# Bromelain Limits Airway Inflammation in an Ovalbumin-induced Murine Model of Established Asthma

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## ABSTRACT

**Context** • Allergic asthma continues to increase despite new pharmacological advances for both acute treatment and chronic-disease management. Asthma is a multifactorial disease process with genetic, allergic, infectious, environmental, and dietary origins. Researchers are investigating the benefits of lifestyle changes and alternative asthma treatments, including the ability of bromelain to inhibit inflammation. Bromelain is a commonly used, proteolytically active pineapple extract.

**Objective** • The present study intended to determine the ability of bromelain to reduce the inflammation of preexisting asthma via an ovalbumin (OVA)-induced murine model of allergic airway disease (AAD).

**Design** • The research team designed a study examining the effects of bromelain in a control group of mice that received phosphate buffered saline (PBS) only and in an intervention group that received bromelain in PBS.

**Setting** • The study took place in the Department of Immunology at the University of Connecticut's School of Medicine, Farmington.

**Intervention** • The research team sensitized female C57BL/6J mice with intraperitoneal OVA/alum and then challenged them with OVA aerosolization for 10 consecutive days. On day 4, the team began administering daily doses of PBS to the control group (n = 10) and bromelain (6mg/kg) in PBS to the bromelain (intervention) group (n = 10).

**Outcome Measures** • The primary measures included bronchoalveolar lavage (BAL) cellular differential, cellular phenotype via flow cytometry, and lung histology. Additional outcomes included testing for serum cytokines and immunoglobulin.

**Results** • Bromelain treatment of AAD mice (bromelain group) resulted in significant anti-inflammatory activity as indicated by reduced BAL total leukocytes ($P < .05$), eosinophils ($P < .05$), and cellular infiltrates via lung pathology ($P < .005$), as compared to the control group. In addition, bromelain significantly reduced BAL CD4^{+} and CD8^{+} T cells without affecting cell numbers in the spleen or hilar lymph node. The study found decreased interleukins IL-4, IL-12, IL-17, as well as IFN-$\alpha$ in the serum of bromelain-treated animals.

**Conclusions** • The results suggest that bromelain has a therapeutic effect in established AAD, which may translate into an effective adjunctive therapy in patients with similar conditions, such as allergic asthma, who have chosen to initiate treatment after the onset of symptoms. (*Altern Ther Health Med.* 2012;18(5):9-17.)

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Allergic asthma continues to increase despite new pharmacological advances for both acute treatment and chronic-disease management. The keystones of asthma therapy include (1) avoidance of triggers, (2) relief of symptoms with inhaled β-adrenergic agonists, and (3) control of airway inflammation with inhaled and oral corticosteroids, inhaled chromones, and oral leukotriene antagonists.

Asthma is a multifactorial disease process with genetic, allergic, infectious, environmental, and dietary origins.[1] Researchers are investigating the benefits of lifestyle changes and alternative asthma treatments.[2] They seek evidenced-based treatments that might become safe and efficacious therapies adjunctive to the current treatment formulary.

According to the 2007 National Health Interview Survey, roughly 40% of adults in the US population use some form of complementary or alternative medicine (CAM).[3] Several studies have examined the use of CAM by patients with asthma, with use estimates ranging from 4% to 79% in adults and from 33% to 89% in children.[4-7] Among the most commonly used CAMs are breathing techniques, herbal products, homeopathy, and acupuncture.[4,8] Nevertheless, researchers have not found strong evidence for the effectiveness of these modalities.[9] Lack of evidence has been attributed to clinical trials with small sample sizes, poor methodology, and a paucity of mechanistic data generated in well-characterized preclinical models.[10,11]

Researchers have established several murine models of asthma or allergic airway disease with the aim of understanding the immunological mechanisms that underlie this complex disease.[12-15] These models share several features that include (1) a sensitization phase with an adjuvant (alum) and a model antigen ovalbumin (OVA), (2) an antigen challenge phase via tracheal instillation or nose-only aerosolization, and (3) the generation of lung inflammation characterized by infiltration of white blood cells (eosinophils), lung pathology, and inflammatory Th2-skewed cytokines (interleukins IL-4, IL-5, IL-3).[12-14,16,17]

Modeling allows for investigation of the effectiveness of numerous, novel, antiasthma therapeutics that have a rich history of traditional use, such as propolis[18]; *Camellia sinensis*[19]; Chinese-patent botanical formulas[20]; *Boswellia serrata* (frankincense), *Glycyrrhiza glabra* (licorice), and *Curcuma longa* (turmeric)[21]; *Mimosa pudica*[22]; and *Ananas comosus* (common pineapple).[12-14] A critical distinction exists, however, between the models and the actual use of the therapies (ie, the schedule at which practitioners deliver the therapy). This schedule includes the periods before or during sensitization, after sensitization, before the acute antigen challenge, or after the development of inflammatory disease.

Several investigators have been interested in the anti-inflammatory mechanisms of specific extracts of *A comosus*, such as the commonly used, proteolytically active pineapple-derived bromelain. Researchers are evaluating the extracts in a variety of both in vivo and in vitro models. Bromelain is a combination of sulfur-containing cysteine endopeptidases that have broad specificity for the cleavage of proteins. Some researchers have proposed one mechanism of action to account for bromelain's therapeutic activity: the cleavage of lymphocyte-cell surface receptors, such as CD4, CD8, CD44, and CD62L. Receptor cleavage can result in altered cell communication, cell trafficking, and cell signaling pathways, leading to the modulation of pro- and anti-inflammatory cytokines such as IL-2, IL-4, IL-6, and TNF-α[23-27]. This modulation makes it a unique choice for modulating allergic asthma.

