

Prolonged Treatment of Peanut-Allergic Mice with Bortezomib Significantly Reduces Serum Anti-Peanut IgE but Does Not Affect Allergic Symptoms

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Key Words

Anaphylaxis · Antibody-secreting cell · Immunoglobulin E · Mast cell · Proteasome inhibitor

Abstract

Background: Anti-peanut immunoglobulin E (anti-Pn IgE) can persist throughout life, suggesting that this condition could be maintained by long-lived antibody-secreting cells (ASCs). To determine the role of long-lived ASCs, peanut-allergic mice underwent prolonged treatment with the proteasome inhibitor, bortezomib (Bz). **Methods:** Intravenous Bz was given twice weekly for 21 weeks to peanut-allergic mice. During treatment, serum anti-Pn IgE was measured, and the mice were rechallenged at the end of treatment. Cell populations were measured, and Pn-specific IgG, total IgG, and total IgE ASCs were enumerated in the bone marrow (BM) and spleen (SPL). **Results:** Prolonged treatment with Bz significantly reduced serum anti-Pn IgE and IgG1 but did not affect symptoms following challenge with Pn, even in mice with undetectable serum anti-Pn IgE. Numbers of CD138+ cells were significantly reduced in the BM but were unaffected in the SPL. Unexpectedly, Bz did not affect numbers of Pn-specific IgG, total IgG, or total IgE ASCs in either the BM or SPL. **Conclusions:** Cells that maintain long-lived serum

anti-Pn IgE are sensitive to Bz. However, prolonged depletion of serum Pn-specific IgE does not result in a decrease of symptoms following challenge with Pn.

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Introduction

Since peanut allergy, often life-long, is mediated by immunoglobulin E (IgE), targeting plasma cells (PCs) as an approach to decrease IgE seems reasonable. Recent studies tracking IgE+ cells show IgE+ PCs to be short-lived [1–4]. This could be confounded by the model in which the IgE was generated (by *Nippostrongylus brasiliensis*), which typically persists for only 4 weeks [4–6].

Bortezomib (Bz) is a modified, dipetidyl boronic acid that binds to the proteasome [7, 8] and induces apoptosis through the unfolded protein response [9–11]. In a murine model of atopic dermatitis, Bz decreased serum IgE and depleted antibody-secreting cells (ASCs), CD4+, and CD8+ cells in the atopic dermatitis lesions, but the atopic dermatitis persisted [12]. In a murine model of asthma, Bz significantly reduced anti-ovalbumin IgE without reducing anti-ovalbumin IgG1 or total IgG [13]. Despite the decrease in anti-ovalbumin IgE, airway hyperrespon-

siveness to methacholine, and PC numbers were not reduced [13].

The C3H/HeJ murine model of peanut allergy generates a high level of serum anti-peanut IgE (anti-Pn IgE) that is long-lived, persisting for at least 21 weeks after challenge [14]. In a previous study, we have shown that CD20+ B cells do not maintain long-lived murine anti-Pn IgE [14]. Therefore, we hypothesized that anti-Pn IgE is maintained by ASCs. To test this, we treated peanut-allergic mice with intravenous Bz and monitored serum anti-Pn IgE for 21 weeks. We rechallenged mice after 22 weeks of treatment and measured numbers of CD138+ cells and IgG and IgE ASCs in the BM and SPL.

Materials and Methods

Crude Peanut Extract

Crude peanut extract (CPE) was purified as previously described [15], dialyzed into PBS, and sterile-filtered.

Murine Model of Peanut Allergy and Treatment with Bz

Murine procedures were conducted with the approval of the University of Colorado Denver Institutional Animal Care and Use Committee. Female C3H/HeJ mice were acquired, housed, fed, sensitized, challenged, and scored as described [14]. Sensitization occurred from weeks -8 to -5, and the first challenge occurred at week -3. Bz and diluent were prepared and dosed [12, 13], and given 2× a week for 21 weeks, beginning 3 weeks after challenge. The duration of dosing was designed to allow 5 half-lives of tissue IgE (approx. 2 weeks) to elapse once serum IgE became undetectable. Mice were rechallenged in week 22.

