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Prolonged treatment of peanut-allergic mice with bortezomib significantly reduces serum anti-peanut IgE but does not affect allergic symptoms

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Abstract

Background—Anti-peanut (Pn) immunoglobulin (Ig) E 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, Pn-allergic mice underwent prolonged treatment with the proteasome inhibitor, bortezomib (Bz).

Methods—Intravenous Bz was given twice weekly for 21 weeks to Pn-allergic mice. During treatment, serum anti-Pn IgE was measured, and mice were re-challenged 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 in symptoms following challenge with Pn.

Keywords

anaphylaxis; antibody secreting cell; IgE; mast cell; proteasome inhibitor

Introduction

Since Pn allergy, often life-long, is IgE-mediated, 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

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Conflict of Interest

The authors declare no conflict of interest.

generated (by *Nippostrongylus brasiliensis*), which typically persists for only four 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 (Ad), Bz decreased serum IgE, and depleted ASCs, CD4⁺, and CD8⁺ cells in Ad lesions; however Ad persisted [12]. In a murine model of asthma, Bz significantly reduced anti-OVA IgE without reducing anti-OVA IgG1 or total IgG [13]. Despite the decrease in anti-OVA IgE, airway hyperresponsiveness to methacholine and numbers of PCs were not decreased [13].

The C3H/HeJ murine model of Pn allergy generates high serum 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 was maintained by ASCs. To test this, we treated Pn-allergic mice with intravenous (i.v.) Bz and monitored serum anti-Pn IgE for 21 weeks. We re-challenged mice after 22 weeks of treatment and measured CD138⁺ cell and IgG and IgE ASC numbers in the BM and SPL.

Materials and Methods

Crude Peanut extract (CPE)

CPE was purified as previously described [15], dialyzed into PBS, and sterile filtered.

Murine model of Pn allergy and treatment with Bz

Murine procedures were conducted in accordance to and approved by 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, dosed [12,13], and given twice per week beginning 3 weeks after challenge for 21 weeks. The duration of dosing was designed to allow 5 half-lives of tissue IgE (~2 weeks) to elapse once serum IgE became undetectable. Mice were re-challenge on 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 [14], and serum anti-Pn IgE and IgG1 antibodies were detected as previously described [14,16].

Statistical analysis

Graphpad Prism 5 (La Jolla, CA, USA) was used throughout. A p-value of less than 0.05 was considered to be significant. The Mann-Whitney 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 were challenged with i.p. CPE. Three weeks following challenge, mice were divided into two groups without significant differences in serum anti-Pn IgE (supplementary fig. 1A), symptom scores (supplementary fig. 1B), or temperature changes (supplementary fig. 1C). Bz was given i.v. twice per week for 21 weeks, and anti-Pn IgE was measured every two weeks starting at week 3 (fig. 1A). During the course of these experiments (30 weeks), three out of 15 mice in the experimental group, and one out of 13 mice in the control group, died. This is likely due to the toxicity associated with long-term (22 weeks) Bz treatment.

Treatment for three weeks resulted in 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 limits of detection: 0.25 ng/mL) (fig. 1B). Anti-Pn IgG1 and total IgG were significantly reduced as well (fig. 1C and D, respectively).

Prolonged treatment with Bz does not affect symptoms of murine Pn allergy

After 22 weeks of treatment, mice were re-challenged. 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) compared to controls.

Prolonged treatment with Bz does not affect ASC numbers

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

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 PC [7], we hypothesized that treatment would significantly decrease total CD138⁺ cells and subsets. Although 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 (iIgκ and sIgκ, respectively) expression. We found no significant differences in the numbers of: i) mature PCs (CD138⁺sIgκ⁻iIgκ⁺), ii) late plasmablasts (CD138⁺sIgκ⁺iIgκ⁺), or iii) plasmablasts (CD138⁺sIgκ⁺iIgκ⁻) in both the BM and SPL of mice treated with Bz (fig. 3B–D, respectively). Additionally, numbers of: i) B220⁺CD19⁺,

ii) IgG1⁺, iii) CD3⁺, and 4) CD11c⁺ cells in both the BM and SPL were not affected (supplementary fig. 3A–D, respectively).

Discussion

In a previous study, treatment of Pn-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; however, anti-Pn IgE and symptoms were not affected [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 significantly decreased anti-Pn IgG1 and IgE, symptoms were unaffected and also did not result in decreased BM or SPL ASCs.

It is unclear why Pn-specific Ig is decreased, while 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 be secreted by ASCs in the presence of Bz and still be detected by our assay, or that the population of ASCs that is affected by treatment may reside elsewhere in the mouse such as in the gut-associated lymphoid tissue such as the 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. who found that Bz did not affect numbers of CD138⁺ cells in the BM [13].

