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Article type : Letter to the Editor

An injectable excipient prevents anti-polyethylene glycol antibody mediated hypersensitivity

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To the Editor,

Hypersensitivity to polyethylene glycol (PEG) is on the rise.^{1, 2} Sporadic allergy to Covid-19 vaccines have been reported and are suspected to be PEG-related.³ So far, conventional premedication using anti-histamine, steroids, NSAIDs, and leukotriene receptor antagonists are not effective in preventing allergy.⁴

Reactions to PEG are molecular weight (MW) dependent.¹ High MW (HMW, ≥ 1 kDa) PEGs are more likely to trigger reactions than low MW (LMW) PEGs. We investigated the effectiveness of preventive administration of LMW PEG as a decoy to prevent hypersensitivity to HMW PEG mediated by anti-PEG antibodies. Monomeric ethylene and diethylene glycol can inhibit histamine-release in an *ex vivo* HMW PEG allergy model.² However, these compounds are too toxic to use *in vivo*. Fortunately, PEG of MW 300-600 Da are safe additives for intravenous injection. Herein, we tested the effectiveness of non-toxic LMW PEG in preventing reactions to HMW PEG mediated by anti-PEG antibodies in a mouse model, using PEG 400 Da as the candidate LMW PEG and PEGylated asparaginase (pegaspargase, PEG MW = 5 kDa) as the cause of HMW PEG hypersensitivity.

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To induce anti-PEG antibodies, female Balb/c mice were sensitized with PEG-catalase (prepared with adjuvant); mice sensitized with adjuvant alone served as negative controls (Fig 1A). Sensitization with PEG-catalase successfully induced high levels of anti-PEG antibodies ($P = 6.2 \times 10^{-5}$, negative control vs. PEG-catalase sensitized, supplemental Fig 1A). PEG-catalase sensitized mice were further assigned to one of the three challenge regimens (Fig 1A, treatment groups 2-4). Negative control mice were further challenged with pegaspargase (Fig 1A, treatment group 1). Temperature drop measured longitudinally at the center of the head (black cross in Fig 1C&E) using an infrared camera was the indicator for hypersensitivity. Pre-challenge baseline temperature and appearance of PEG-catalase sensitized vs. negative control mice were not different ($P = 0.37$ for temperature). Sensitized mice challenged with pegaspargase (pegaspargase group) had clear allergic reactions, becoming scruffy, less active, and displaying about a 5°C temperature decrease within 30 min after challenge (Fig 1D&E, red line in Fig 2A). Serum mMCP-1 and IgE were not informative for allergy (Supplemental Methods and Results). Negative control mice stayed groomed and active with no temperature drop from baseline ($P \geq 0.73$ between all post-challenge time points and baseline, Fig 1B&C, gold line in Fig 2A).

PEG-catalase sensitized mice administered PEG 400Da either alone or as pretreatment had similar pre-challenge antibody levels to the pegaspargase group ($P = 0.94$ and 0.72 for PEG 400Da and PEG 400Da + pegaspargase vs. pegaspargase, Fig 2B). PEG 400Da alone did not induce hypersensitivity ($P \geq 0.49$, temperature of PEG 400 (blue line) vs. negative control (gold line) at all post-challenge time points, Fig 2A). When sensitized mice were pre-treated with PEG 400Da and then challenged with pegaspargase, no change in their temperature was observed ($P \geq 0.92$, temperature of PEG 400Da + pegaspargase (green line) vs. negative control (gold line) at all post-challenge time points, Fig 2A), indicating successful prevention of hypersensitivity. These mice also remained active and alert. Temperature AUC of PEG 400 and PEG + pegaspargase mice did not differ from negative control mice ($P = 0.95$ and 0.99 for PEG 400Da and PEG 400Da + pegaspargase vs. negative control, Fig 2C), while the AUC of pegaspargase group was significantly lower than the rest ($P = 3.0 \times 10^{-7}$, Fig 2C). Asparaginase activity was below the detection limit in the pegaspargase group. Although PEG 400Da prevented anti-PEG antibody-mediated pegaspargase hypersensitivity, it did not rescue pegaspargase from increased clearance mediated by anti-PEG antibody ($P = 0.94$, Fig 2D). Pathologic

examination of PEG 400Da treated mice did not find signs of toxicity except for microgranulomas at the injection site, which is not considered a safety issue.⁵