Researchers have shown that bromelain has potential beneficial effects in treating infections, cancer, cardiovascular disease, musculoskeletal injuries, and arthritis.[28] The research team for the current study has shown previously that bromelain, injected intraperitoneally and administered orally, can reduce airway inflammation in a mouse model of asthma,[29,30] at least in part through enzymatic cleavage of CD25 from activated T cells.[31] The current team notes, however, that its previous studies addressed the ability of bromelain to attenuate the development of asthma when given to sensitized mice prior to and during exposure to an aerosol antigen. While this model may have merit for preventing the development of asthma in previously sensitized allergic individuals, it did not assess whether bromelain could modulate existing asthma when practitioners apply treatment after onset. The outcomes for a trial studying the second circumstance hold more relevance in a clinical setting, and as the goal of its present study, the research team has chosen to examine that second circumstance.

## METHODS
### Animals
The research team purchased female C57BL/6J mice, 3 to 6 months of age with weights from 17 g to 20 g (Jackson Laboratory, Bar Harbor, Maine), and housed them conventionally in plastic cages with corncob bedding. The team maintained the mouse room at 22°C to 24°C with a daily light/dark cycle (light from 0600 to 1800 hours). The study supplied food and water *ad libitum*. The Animal Care Committee at the University of Connecticut Health Center approved all protocols for mouse use.

### Interventions
**Ovalbumin Sensitization and Aerosol Exposure Protocol.** The research team immunized mice with three weekly intraperitoneal injections of a suspension containing 25 μg of OVA (grade V; Sigma Chemical, St Louis, Missouri) and 2 mg of aluminum hydroxide (alum) in 0.5 mL of 0.9% sodium chloride (pH 5.5, 308 mOsmol/L; Baxter Healthcare, Deerfield, Illinois). One week after the last injection, the research team exposed the mice to aerosolized 1% OVA in normal saline, 1 hour per day for 10 days.[29,30] A BANG nebulizer (CH Technologies, Westwood, New Jersey) generated the aerosols into a 7.6-L inhalation-exposure chamber with attached restraint tubes. The mass-median aerodynamic diameter and geometric standard deviations were 1.4 μm and 1.6 μm, respectively. The estimated daily inhaled OVA dose approximated 30 μg to 40 μg per mouse.

**Control and Bromelain Treatment.** The research team made a stock solution of stem bromelain, Lot no. 2890 (Vital Nutrients, Middletown, Connecticut), using 60 mg of bromelain dissolved in 250 mL of phosphate buffered saline (PBS). For the bromelain group (n = 10), the research team administered 6 mg/kg of bromelain in 0.5 mL of PBS. The control group (n = 10) received 0.5 mL of PBS only. The team delivered the treatments intraperitoneally twice daily for 7 consecutive days, beginning on day 4 of the OVA aerosol challenge (Figure 1). The research team optimized the bromelain dose based on previous in vivo dose-response studies that its laboratory had performed.[29,30] As previously report-

**Figure 1.** Bromelain Treatment Protocol

The research team sensitized mice (n = 20) with three ovalbumin-alum intraperitoneal injections 1 week apart (day −21, day −14, day −7). From days 1 to 10, animals underwent aerosol challenge with 1% OVA in saline for 1 hour daily. From days 4 to 10, animals received treatment with either bromelain (6 mg/kg in 0.5 mL phosphate buffered saline [PBS]) or PBS only (0.5 mL) twice daily. The research team sacrificed the animals 24 h after the last treatment and harvested bronchoalveolar lavage, lung tissue, hilar nodes, and spleen from each mouse to assess the outcomes of allergic airway disease.
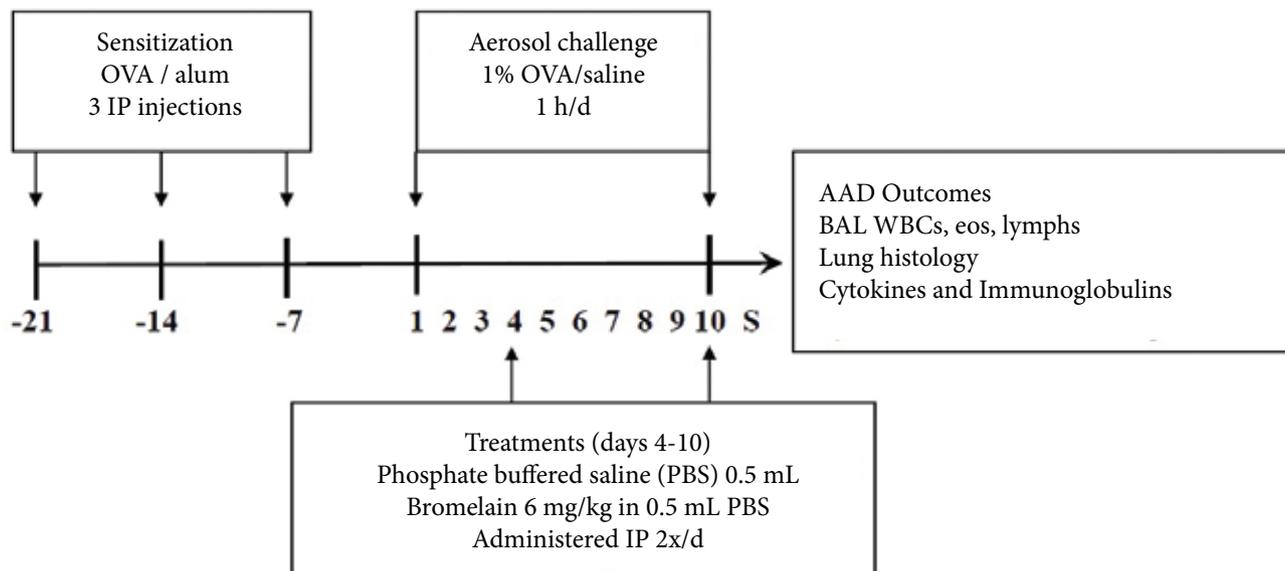