We found that Bz significantly decreased IgE but did not affect symptoms. The half-life of murine IgE in the plasma is less than one day [19–21] and is unknown in the tissue. If we assume that the tissue half-life is the same as that in humans (~2 weeks), serum anti-Pn IgE in the majority of treated mice were undetectable for at least 5 half-lives [22]. However, since treated mice still reacted, it is likely that sufficient IgE remains 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., using B cell deficient (C57BL/6-μm^{-/-}) mice, found that passively transferred trinitrophenyl (TNP)-specific IgE became undetectable at 6 days, but treatment of recipients with TNP-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 (this report) and others [12,13,19] have shown that depletion of serum IgE does not result in a correlated decrease in symptoms, even after prolonged depletion. IgE bound to mast cells in the murine system likely acts as a reservoir [19]. Therefore, efforts to deplete ASCs to treat Pn and other allergies need to consider the persistence of sensitization in the absence of serum IgE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this article

ASCs	antibody secreting cells
Ad	atopic dermatitis
BM	bone marrow
Bz	bortezomib
CPE	crude peanut extract
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
FACS	fluorescence-activated cell sorting
FcεRI	Fc epsilon receptor I
Ig	immunoglobulin
ιIgκ	intracellular immunoglobulin kappa light chain
i.p	intraperitoneal
i.v	intravenous
OVA	ovalbumin
Pn	peanut
PC	plasma cells
SPL	spleen
σIgκ	surface Ig kappa light chain
wk	week

References

1. Yang Z, Sullivan BM, Allen CD. Fluorescent in vivo detection reveals that ige(+) b cells are restrained by an intrinsic cell fate predisposition. *Immunity*. 2012; 36:857–872. [PubMed: 22406270]
2. He JS, Meyer-Hermann M, Xiangying D, Zuan LY, Jones LA, Ramakrishna L, de Vries VC, Dolpady J, Aina H, Joseph S, Narayanan S, Subramaniam S, Puthia M, Wong G, Xiong H, Poidinger M, Urban JF, Lafaille JJ, Curotto de Lafaille MA. The distinctive germinal center phase of ige+ b lymphocytes limits their contribution to the classical memory response. *The Journal of experimental medicine*. 2013; 210:2755–2771. [PubMed: 24218137]
3. Erazo A, Kutchukhidze N, Leung M, Christ AP, Urban JF Jr, Curotto de Lafaille MA, Lafaille JJ. Unique maturation program of the ige response in vivo. *Immunity*. 2007; 26:191–203. [PubMed: 17292640]
4. Talay O, Yan D, Brightbill HD, Straney EE, Zhou M, Ladi E, Lee WP, Egen JG, Austin CD, Xu M, Wu LC. Ige(+) memory b cells and plasma cells generated through a germinal-center pathway. *Nature immunology*. 2012; 13:396–404. [PubMed: 22366892]
5. Watanabe N, Katakura K, Kobayashi A, Okumura K, Ovary Z. Protective immunity and eosinophilia in ige-deficient sj/9 mice infected with *nippostrongylus brasiliensis* and *trichinella spiralis*. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:4460–4462. [PubMed: 3380800]
6. Pochanke V, Koller S, Dayer R, Hatak S, Ludewig B, Zinkernagel RM, Hengartner H, McCoy KD. Identification and characterization of a novel antigen from the nematode *nippostrongylus brasiliensis* recognized by specific ige. *European journal of immunology*. 2007; 37:1275–1284. [PubMed: 17429848]
7. Paramore A, Frantz S. Bortezomib. *Nature reviews Drug discovery*. 2003; 2:611–612.
8. Chauhan D, Catley L, Li G, Podar K, Hideshima T, Velankar M, Mitsiades C, Mitsiades N, Yasui H, Letai A, Ova H, Berkers C, Nicholson B, Chao TH, Neuteboom ST, Richardson P, Palladino MA, Anderson KC. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from bortezomib. *Cancer Cell*. 2005; 8:407–419. [PubMed: 16286248]
9. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, Anderson KC. The proteasome inhibitor ps-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer research*. 2001; 61:3071–3076. [PubMed: 11306489]
10. Landowski TH, Megli CJ, Nullmeyer KD, Lynch RM, Dorr RT. Mitochondrial-mediated dysregulation of ca2+ is a critical determinant of velcade (ps-341/bortezomib) cytotoxicity in myeloma cell lines. *Cancer research*. 2005; 65:3828–3836. [PubMed: 15867381]
11. Neubert K, Meister S, Moser K, Weisel F, Maseda D, Amann K, Wiethe C, Winkler TH, Kalden JR, Manz RA, Voll RE. The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. *Nature medicine*. 2008; 14:748–755.
12. Mudnakudu Nagaraju KK, Babina M, Worm M. Opposing effects on immune function and skin barrier regulation by the proteasome inhibitor bortezomib in an allergen-induced eczema model. *Experimental dermatology*. 2013; 22:742–747. [PubMed: 24118387]
13. Wegmann M, Lunding L, Orinska Z, Wong DM, Manz RA, Fehrenbach H. Long-term bortezomib treatment reduces allergen-specific ige but fails to ameliorate chronic asthma in mice. *International archives of allergy and immunology*. 2012; 158:43–53. [PubMed: 22212634]
14. Moutsoglou DM, Dreskin SC. B cells establish, but do not maintain, long-lived murine anti-peanut ige(a). *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2016; 46:640–653. [PubMed: 27021119]
15. Porterfield HS, Murray KS, Schlichting DG, Chen X, Hansen KC, Duncan MW, Dreskin SC. Effector activity of peanut allergens: A critical role for ara h 2, ara h 6, and their variants. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2009; 39:1099–1108. [PubMed: 19438581]
16. Kulis M, Pons L, Burks AW. In vivo and t cell cross-reactivity between walnut, cashew and peanut. *International archives of allergy and immunology*. 2009; 148:109–117. [PubMed: 18802355]