We established an anti-PEG mediated HMW PEG hypersensitivity model using noninvasive infrared imaging with no anesthesia needed. Pre-treatment using LMW PEG as an immune decoy prevented hypersensitivity mediated by HMW PEG-conjugated therapeutics. Additional study of this strategy could include consideration of the amount of anti-PEG antibodies in humans, other measures of allergy mediators and antibody subclasses, and the PEG load in different drugs, including those in Covid-19 vaccines made by Pfizer-BioNTech and Moderna.

Reactions to other excipients, such as polysorbates, might also be of interest.

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Key words

polyethylene glycol, hypersensitivity reaction, allergy, hapten inhibition, Covid-19 vaccine

FIGURE LEGENDS

FIGURE 1. Mouse model for anti-PEG antibody-mediated HMW PEG hypersensitivity reactions. Alum was used as adjuvant. Blood was collected one day before challenge for anti-PEG antibody measurement and 2 hours post-challenge for pegaspargase activity measurement. **A**, treatment regimen and groups; **B-E**, Mouse temperature monitoring with infrared imaging; **B-C**, negative control mouse; **D-E**, Mouse sensitized with PEG-catalase and challenged with pegaspargase.

Figure 2. Prevention of HMW PEG hypersensitivity reactions with PEG 400Da. **A**, Temperature change in different treatment groups up to 120 min post-challenge; **B**, Pre-challenge anti-PEG IgG antibody level in different treatment groups; **C**, Post-challenge 120 min temperature AUC in different treatment groups; **D**, Asparaginase activity 120 min post-challenge in groups challenged with pegaspargase. Red dots indicate measurements below detection limit (0.01U/ml).

FIGURE 1

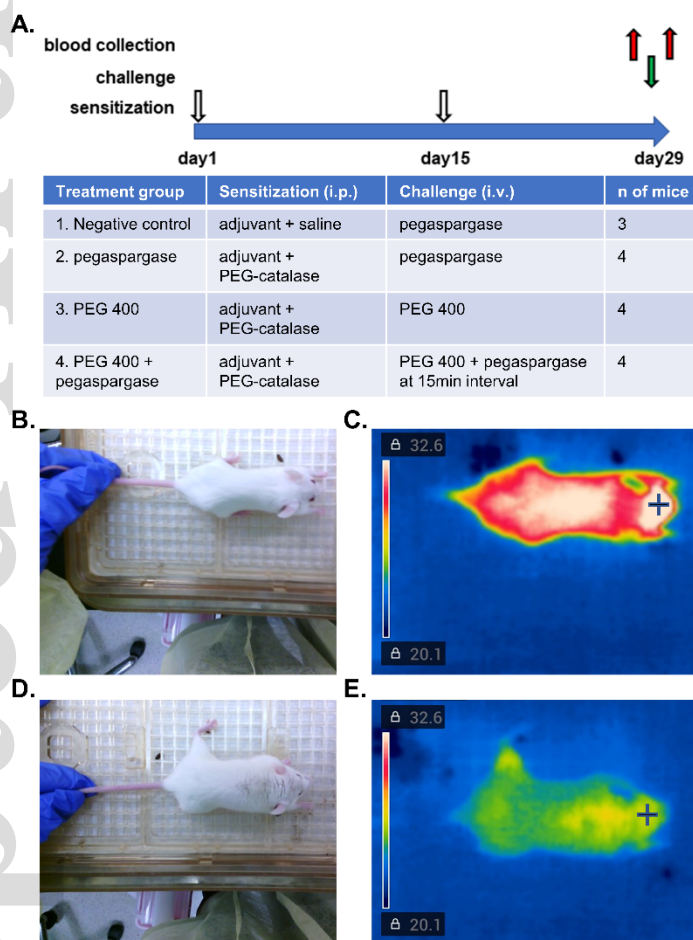
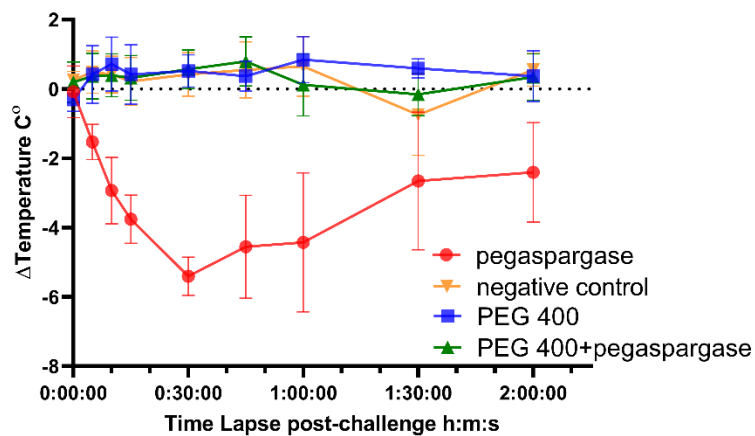
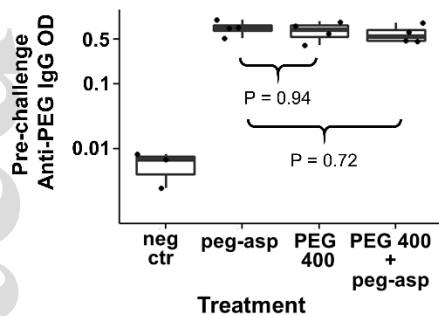