Flow Cytometry, Enumeration of ASCs, and Measurement of Serum Anti-Pn IgE and IgG1 and Total IgG

Cells from the BM and SPL were stained as previously described [14]. Anti-Pn IgG, total IgG, and total IgE ASCs were enumerated with ELISPOT, and serum anti-Pn IgE and IgG1 antibodies were detected as previously described [14, 16].

Statistical Analysis

Graphpad Prism 5 (La Jolla, Calif., USA) was used throughout. $p < 0.05$ was considered to be significant. The Mann-Whitney U test was used to compare symptom scores. A two-tailed, unpaired Student's *t* test was used for the remaining statistical comparisons.

Results

Prolonged Treatment with Bz Significantly Depletes Serum Ig Titers

Prior to treatment, mice were sensitized with intragastric CPE and cholera toxin and challenged with intraperitoneally administered CPE. Three weeks after challenge, they were divided into 2 groups with no significant differ-

ences in serum anti-Pn IgE (online suppl. fig. 1A: see www.karger.com/doi/10.1159/000449247 for all online suppl. material), symptom scores (online suppl. fig. 1B) or temperature changes (online suppl. fig. 1C). Bz was given intravenously 2× a week for 21 weeks, and anti-Pn IgE was measured every 2 weeks, starting at week 3 (fig. 1a). During the course of these experiments (30 weeks), 3/15 mice in the experimental group and 1/13 mice in the control group died. This is likely due to the toxicity associated with long-term (22 weeks) Bz treatment.

Treatment for 3 weeks resulted in a significant reduction in anti-Pn IgE (fig. 1b) and was continued twice weekly for an additional 18 weeks. Anti-Pn IgE continued to decrease in the treatment group and by week 11, the majority (75%) of these mice did not have detectable anti-Pn IgE (lower limit of detection: 0.25 ng/ml; fig. 1b). Anti-Pn IgG1 (fig. 1c) and total IgG (fig. 1d) were significantly reduced as well.

Prolonged Treatment with Bz Does Not Influence the Symptoms of Murine Peanut Allergy

After 22 weeks of treatment, the mice were rechallenged. Unexpectedly, even though most mice did not have detectable serum anti-Pn IgE, they still reacted upon challenge and did not have significantly different symptoms (fig. 2a) or temperature drops (fig. 2b) when compared to controls.

Prolonged Treatment with Bz Does Not Affect ASC Numbers

In the treated mice, serum anti-Pn IgE was largely absent, and serum anti-Pn IgG1 and total IgG were significantly reduced, so we hypothesized that treatment would significantly reduce ASC numbers. Unexpectedly, Pn-specific and total IgG ASCs in the BM and SPL of treated mice did not significantly differ compared to controls (online suppl. fig. 2A, B). We were unable to enumerate the IgE ASCs in the mice (after 22 weeks of treatment) due to the insufficient sensitivity of our assay. However, total IgE ASC numbers in a group treated for 10 weeks did not significantly differ from controls (online suppl. fig. 2C, D).

Prolonged Treatment with Bz Reduces Numbers of CD138+ Cells in the BM but Not in the SPL and Does Not Affect CD138+ Cell Subsets

Because Bz is thought to deplete long-lived PCs [7], we hypothesized that treatment would significantly decrease the total CD138+ cells and subsets. Although

Fig. 1. Treatment with Bz significantly reduces serum anti-Pn IgE, anti-Pn IgG1, and total IgG. **a** Timing of sensitization, challenge, and treatment of Pn-allergic mice with Bz and diluent, and rechallenge following treatment (week 0) and every 2 weeks during treatment (weeks 3–21) with either Bz or diluent (Dil). Anti-Pn IgE prior to treatment (week 0) and every 2 weeks during treatment (weeks 3–21) with either Bz or diluent (Dil). Anti-Pn IgG1 (**c**) and total IgG (**d**) levels in the serum in week 21. Data represent 1/3 independent experiments with 10–15 mice/group. Error bars denote mean $\pm$ SD. n.s. = Not significant, wk = week. * $p < 0.05$, *** $p < 0.001$ and **** $p < 0.0001$.