17. Markovina S, Callander NS, O'Connor SL, Kim J, Werndli JE, Raschko M, Leith CP, Kahl BS, Kim K, Miyamoto S. Bortezomib-resistant nuclear factor-kappaB activity in multiple myeloma cells. *Mol Cancer Res.* 2008; 6:1356–1364. [PubMed: 18708367]
18. Oerlemans R, Franke NE, Assaraf YG, Cloos J, van Zantwijk I, Berkers CR, Scheffer GL, Debipersad K, Vojtekova K, Lemos C, van der Heijden JW, Ylstra B, Peters GJ, Kaspers GL, Dijkmans BA, Scheper RJ, Jansen G. Molecular basis of bortezomib resistance: Proteasome subunit beta5 (psmb5) gene mutation and overexpression of psmb5 protein. *Blood.* 2008; 112:2489–2499. [PubMed: 18565852]
19. Kubo S, Nakayama T, Matsuoka K, Yonekawa H, Karasuyama H. Long term maintenance of ige-mediated memory in mast cells in the absence of detectable serum ige. *Journal of immunology.* 2003; 170:775–780.
20. Haba S, Ovary Z, Nisonoff A. Clearance of ige from serum of normal and hybridoma-bearing mice. *Journal of immunology.* 1985; 134:3291–3297.
21. Vieira P, Rajewsky K. The half-lives of serum immunoglobulins in adult mice. *European journal of immunology.* 1988; 18:313–316. [PubMed: 3350037]
22. Ishizaka K, Ishizaka T. Ige and reaginic hypersensitivity. *Annals of the New York Academy of Sciences.* 1971; 190:443–456. [PubMed: 4109645]

Fig. 1. Treatment with Bz significantly reduces serum anti-Pn IgE, anti-peanut IgG1, and total IgG

A Timing of sensitization, challenge, and treatment of Pn-allergic mice with Bz and diluent, with re-challenge following treatment. **B** Serum anti-Pn IgE is shown prior to treatment (week 0) and every two weeks during treatment from weeks 3 to 21 with either Bz or diluent (Dil). **C** Anti-Pn IgG1 and **D** total IgG levels in the serum on week 21. Data represent one of three independent experiments with 10–15 mice per group. Error bars denote mean $\pm$ SD. NS-not significant, wk-week, * $p < 0.05$, *** $p < 0.001$, and **** $p < 0.0001$.

Fig. 2. Symptoms of Pn allergy are not affected by prolonged treatment with Bz

A Symptom scores and **B** body temperatures were measured at 30, 45, and 60 minutes post-challenge with CPE following treatment with Bz or diluent (Dil) on week 22. Data represent one of three independent experiments with 10–15 mice per group. Error bars denote mean $\pm$ SD. min-minute.

Fig. 3. Numbers of CD138⁺ cell subsets in the bone marrow and spleen after prolonged treatment with Bz

Total numbers of **A** CD138⁺, **B** CD138⁺ sIgκ⁻ iIgκ⁺, **C** CD138⁺ sIgκ⁺ iIgκ⁺, and **D** CD138⁺ sIgκ⁺ iIgκ⁻ cells in the BM and SPL following treatment with 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 ± SD. iIgκ-intracellular Ig kappa light chain, sIgκ-surface Ig kappa light chain, **p<0.01.