**Abbreviations:** AAD, allergic airway disease; BAL, bronchoalveolar lavage; eo, eosinophil; IP, intraperitoneal injections; lymph, lymphocyte; OVA, ovalbumin; PBS, phosphate buffered saline; S, sacrificed; WBC, white blood cell (leukocyte).

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ed, the research team had tested bromelain independently for authenticity, potency (2400-2660 GDU/g), microbial contamination, residual solvents, heavy metals, aflatoxins, and endotoxin (Vital Nutrients, Middletown, Connecticut; ChromaDex, Clearwater, Florida; and Pharmline, Florida, New York).[31] Twenty-four hours after the final aerosol exposure and the final bromelain treatment, the research team sacrificed the mice for tissue analysis.

## OUTCOME MEASURES
### Bronchoalveolar Lavage and Tissue Processing

At sacrifice, the research team harvested bronchoalveolar lavage (BAL) fluid, hilar lymph nodes (HLN), and spleens and processed them for the isolation and enumeration of leukocytes. For collection of BAL, the team lavaged the lungs *in situ,* with five 1.0-mL aliquots of sterile saline. The team determined the total protein concentrations in BAL fluid using the Pierce Bicinchoninic Acid Protein Assay Kit (Thermo Scientific Pierce, Rockford, Illinois) with OVA as the standard. The team harvested the lymph nodes and spleens and mechanically disrupted them into single-cell suspensions, using lysis of splenic erythrocytes Tris ammonium chloride (TAC) lysis buffer (9 parts 0.83% w/v $NH_4Cl$; 1 part 2.57% w/v Tris, pH 7.0; TAC solution). For all tissue samples, the team obtained counts of the total nucleated

cells using a hemocytometer with nigrosin dye exclusion as a measure of viability.

### Flow Cytometry

Post centrifugation (1500 rpm x 10 min) the research team resuspended cell pellets via manual disruption in PBS containing 0.2% bovine serum albumin (BSA) and 0.1% $NaN_3$ at a concentration of $1 \times 10^5$ to $1 \times 10^6$ white blood cells/mL. The team then incubated 100 μL of the cells with 100 μL mAb (diluted per manufacturers' recommendations for 30 min at 4°C). After staining the cells, the team washed them twice with PBS-0.2% BSA-0.1% $NaN_3$ solution and measured their relative fluorescence intensities on a 4-decade log scale. For the measurement, the research team used flow-cytometric analysis with a LSR II (Becton-Dickinson, San Diego, California) and BD FACSDiva Software v 4.1 (Becton Dickinson, San Jose, California). The team analyzed the results with FlowJo 7.6.1 (Tree Star, Ashland, Oregon) and used the following fluorescence-labeled monoclonal antibodies: CD4-PacOrange (RM4-5); CD8a-APC-780 (53-6.7); CD19-PE (1D3); CD25-AF-700 (PC61.5); F4/80-PECy7 (BM8); CDllc-APC (N418); and MHCII-FITC (2G-9) (Pharmingen, San Jose, California, or eBioscience, San Diego, California).

### Lung Histology

For animals that did not undergo BAL (n = 4 per group), the research team fixed the removed lungs with a 10% buffered formalin and processed them in a standard manner. The team stained tissue sections with hematoxylin and eosin and evaluated all specimens with a microscope-mounted Nikon Eclipse 400camera (Tokyo, Japan). The team created digital images using Spot RT Slider Software (Sterling Heights, Michigan) and evaluated them in Microsoft Photo Editor (Redmond, Washington). Five separate individuals graded the degree of cellular infiltration (0-5) in a blinded manner.

### Serum Immunoglobulin and Cytokine Measurements

The research team thawed all serum samples and vortexed them prior to immunoglobulin and cytokine measurement. The team determined immunoglobulin concentrations using Milliplex Mouse Immunoglobulin Isotype Kit Panels (Cat #MGAM-300; Millipore, Billerica, Massachusetts) following the manufacturer's instructions. Assay sensitivities (minimum detectable concentrations) were IgM 0.3 ng/mL; IgG1 0.3 ng/mL; IgG3 0.4 ng/mL; IgG2a 0.4 ng/mL; IgG2b 0.4 ng/mL; and IgA 0.7 ng/mL. For assessment of cytokines and chemokines, the team processed samples with a Milliplex Mouse Cytokine/Chemokine kit (Cat #MPXMCYTO70KPMX22; Millipore, Billerica, Massachusetts) following manufacturer's instructions. Assay sensitivities were IL-1α 5.1 pg/mL; IL-1β 2.0 pg/mL; IL-2 0.8 pg/mL; IL-4 0.4 pg/mL; IL-5 0.7 pg/mL; IL-6 1.8 pg/mL; IL-12(p40) 4.9 pg/mL; IL-13 6.3 pg/mL; IL-15 6.5 pg/mL; IL-17 0.5 pg/mL; and TNFα 1.0 pg/mL.

