondary to the control of other IL-17–producing immune cells with a potential role in disease development, such as IL-17–producing γδ T cells and/or group 3 innate lymphoid cells (37).

We have previously reported that BAMBI deficiency increases Treg cell numbers through the control of both CD25 expression and IL-2 signaling strength by a Smad-3–dependent mechanism (17). The increase in the expression of CD25 in Treg cells and in their proliferative responses to IL-2 ICs observed in WT mice after treatment with B101.37 indicates that this may also be the mechanism through which this anti-BAMBI mAb operates in Treg cells. In addition, we show herein that BAMBI inhibition modulated MIP development through a TGFβ3–dependent mechanism. This is particularly relevant since BAMBI also inhibits BMP in the...
activin pathway and potentiates Wnt/β-catenin signaling (18,25). The role of TGFβ in psoriasis development is controversial. TGFβ possesses antiproliferative effects in keratinocytes and promotes their maturation (38,39). Furthermore, a decrease in TGFβ2, TGFβ3, and Smad-2 expression has been reported in psoriatic skin lesions (40), and the clinical improvement of psoriatic lesions after treatment with methotrexate is associated with an increase in the expression of TGFβ1 (41). On the other hand, an increase in circulating levels of TGFβ1 has been associated with a severe disease (42), and transgenic mice overexpressing TGFβ1 in keratinocytes develop skin psoriasis (43–45). The reason for the apparent discrepancies between these studies and our observations is probably related to the cell type in which BAMBI inhibition alters TGFβ signaling during the development of MIP. In this regard, the absence of BAMBI in non-hematopoietic cells does not appear to affect disease development in chimeric mice.

In the initial description of the MIP model, it was demonstrated that disease severity was greatly dependent on the strain of mice analyzed, and that only B10Q mice with a mutation in the Ncf1 gene, which caused a reduction in macrophage reactive oxygen species production, developed histologic joint lesions (20). In the present study, we used mice with a B10.RIII genetic background, and despite the presence of an evident macroscopic paw swelling in IgG1-C–treated control WT mice, no histologic signs of joint destruction were observed after SCM injection. However, paw swelling in these controls could be attributed to the co-presence of psoriatic-like skin lesions with periostitis and a discrete Achilles tendon thickening. The severity of all of these histologic lesions was significantly lower in either BAMBI-KO mice or WT mice after the pharmacologic blockade of BAMBI with B101.37 mAb. Furthermore, the beneficial effects of B101.37 mAb treatment against CIA development clearly emphasize the potential efficacy of this treatment, not only in chronic inflammatory skin lesions but also in autoimmune arthritis development.

Multiple mAbs against cytokines or soluble receptors for these factors are used for the treatment of chronic inflammatory/autoimmune diseases (46,47). These compounds are very specific, and the results obtained with each mAb have been very positive. However, ~30% of patients with severe diseases remain unresponsive to existing therapies, and the appearance of drug resistance is frequent (48,49). Our present results describe a potentially useful molecular target candidate, BAMBI, and a novel mAb against it, the B101.37 mAb. Experiments are in progress to determine whether B101.37 mAb also modulates BAMBI activity in humans.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. R. Merino had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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