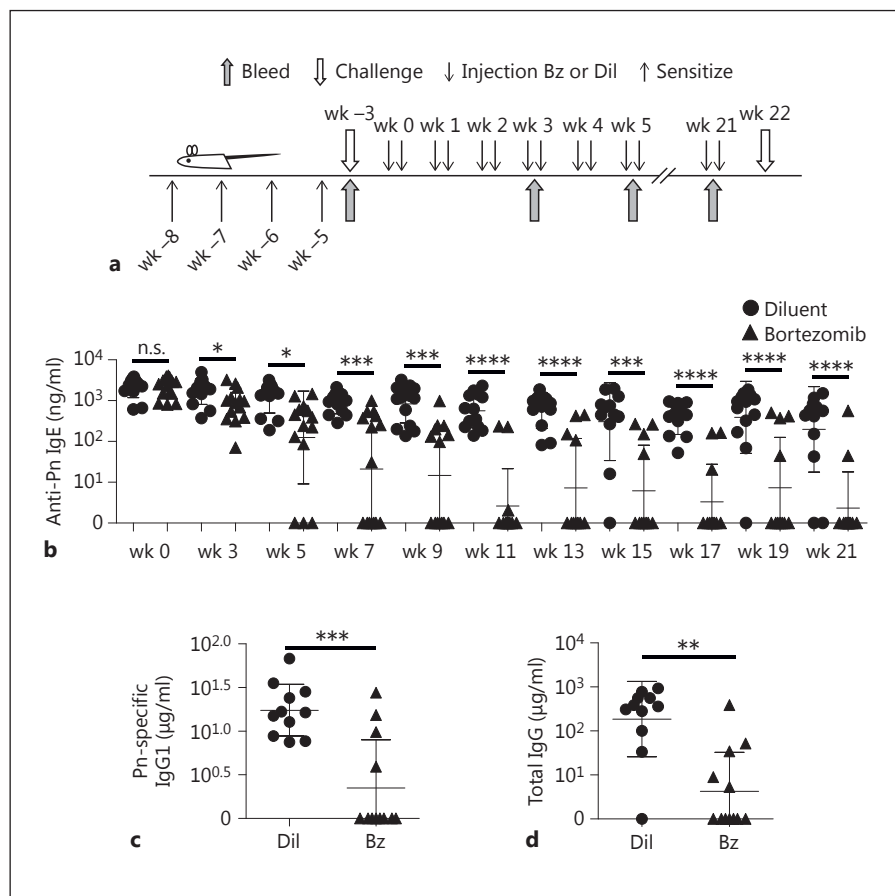
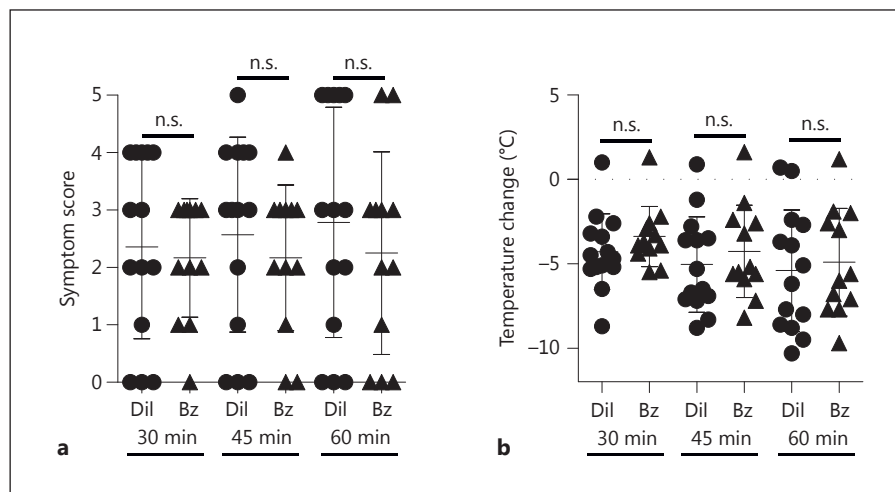


Fig. 2. Symptoms of peanut allergy are not affected by prolonged treatment with Bz. Symptom scores (**a**) and body temperatures (**b**) were measured 30, 45, and 60 min after challenge with CPE following treatment with Bz or diluent (Dil) in week 22. Data represent 1/3 independent experiments with 10–15 mice/group. Error bars denote mean $\pm$ SD. n.s. = Not significant.