### Statistical Analysis

The research team made statistical comparisons between groups with analysis of variance and unpaired $t$ tests using JMP Software (SAS Institute, Cary, North Carolina). Serum cytokine levels were not normally distributed, and the team log-transformed them for statistical analysis. The team expressed all data as means ± standard error of the mean and considered differences to be significant at $P \leq .05$.

## RESULTS
### Bromelain Treatment Was Nontoxic

As the research team has reported previously,[29] intraperitoneal bromelain was nontoxic, as assessed by body weight and BAL protein concentration. The team noted no

**Figure 2.** Effect of Bromelain Treatment on Total Leukocytes

For mice with allergic airway disease, bromelain significantly reduced total bronchoalveolar (BAL) lavage leukocytes and eosinophils in the bromelain group (black bars) as compared to the control group (gray bars) without change in BAL macrophages, neutrophils, or lymphocytes. The research team saw no significant reduction in leukocytes in the tissue of the spleen or hilar lymph nodes. The data represent mean ± standard error of the mean values.
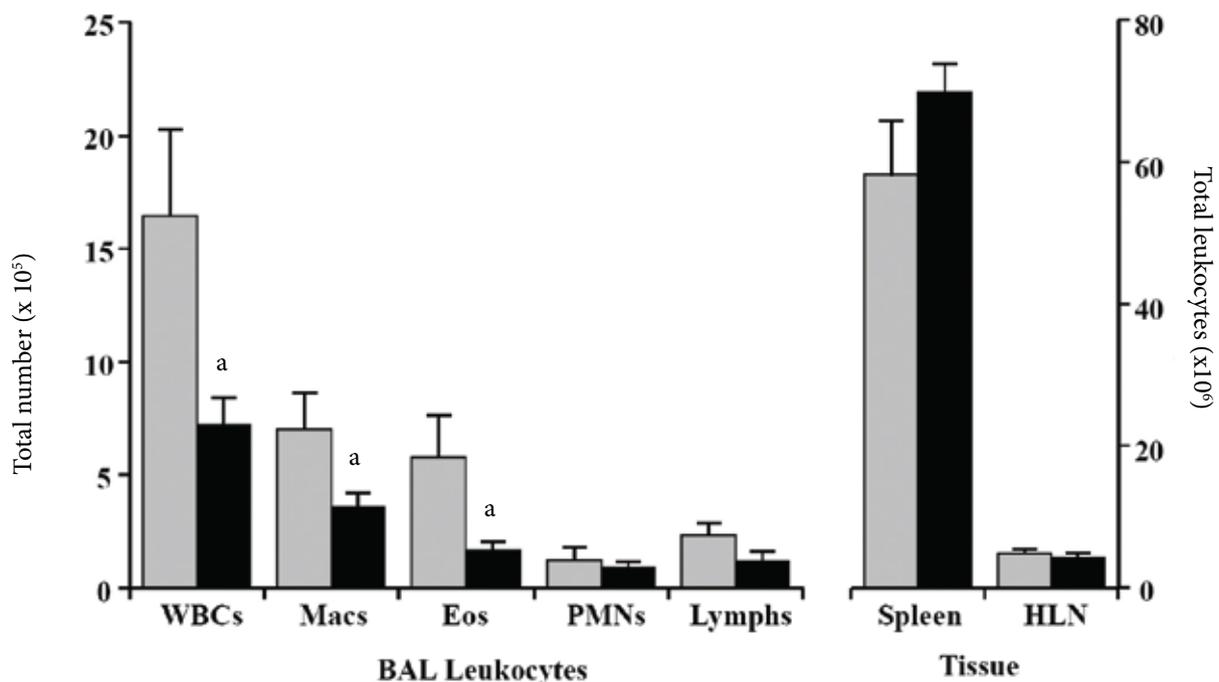


**Abbreviations:** BAL, bronchoalveolar lavage; eo, eosinophil; HLN, hilar lymph node; lymph, lymphocyte; mac, macrophage; PMN, neutrophil; WBC, white blood cell (leukocyte).

[a]Indicates $P < .05$ for the bromelain vs the control group; n = 10 animals per group.

difference in body weight in bromelain-treated mice as compared to PBS-treated control animals (19.1 ± 0.26 g vs 18.8 ± 0.35 g; $P = .54$). Also, no significant change in BAL total protein concentrations occurred between bromelain-treated mice and PBS controls (143 ± 10 μg/mL vs 174 ± 17 μg/mL; $P = .18$; n = 10 animals per group).

**Bromelain Decreased Pulmonary Eosinophilia in Established Asthma**

Exposure of OVA-sensitized mice to daily OVA aerosols resulted in AAD, characterized by robust increases in BAL leukocytes and eosinophils (Figure 2). Treatment with bromelain significantly inhibited OVA-induced increases in BAL leukocytes ($7.22 ± 1.20$ vs $16.41 ± 3.84$ x $10^5$ cells in control mice; $P = .035$; n = 10 each) and eosinophils ($1.65 ± 0.40$ vs $5.80 ± 1.87$ x $10^5$ cells; $P = .044$), without change in other cell types. In contrast to leukocyte numbers in BAL fluid, bromelain did not change those numbers in the spleens and HLNs of the sensitized and challenged mice (Figure 2).