FIGURE 2

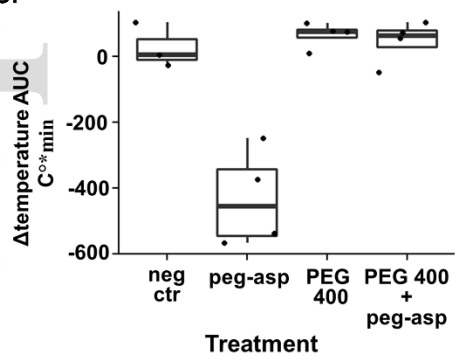
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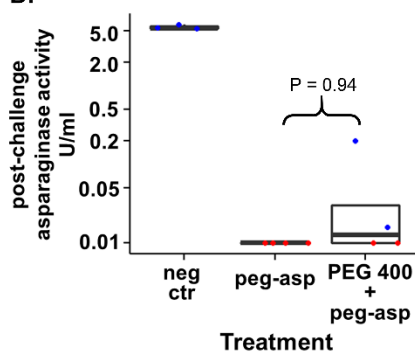
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C.



D.



1 Supplemental Methods

2 Anti-PEG antibodies mediated HMW PEG hypersensitivity mouse model and treatment regimens

3 Female Balb/c mice 8-10-week-old (JAX) were sensitized via intraperitoneal injection with 30µg

4 PEGylated bovine catalase (Sigma-Aldrich, PEG MW = 5kDa) using Alum (Thermo Scientific)

5 as adjuvant twice at 2-week intervals (groups 2, 3, and 4; Fig 1A). Sensitized mice were challenged

6 with 300 µg pegaspargase (Servier, PEG MW = 5kDa) intravenously on day 29 after the first

7 sensitization (pegaspargase group and PEG 400 + pegaspargase group, groups 2 and 4) or with

8 PEG 400 alone (group 3) (Fig 1A). Negative control mice (group 1) received only Alum for

9 sensitization and were then challenged with pegaspargase. PEG-catalase and PEG-asparaginase

10 were used for this model because 1) both catalase (bovine) and asparaginase (E.coli) are foreign

11 proteins for mice, and there is no amino acid sequence similarity between these two proteins and

12 any known proteins in mice; and 2) These two PEGylated proteins were readily available due to

13 long-standing investigations in hypersensitivity to PEGylated therapeutics. PEG-catalase is what

14 we use to identify anti-PEG antibodies in patients treated with pegaspargase (used for pediatric

15 acute lymphoblastic leukemia) in a CLIA-approved clinical assay.¹ We used PEG-catalase as the

16 sensitization reagent and pegaspargase as the challenge, not the other way around, because of the

17 toxicity of pegaspargase. PEG-catalase has been tested preclinically for a wide range of diseases