treatment did not significantly affect IgG and IgE ASC numbers, B220+ and B220- CD138+ cells were significantly decreased in the BM but were unaffected in the SPL (fig. 3A). We also analyzed subsets of B220+ and B220- CD138+ cells based on intracellular and surface Ig

kappa light chain (iIg κ and sIg κ) expression. We found no significant differences in the numbers of: (1) mature PCs (CD138+ sIg κ - iIg κ +), (2) late plasmablasts (CD138+ sIg κ + iIg κ +), or (3) plasmablasts (CD138+ sIg κ + iIg κ -) in both the BM and SPL of mice treated with

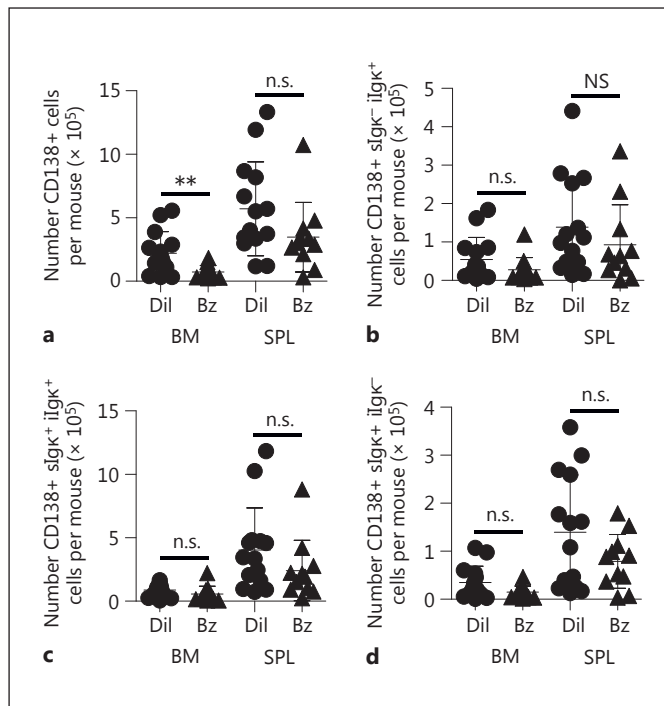


Fig. 3. Numbers of CD138+ cell subsets in the BM and SPL after prolonged treatment with Bz. Total numbers of CD138+ (a), CD138+ sIgk- iIgk+ (b), CD138+ sIgk+ iIgk+ (c), and CD138+ sIgk+ iIgk- (d) cells in the BM and SPL following treatment with Bz or diluent (Dil) in week 22. Numbers of cells analyzed by FACS were normalized to the total numbers of cells enumerated in the BM hind legs or SPL of each mouse, indicating the total number of each cell type per compartment. Data represent 1/2 independent experiments with 10–15 mice/group. Error bars denote mean $\pm$ SD. ** $p < 0.01$.

Bz (fig. 3b–d). Additionally, numbers of: (1) B220+ CD19+, (2) IgG1+, (3) CD3+, and (4) CD11c+ cells in both the BM and SPL were not affected (online suppl. fig. 3A–D).

Discussion

In a previous study, treatment of peanut-allergic mice with anti-CD20 antibody led to prolonged depletion of CD19+ cells in the peripheral blood, peritoneum, and SPL as well as depletion of mature, but not immature CD19+ cells in the BM, without affecting anti-Pn IgE and symptoms [14]. These results suggest that long-lived PCs, rather than CD20+ cells, may be central to maintaining long-lived IgE.

In this study, we investigated the role of ASCs in maintaining persistently elevated anti-Pn IgE. Whereas Bz sig-

nificantly decreased anti-Pn IgG1 and IgE, the symptoms were unaffected and also did not result in decreased BM or SPL ASCs.

It is unclear why Pn-specific Ig is reduced while the ASC numbers remain unaffected. Resistance of multiple myeloma cells to Bz treatment has been shown to occur in vitro [17, 18]; therefore, it is possible that a prolonged treatment course results in resistance to Bz-induced death. Alternatively, quantitatively less IgE may have been secreted by ASCs in the presence of Bz and was still detected by our assay, or the population of ASCs that was affected by treatment may reside elsewhere in the mouse, such as in the gut-associated lymphoid tissue, i.e. Peyer's patches and mesenteric lymph nodes (not examined in this study). While we found that antigen-specific ASCs were not affected by Bz, total CD138+ cells were significantly decreased in the BM only, and not in the SPL. Our findings only slightly differ from those of Wegmann et al. [13], who found that Bz did not affect numbers of CD138+ cells in the BM.