**Bromelain Selectively Decreased CD4⁺ T and CD8⁺ T Cells in Bronchoalveolar Lavage From Mice With Asthma**

Although the bromelain treatment did not significantly reduce the total number of BAL lymphocytes (Figure 2; control [$2.3x10^6 ± 0.6$]; bromelain [$1.2x10^6 ± 0.4$, $P = .13$]), bromelain did reduce the distribution of BAL CD4⁺ T cells and CD8⁺ T cells in the treated animals of the intervention group (Table 1). BAL CD19⁺ B cells were trended toward significance between the two groups $P = .05$. Nevertheless, bromelain did not affect the relative percentages of (1) CD4⁺ (control [$10.3 ± 1.8\%$], bromelain [$7.6 ± 1.4\%$], $P = .26$); (2) CD8⁺ (control [$10.8 ± 2.7\%$], bromelain [$7.8 ± 1.4\%$], $P = .35$); and (3) CD19⁺ lymphocytes (control [$9.3 ± 2.3\%$], bromelain [$11.3 ± 2.7\%$], $P = .57$) (Table 1). Similarly, bromelain exerted no effects on the number or percentages of splenic or HLN lymphocytes (Table 1). In contrast, both the absolute numbers and percentages of BAL CD4⁺ T cells that expressed the surface activation marker CD25⁺ were lower in the bromelain group compared to control animals ($P = .012$; Figure 3). The percentages of CD4⁺ T cells expressing CD25 were unchanged in the spleen ($P = .27$) and HLNs ($P = .33$; Figure

**Figure 3.** Effect of Bromelain Treatment on CD4⁺CD25⁺ T Lymphocytes
In bronchoalveolar lavage fluid, bromelain treatment significantly decreased the percentage of CD4⁺CD25⁺ T cells (black bars) as compared to phosphate buffered saline–treated control mice (gray bars). In contrast, bromelain had no effect on CD4⁺CD25⁺ T cell percentages in the spleens or hilar lymph nodes. The data represent mean ± standard error of the mean values; n = 10 animals per group.
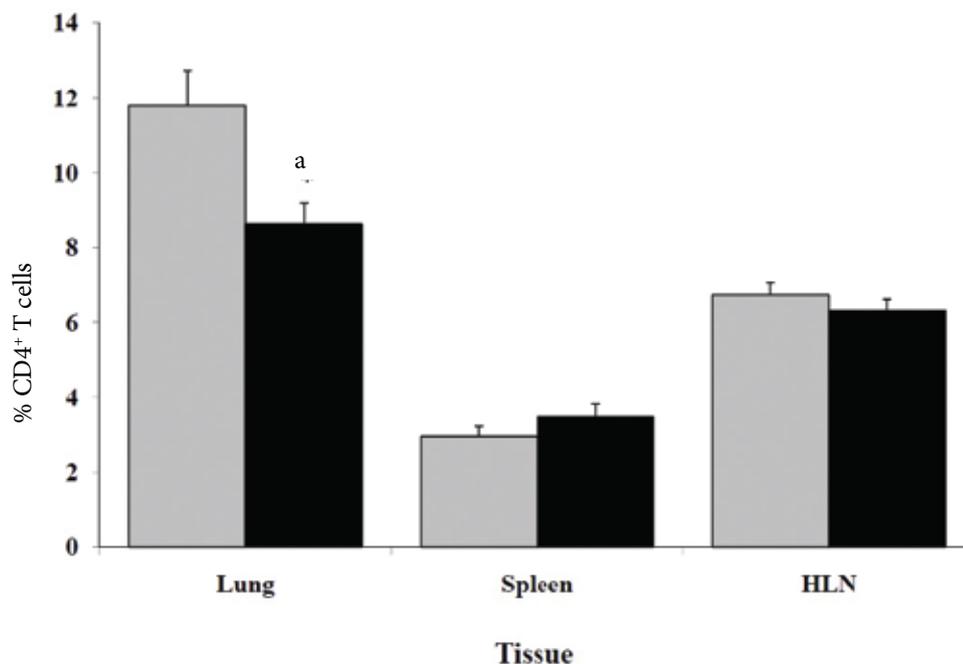


**Abbreviation:** HLN, hilar lymph node.

ᵃIndicates $P < .05$ for the bromelain vs the control group.

