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Pre-existing anti-polyethylene glycol antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer

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To the Editor

Nucleic acid aptamers are a novel class of drugs that can be selected to inhibit targets of interest, including protein-protein interactions.¹ Pegnivacogin is a 2'-fluoropyrimidine-modified RNA aptamer that inhibits coagulation factor IXa, coupled to an approximately 40-kDa branched molecule of methoxypolyethylene glycol (mPEG), to increase its concentration and half-life in plasma.² During the RADAR³ phase 2b clinical trial in patients with acute coronary syndrome, allergic reactions occurred within minutes of a first dose of pegnivacogin in 3 of 640 patients (Table I). Two met criteria for anaphylaxis, and 1 was an isolated dermal reaction; each event was deemed serious, and 1 life-threatening, and together they led to early termination of the trial.³

In a broad investigation into a cause for these 3 events (detailed in Methods in this article's Online Repository at www.jacionline.org), a clinical database review found no other serious allergic reactions (SARs) to pegnivacogin; a quality analysis found no aggregation, degradation, or other deviations of the study product from specifications; and a primate pharmacology study found no evidence that pegnivacogin caused an inflammatory response, histamine release, or complement activation. However, blinded testing of more than half of

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all RADAR patients identified an association between high levels of antibody to polyethylene glycol (PEG) and the first-exposure allergic reactions. In addition to the immediate relevance, our findings are the first to document the potential clinical significance of pre-existing antibody to PEG, a component of numerous consumer and medicinal products.

Initially, coded samples from 31 RADAR patients were tested for anti-PEG antibody (see Analytical methods and Fig E1 in this article's Online Repository at www.jacionline.org) in 2 ELISAs to detect IgG binding to pegloticase, a PEGylated urate oxidase (a protein not expressed in humans), and to the 40-kDa mPEG component of pegnivacogin. Unblinding of the data revealed that samples giving the highest signals in both ELISAs were from the 3 patients with SARs (predose from patients 418-008 and 406-003; 88-day postinfusion from patient 602-004 from whom no predose sample was available). Direct (Fig 1, A) and competition ELISAs (Fig 1, B) showed that antibody from each patient could bind to linear and branched PEGs of 5 to 40 kDa, presented as free mPEG or PEG-diol (lacking methoxy termini), or when conjugated via different linkages to 2 proteins and pegnivacogin; importantly, they did not recognize the un-PEGylated aptamer. The predominant heavy-chain isotype was IgG in all 3 patients, and for patient 418-008, who had the highest anti-PEG antibody level, IgG₂ was the predominant IgG subtype (see Fig E2 in this article's Online Repository available at www.jacionline.org).

To assess the overall prevalence and levels of pre-existing anti-PEG antibody, the sponsor submitted coded pretreatment plasma samples from 328 other RADAR patients who had not experienced allergic reactions (all remaining samples available). Recoded samples available from 26 of the initial 31 patients were added, to give a set of 354 unique patient samples (353 pretreatment and 1 posttreatment). In the pegloticase ELISA, 139 of these exceeded the A405 cutoff point of 0.15, with about a third having an A405 value of less than 0.2. Ninety samples with an A405 value of more than 0.2 were then retested with a validated automated competition ELISA, in which pegloticase was the plate coating and 10-kDa PEG-diol was the competitor.⁴ Of these, 82 retested as positive with confirmed specificity for PEG (Fig 1, C). Applying this false-positive rate of 9%, we estimate that 36% of all RADAR patients tested were positive for anti-PEG antibody, with 23% having an A405 value of more than 0.2 in the pegloticase ELISA. The 3 patients with SARs were among only 8 with an A405 value of 1.0 or more (top 2.3%), with patient 418-008 having the highest A405 value of 1.9. Two of these 8 patients received heparin in the trial; of the other 6, 3 (50%) experienced allergic SARs. In further testing of 15 samples with A405 values within the 90th percentile, anti-PEG IgG titers for the 3 patients with SARs ranged from 1:120 to 1:3100 (patient 418-008), compared with approximately 1:110 to 1:950 for the 12 patients without allergies.