We found that Bz significantly decreased IgE but did not affect the symptoms. The half-life of murine IgE in the plasma is less than 1 day [19–21] and is unknown in the tissue. If we assume that the tissue half-life is the same as that in humans (approx. 2 weeks), serum anti-Pn IgE in the majority of treated mice was undetectable for at least 5 half-lives [22]. However, since the treated mice still reacted, it is likely that sufficient IgE remained bound to the high-affinity Fc epsilon receptor I (FcεRI) to allow for allergen-induced activation of mast cells, even with prolonged, undetectable serum IgE. In support of this explanation, Kubo et al. [19], using B-cell-deficient (C57BL/6- μ m^{-/-}) mice, found that passively transferred trinitrophenyl-specific IgE became undetectable at 6 days, but that treatment of recipients with trinitrophenyl BSA as long as 42 days after passive transfer induced anaphylaxis. Alternatively, our mice may have reacted due to IgG-mediated anaphylaxis. However, this is unlikely, given that serum anti-Pn IgG1 was significantly decreased by Bz.

Our results show that long-lived anti-Pn IgE in mice is maintained by cells sensitive to Bz. However, we have shown here, confirming the reports of others [12, 13, 19], that a depletion of serum IgE does not result in a correlated decrease of symptoms, even after prolonged depletion. IgE bound to mast cells in the murine system likely acts as a reservoir [19]. Therefore, when efforts to deplete ASCs to treat peanut and other allergies are made, the persistence of sensitization in the absence of serum IgE needs to be taken into consideration.

Acknowledgements

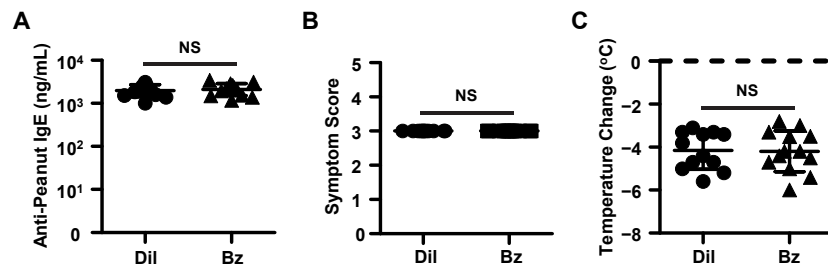
We thank Drs. John C. Cambier, Philippa Marrack, Rafeul Alam, Angie Ribera, and Andrew Fontenot for suggestions on analysis and experimentation. Sources of support: a mini-grant from the American Academy of Allergy, Asthma, and Immunology (AAAAI) (to S.C.D.), a predoctoral grant from the Colorado Clinical & Translational Sciences Institute (NIH/NCATS Colorado CTSI grant No. TL1 TR000155) (to D.M.M.) and funding by the University of Colorado Division of Allergy and Clinical Immunology.

Disclosure Statement

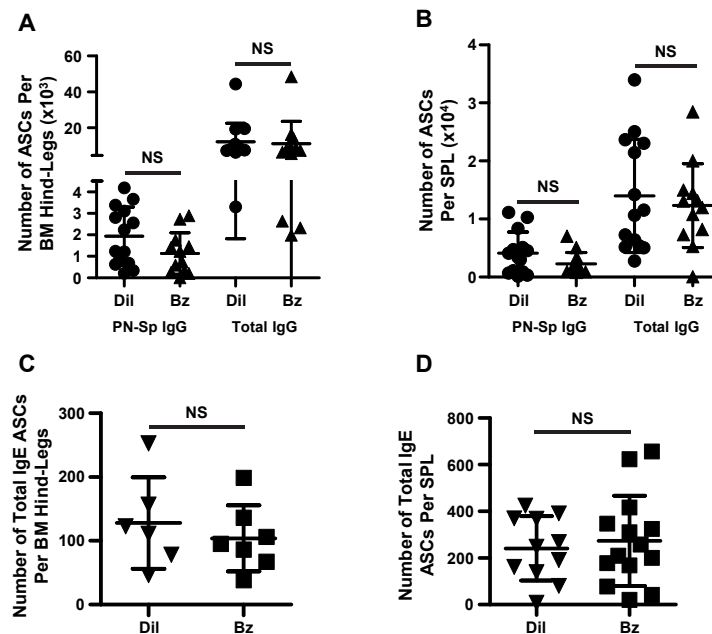
The authors declare no conflict of interests.