18 including ischemia/reperfusion injury and cancer metastasis.^{2,3} Serum samples were collected one

19 day before challenge (retro-orbital) and 2 hours post-challenge (cardiac puncture). To test the

20 effectiveness of PEG 400 on preventing anti-PEG antibody-mediated hypersensitivity, mice were

21 challenged with 15 mg PEG 400Da (Sigma Aldrich) or pegaspargase with 15 mg PEG 400Da

22 pretreatment. When used as pretreatment, PEG 400Da was administered 15 minutes before

23 pegaspargase, both intravenously. Pre-challenge sera were used for anti-PEG IgG antibody

measurement. Post-challenge sera were used for asparaginase activity measurement. Mouse body temperature was measured at baseline before challenge and at 5, 10, 15, 30, 45, 60, 90, and 120 min post-challenge. Infrared images were captured using a FLIR camera placed 7.5 inches above the mice. All post-challenge temperature measurements were relevelled to the mean pre-challenge value of all mice. Area-under-the-curve (AUC) of temperature change vs time was estimated from 0 to 120 min post-challenge for each mouse.

Measurement of anti-PEG IgG/IgE and mMCP-1

Blood was collected retro-orbitally one day before challenge in all mice and assayed for anti-PEG IgG using an ELISA assay reported before.⁴ NH₂-PEG-5k (Creative PEGworks) was used as coating antigen. All samples were measured at 1:50 dilution first. If the measurement yielded an OD above 1.0, the sample was re-assayed at 1:100 dilution. OD at 1:100 dilution was converted to 1:50 OD by taking into consideration the difference in dilution factor ($OD_{1:100} * 2 = OD_{1:50}$). The OD shown in results was 1:50 OD.

Anti-PEG IgE was also measured in these samples using a method modified from mouse anti-ovalbumin IgE measurement published by others using reagents from Cayman Chemical.⁵ Briefly, pre-challenge serum samples were diluted at 1:25 then incubated in a plate pre-coated with anti-mouse IgE. The plate was then incubated with biotin labeled PEG 5kDa, then HRP-conjugated streptavidin. TMB was used as HRP substrate. Results were read at wavelength 650nm.

Mucosal mast cell protease-1 (mMCP-1) was measured as described in our previous publication.⁶

Statistical analysis

Area-under-the-curve (AUC) of temperature change curve was calculated using trapezoidal method. Statistical analyses and graphics were done with R and GraphPad Prism. For all analyses, one-way ANOVA with post-hoc Tukey HSD Test was used.

Further details on materials and methods are available upon request.

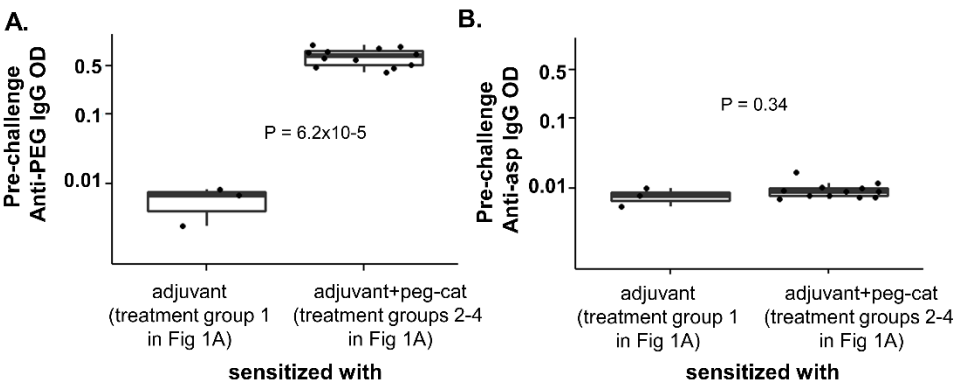
Supplemental Results

PEG-catalase sensitization successfully induced anti-PEG IgG ($P = 6.2 \times 10^{-5}$, Supplemental Figure 1).