**Table 1.** Lymphocyte Distributions in Tissues From Bromelain-treated and Control Mice[a]

| BAL (total cells x 10⁶) | Control | Bromelain | P-value |
|---|---|---|---|
| CD4⁺ T cell | 2.41 ± 0.6 | 0.83 ± 0.16 | .028 |
| CD8⁺ T cell | 2.29 ± 0.6 | 0.87 ± 0.19 | .046 |
| CD19⁺ B cell | 2.81 ± 0.8 | 1.41 ± 0.44 | .050 |
| Spleen (total cells x10⁶) | | | |
| CD4⁺ T cell | 602.1 ± 53 | 578.8 ± 36.2 | .98 |
| CD8⁺ T cell | 425.3 ± 34 | 428.4 ± 28.0 | .99 |
| CD19⁺ B cell | 3964 ± 1150 | 4989 ± 1170 | .29 |
| HLN (total cells x10⁶) | | | |
| CD4⁺ T cell | 48.8 ± 7.1 | 43.2 ± 5.8 | .55 |
| CD8⁺ T cell | 42.5 ± 6.2 | 42.8 ± 6.0 | .98 |
| CD19⁺ B cell | 42.5 ± 7.1 | 43.5 ± 7.3 | .98 |
| BAL (%) | | | |
| CD4⁺ T cell | 10.3 ± 1.8% | 7.6 ± 1.4% | .26 |
| CD8⁺ T cell | 10.8 ± 2.7% | 7.8 ± 1.4% | .35 |
| CD19⁺ B cell | 9.3 ± 2.3% | 11.3 ± 2.7% | .57 |

**Abbreviations:** BAL, bronchoalveolar lavage; HLN, hilar lymph node.

[a]The distribution of lymphocyte subsets (CD4+ and CD8+ T cells and CD19+ B cells) was measured in the bronchoalveolar lavage (BAL), spleen, and hilar lymph nodes (HLNs) and % of cells in the BAL after 10 days of ovalbumin aerosol exposures in mice receiving phosphate buffered saline (control group) and as compared to bromelain treatment on days 4-10. Significant reductions were observed in total cell numbers in the BAL between treatments. No differences were noted in total cells in the spleen and HLN or in % BAL in any cells subsets. Data represent mean ± standard error of the mean; n = 10 animals in each group.

3).

## Bromelain Attenuated Lung Histopathologic Changes During Allergic Airway Disease

The research team performed histological evaluations and obtained pathology scores on unmanipulated, uninflated, formalin-fixed lungs from separate groups (n = 4 each) of PBS-treated control animals and bromelain-treated mice with AAD. The team stained the samples with hematoxylin and eosin (Figure 4). As demonstrated in the top panels, the AAD mice in the control group (Figure 4A) showed substantial peribronchial and perivascular inflammation comprising lymphocytes, plasma cells, and eosinophils. Bromelain-treated AAD mice had less histological injury (Figure 4B). Statistical comparison of pathological scoring by five blinded reviewers demonstrated significantly less pathol-

ogy in the bromelain-treated mice than in the control animals (mean score 1.40 ± 0.27 vs 2.35 ± 0.31; P = .0077; Figure 4C).

## Bromelain Did Not Affect Serum Immunoglobulin Levels but Decreased Selective Serum Cytokines

Bromelain treatment did not elicit generalized immune suppression, as evidenced by lack of a direct effect on serum immunoglobulin levels (Table 2) or generalized effect on serum cytokines. As seen in Figure 5, however, bromelain-treated animals did demonstrate selective decreases in key cytokines, including IL-4 (0.33 vs 1.38 pg/mL; P = .0002), IL-12 (8.67 vs 36.8 pg/mL; P = .003), IL-17 (0.54 vs 3.88 pg/mL; P = .0004), IFN-g (1.56 vs 86.5 pg/mL; P = .016). In contrast, the chemokine interferon, gamma-induced protein (IP-10) increased in bromelain-treated animals (61.6 vs 39.2 pg/mL; P = .02).

## DISCUSSION

The current study is a continued characterization of the use of bromelain, a cysteine protease extracted from pineapple, in asthma. The research team has previously shown that intraperitoneal and oral administration of bromelain can prevent the development of asthma in sensitized mice when given prior to aerosolized antigen exposure.[29,30] Since most people taking bromelain for asthma, however, would have an established disease already and may be experiencing symptoms, a more relevant question is whether bromelain can attenuate existing asthma. To the research team's knowledge, few reports exist that study the effects of botanicals in models of established or chronic asthma.[20] The present study demonstrated a beneficial effect of bromelain when administered 3 days after consecutive OVA aerosol challenge, a time when eosinophilic airway inflammation and airway hyperresponsiveness were well established.[17,29,31] At that point, bromelain was capable of attenuating the airway leukocytosis and eosinophilia and reducing the histopathological changes in the lung that occur in asthma. This anti-inflammatory effect appeared to be tissue specific, as no changes occurred in the spleen or HLN leukocyte populations or in serum immunoglobulin levels, even though the research team gave the bromelain systemically.

Broad decreases in CD4⁺ and CD8⁺ T cells and CD19⁺ B cells in the BAL fluid accompanied the decrease in airway eosinophilic inflammation. Such changes were also selective to the airways, and the research team did not see them in HLNs or spleens. While cell numbers decreased significantly, no changes occurred in the relative percentages of major lymphocyte subpopulations in BAL for either the control or the bromelain-treated mice. This finding differed from what the research team had observed when it gave bromelain before the initiation of the OVA aero-