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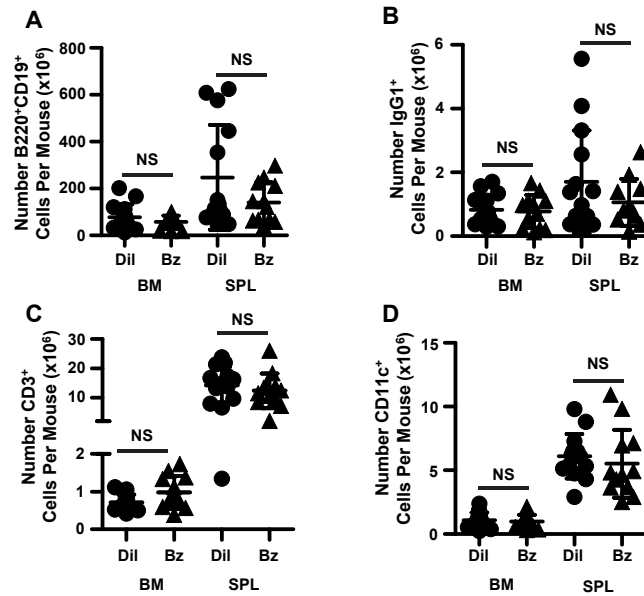
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Supplementary Fig. 1. Anti-Pn IgE, symptom scores, and temperature drops prior to treatment are not significantly different between groups receiving either diluent or Bz. A Serum anti-Pn IgE at week 0 prior to treatment with diluent (Dil) or Bz. **B** Symptom scores and **C** body temperature changes measured during the initial challenge prior to treatment on week -3.



Supplementary Fig. 2. Prolonged treatment with Bz does not affect numbers of Pn-specific IgG, total IgG, and total IgE antibody secreting cells in the bone marrow or spleen. Pn-specific IgG and total IgG ASCs in the **A** BM and **B** SPL of Pn-allergic mice treated with either Bz or diluent (Dil) on week 22. Total IgE ASCs in the **C** BM and **D** SPL on week 10. To determine total IgE ASC numbers in the BM (shown in **C**), cells from BM hind-legs of two mice were pooled. Numbers of ASCs were normalized to the total number of cells enumerated in each pair of BM hind-legs or SPL of each mouse. Data represent one of three independent experiments for IgG ASCs (**A** and **B**) and one of two independent experiments for IgE ASCs (**C** and **D**). Error bars denote mean $\pm$ SD. Sp-specific



Supplementary Fig. 3. Prolonged treatment of peanut-allergic mice with bortezomib does not affect populations of B220⁺CD19⁺, IgG1⁺, CD3⁺, and CD11c⁺ cells in the bone marrow or spleen. **A** B220⁺CD19⁺, **B** IgG1⁺, **C** CD3⁺, and **D** CD11c⁺ cells in the BM and SPL of peanut-allergic mice treated with intravenous bortezomib (Bz) or diluent (Dil) on week 22. Numbers of cells analyzed by FACS were normalized to the total numbers of cells enumerated in the BM hind-legs or SPL of each mouse, indicating the total number of each cell type per compartment. Data represent one of two independent experiments with 10-15 mice per group. Error bars denote mean $\pm$ SD.

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Supplementary Fig. 3 Prolonged treatment with Bz does not affect populations of B220⁺CD19⁺, IgG1⁺, CD3⁺, and CD11c⁺ cells in the BM or SPL. **A** B220⁺CD19⁺, **B** IgG1⁺, **C** CD3⁺, and **D** CD11c⁺ cells in the BM and SPL of Pn-allergic mice treated with i.v. Bz or diluent (Dil) on week 22. Numbers of cells analyzed by FACS were normalized to the total numbers of cells enumerated in the BM hind-legs or SPL of each mouse, indicating the total number of each cell type per compartment. Data represent one of two independent experiments with 10-15 mice per group. Error bars denote mean $\pm$ SD.