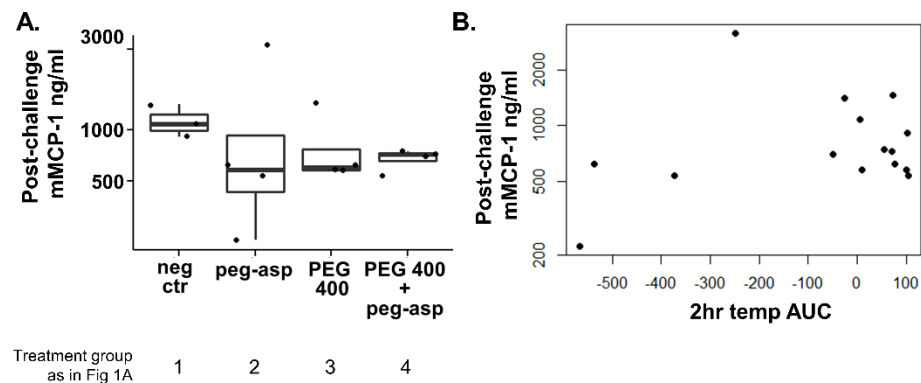
Post-challenge mMCP-1 levels were not associated with treatment group ($P = 0.79$, Supplemental Figure 2A) or temperature AUC ($P = 0.92$, Supplemental Figure 2B).

Anti-PEG IgE was induced in some PEG-catalase sensitized mice with high variability (Supplemental Figure 3A&B), but were not associated with reaction severity in PEG-catalase sensitized mice challenged with PEG-asparaginase ($P = 0.81$, Supplemental Figure 3C).

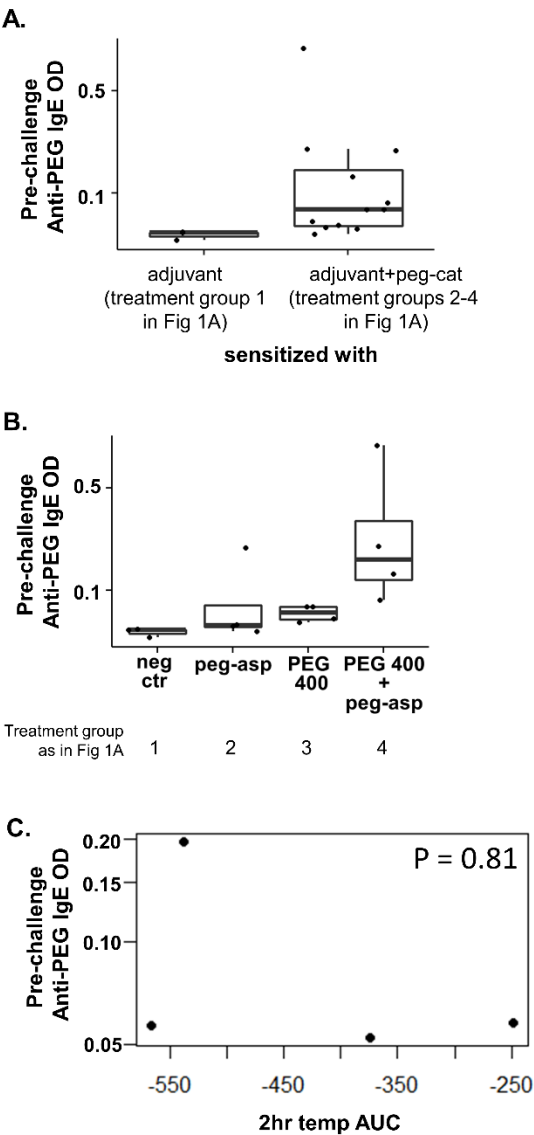
Supplemental Figure 1. Pre-challenge anti-PEG (A), and not anti-asparaginase (B), IgG level was significantly increased by PEG-catalase sensitization ($P = 6.2 \times 10^{-5}$ for anti-PEG, and $P = 0.34$ for anti-asparaginase).



Supplemental Figure 2. Post-challenge mMCP-1 levels were not different by treatment group (A) nor associated with temperature AUC (B).



Supplemental Figure 3. Anti-PEG IgE was induced in some PEG-catalase sensitized mice as measured in pre-challenge serum samples (A&B). Pre-challenge anti-PEG IgE did not associate with temperature AUC (C).



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