**Figure 4.** Bromelain Treatment Reduces Lung Pathology
The research team stained lung sections with hematoxylin and eosin, and five individuals scored the samples blindly on a scale from 0 to 5, based on the level of pathology. Panels A and B illustrate representative pathology from a control, phosphate buffered saline–treated mouse (A) and a bromelain-treated mouse (B). The inflammation (arrows) surrounding airways (aw) and blood vessels (bv) appeared significantly reduced in the bromelain (B) animal as compared to the control animal (A). Figures are at 10X magnification. Panel C demonstrates mean ± standard error of the mean pathology scores for each group (n = 4 each).
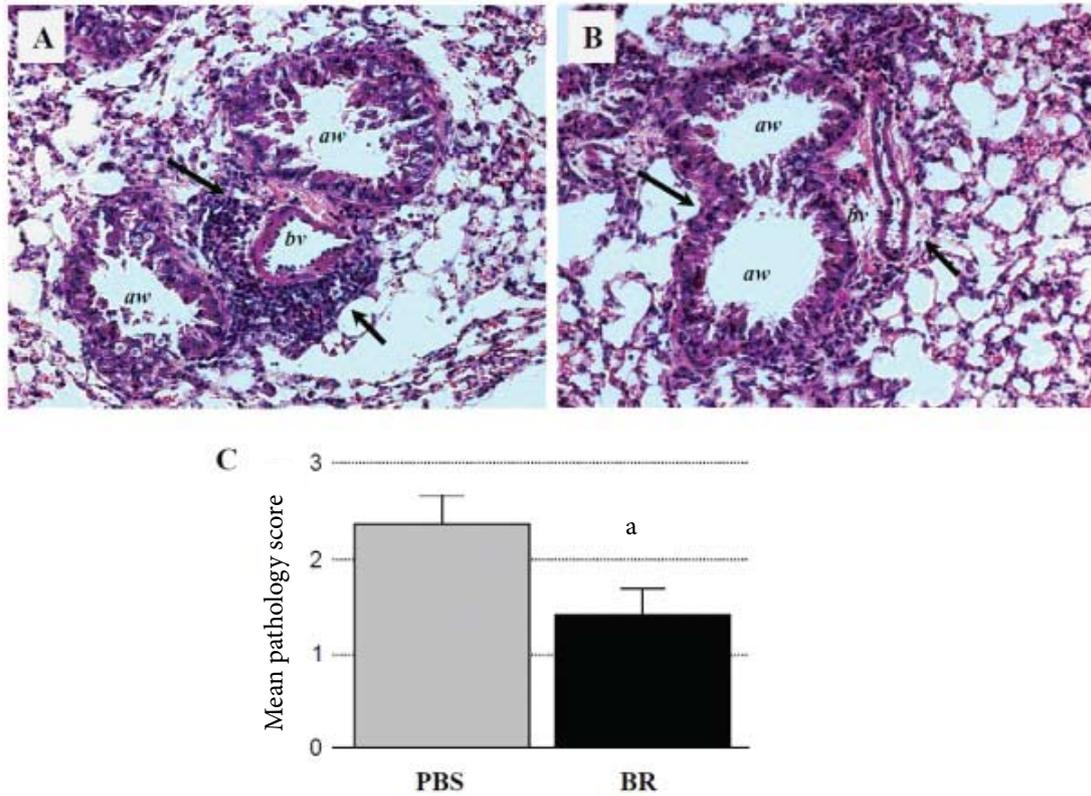


**Abbreviations:** BR, bromelain; PBS, phosphate buffered saline.

[a]Indicates *P* < .05 between groups.

**Table 2.** Serum Immunoglobulin Levels in Control and Bromelain-treated Mice[a]

|  | IgG1 | IgG2[a] | IgG2b | IgG3 | IgA | IgM |
|---|---|---|---|---|---|---|
| PBS | 1836 ± 427 | 15.8 ± 5.1 | 359 ± 128 | 135 ± 49 | 16.7 ± 3.3 | 622 ± 161 |
| BR | 2167 ± 240 | 15.5 ± 2.4 | 380 ± 87 | 106 ± 46 | 16.4 ± 5.4 | 616 ± 214 |
| *P*-value | .57 | .96 | .89 | .48 | .96 | .98 |

**Abbreviations:** BR, bromelain; Ig, immunoglobulin; PBS, phosphate buffered saline.

[a]Serum immunoglobulin levels were measured after 10 days of ovalbumin aerosol exposures in mice receiving phosphate buffered saline (control) and receiving bromelain (intervention) on days 4-10. The team saw no significant differences in any immunoglobulin class. Data represent mean ± standard error of the mean; n = 4 animals in each group.

**Figure 5.** Bromelain Reduces Selected Cytokine Levels
Bromelain treatment selectively decreased specific cytokine levels in serum of mice with allergic airway disease. The data represent mean±standard error of the mean values; n=4-10 samples per group.
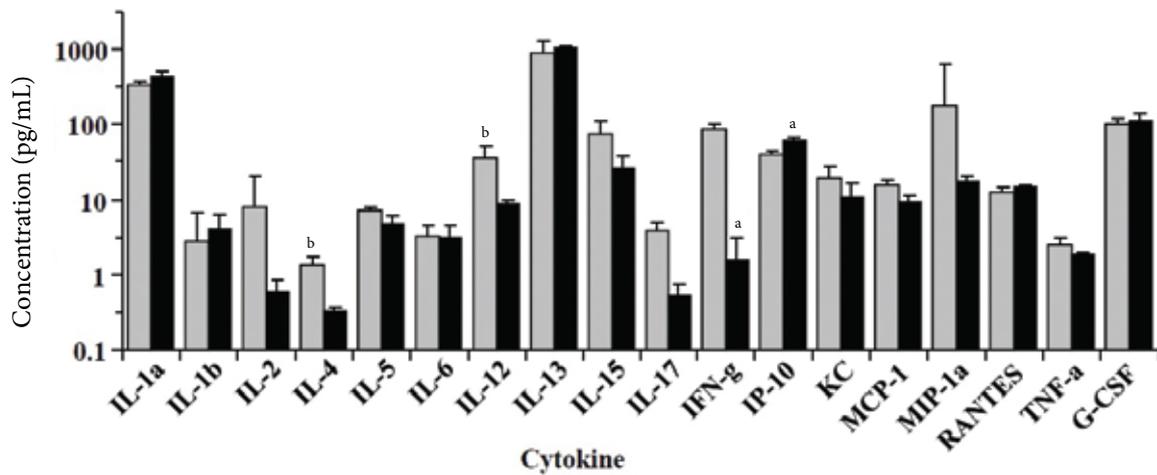