Oligonucleotides can potentially trigger allergic-like events by activating the alternative complement pathway, or an innate immune response via Toll-like receptors.⁵ No evidence of the former mechanism was observed with pegnivacogin in primate studies (see Figs E3–E5 in this article's Online Repository at www.jacionline.org) or in phase 1 patients across a wide range of doses. Activation of Toll-like receptors typically elicits a mitogenic response with flu-like symptoms, which were not observed with the reactions in RADAR. Antibody-mediated mechanisms can also trigger allergy, but humans are not generally exposed to RNA

oligonucleotides, and antibody to bacterial DNA and antinuclear autoantibodies do not react significantly with RNA. In contrast, free PEGs are used as additives in numerous consumer products and topical and parenteral medications, and as osmotic laxatives. Perhaps as a result of this wide exposure, anti-PEG antibodies, of no apparent clinical significance, have been detected by various methods as an incidental finding in several study populations at a prevalence of approximately 3% to more than 40%.⁶ mPEGs have also been used for more than 25 years to covalently modify (PEGylate) biologically active molecules as a means of masking epitopes and slowing elimination. Some PEGylated enzymes and liposomes have recently been found to induce anti-PEG antibodies.⁶ Findings in clinical trials of PEGylated enzymes support a role for high levels of pre-existing anti-PEG antibodies in causing first-exposure reactions to pegnivacogin.

In a phase 2 trial of pegloticase for refractory gout, we found anti-PEG antibodies in pretreatment samples of 5 of 27 (19%) patients. The patient with the highest level had an adverse reaction during his first infusion of pegloticase, and patients in whom high levels of anti-PEG antibodies were induced by pegloticase had more frequent and more severe infusion reactions than did those who remained antibody negative.⁴ Infusion reactions, including anaphylaxis-like SAEs, were also associated with higher levels of pegloticase-induced anti-PEG antibodies during phase 3 investigation.⁷ In a phase 1 trial for treating phenylketonuria, a PEGylated phenylalanine ammonia lyase (rAvPAL-PEG) induced anti-PEG antibodies in all 25 participants, 2 of whom had hypersensitivity reactions to an incidentally prescribed birth control medication formulated with PEG before its use was forbidden.⁸

We postulate that a high level of pre-existing anti-PEG antibodies was a major, but not the sole, factor necessary for triggering first-exposure allergic reaction to pegnivacogin. A likely second factor is the relatively large amount of PEG delivered in a single 1 mg/kg bolus dose of pegnivacogin, for example, approximately 64 mg of PEG for an 80-kg patient. In contrast, with peginterferon alfa2a (PEGASYS), which also contains a single 40-kDa branched mPEG, a standard dose would deliver about 0.12 mg of PEG. Other factors must also be involved, as a few other RADAR patients with similar high levels of pre-existing anti-PEG antibodies did not experience an allergic reaction to pegnivacogin. This could reflect differences in properties of anti-PEG antibodies in individual patients, as well as differences in susceptibility to antibody-triggered allergic reactions, that is, the same factors that underlie the spectrum of responses that antidrug antibodies elicit to some antibiotics and un-PEGylated protein-based biologics.^{9,10}