**Abbreviations:** G-CSF, granulocyte colony-stimulating factor; IL, interleukin; IFN, interferon; KC, keratinocyte chemoattractant; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T-cell expressed and secreted; TNF, tumor necrosis factor.

[a]Indicates $P<.05$.
[b]Indicates $P<.005$ for the bromelain vs the control group.

sol challenges. In that model, bromelain treatment decreased total lymphocytes in addition to BAL lymphocyte subsets.[30] A potential explanation for the discrepancy could be the difference in the time of bromelain administration. In the team's model from its other studies, significant lymphocyte recruitment to the airways occurred by day 3 of OVA exposures.[29,30]

Carson et al found from day 3 to day 10 that the predominant Th2-skewed lymphocyte found in the BAL during AAD was the CD4+CD25+, activated T effector cells.[14] In the research team's other studies, treatment with bromelain prior to aerosol challenge may have altered the pattern of initial CD4+ T-cell recruitment whereas, in the current study, later administration of bromelain (from day 3 to 10), occurred after asthma was well established (eg, CD4 T cells are prominent). While total CD4+ T cells were unchanged in the current study, bromelain treatment significantly reduced the percentage of CD4+ T cells expressing the activation marker CD25. Again, this finding was specific to BAL, and the research team did not see it in the spleen or HLN. The general inhibition of airway inflammation exerted by bromelain may explain the regional reduction in CD4+CD25+ T cells and hence the existence of fewer cells that have an activated (CD25+) phenotype. It may also reflect selected removal of CD25 (the high affinity IL2-Rα) from T cells by the bromelain treatment, which the research team previously has reported occurs with treatment.[31] Reduction of CD25 may limit IL-2 from binding, which corresponds to reduced cell

expansion and differentiation. Amelioration of disease also could occur by selective expansion of regulatory T cells. The research team, however, has shown previously that bromelain treatment also reduced CD25 from regulatory T cells (which constitutively express CD25) while having no effect on expression of Foxp3.

Bromelain did not affect serum immunoglobulin levels, further suggesting a lack of generalized immunomodulatory effect. The bromelain treatment, however, selectively decreased some serum cytokines—specifically, interleukins 4, 12, and 17 as well as IFN-γ. The treatment did not affect other Th2 cytokines, such as IL-5 and IL-13. The role of IL-4 in asthma is well established. The differentiation of naïve T lymphocytes into Th2 cells in the presence of an allergen requires this cytokine, and it is the principal stimulus for B-cell isotype switching to IgE.[32] The research team would expect inhibition of this key Th2 cytokine to attenuate the AAD response. IL-17 expression in the airways is also upregulated after sensitization and challenge with antigen, and it is associated particularly with neutrophil recruitment and more severe asthma.[33] Of interest, Th17 cells are resistant to steroids in vitro, and a recent study indicated that airways hyperreactivity induced by transferred Th17 cells in a mouse model is steroid-resistant.[34] Zhao et al have found that some human participants with steroid-resistant asthma have elevated levels of Th17 cells compared to healthy controls.[35] That bromelain decreased IL-17 in AAD mice raises the intriguing speculation that it could be useful in some

humans with refractory, Th17-driven asthma. Additionally, bromelain significantly increased serum levels of interferon γ-inducible protein (IP-10). Researchers have found that IP-10 expression occurs in bronchial epithelial cells and believe that the effect is associated with human immune defense against pathogens.[36] It is unclear, however, if increasing levels of IP-10 in a nonviral, allergic, airway-disease model would provide added support. Likewise, researchers would not expect bromelain's inhibition of IFN-γ and IL-12 to be a therapeutic target in asthma and that inhibition may demonstrate some broader immunomodulatory effects of the agent.

## CONCLUSION

In summary, administration of bromelain after the onset of AAD (asthma) inhibited progressive airway eosinophilia by ~70%. This effect was similar to the ~55% reduction in airway eosinophils seen when the current research team gave bromelain to sensitized mice before initiation of OVA aerosol challenges in a prior study.[29,30] Thus, bromelain may be as effective in treating existing asthma as it is in preventing the development of allergic airway inflammation. The attenuated airway eosinophilia was associated with fewer airway CD4+ and CD8+ T cells, decreased numbers of activated CD4+CD25+ T cells, lower levels of Th2 cytokines, and reduced lung histopathology. This modulatory role of bromelain appeared to be specific to the tissue or site of inflammation, as treatment did not affect lymphocyte numbers in the spleen or HLN. These observations suggest that bromelain may be effective in human asthmatics with existing disease, perhaps particularly in those with more steroid-resistant asthma.

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