On the basis of our findings, we advise testing for pre-existing anti-PEG antibodies during clinical trials of new PEGylated therapeutic agents, particularly those containing high densities of PEG, or that are given at doses that would expose patients to relatively large amounts of PEG. Further research is needed into the prevalence of “incidental” anti-PEG antibodies, the basis for PEG immunogenicity, and the mechanism(s) by which PEG-anti-PEG immune complexes trigger allergic reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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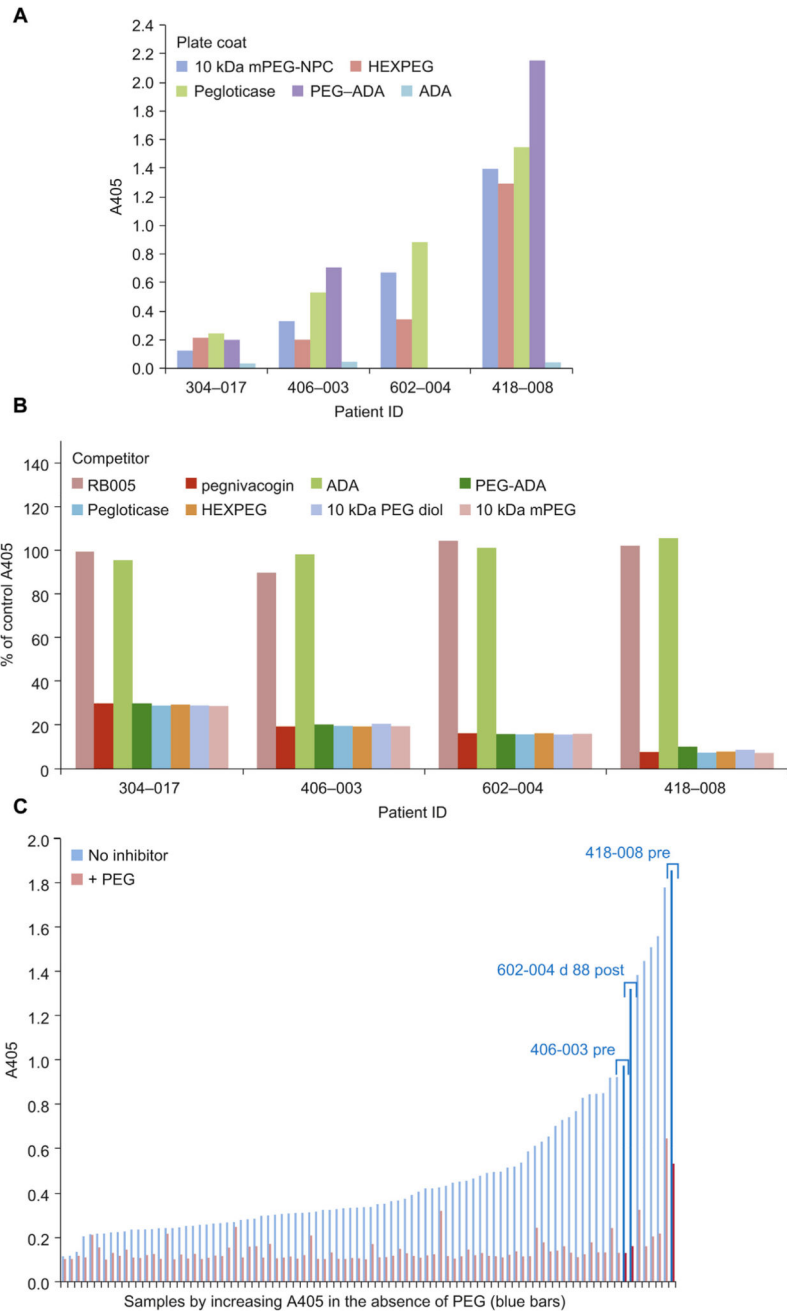


FIG 1. Characterization of anti-PEG antibodies in RADAR patient samples. **A**, ELISAs to assess binding to the antigens indicated in the figure (for description and source of these materials, see this article’s Online Repository at www.jacionline.org). **B**, Competition ELISA. Duplicate aliquots of samples, one spiked with 20 mg of the indicated competing antigen, were tested in the antipegloticase ELISA. IDs 406-003, 602-004, and 418-008 are the 3 patients who experienced SARs to pegnivacogin; ID 304-017 had no reaction to pegnivacogin, but was positive for anti-PEG antibody. **C**, Automated competition ELISA. The 90 samples tested had an A405 value of more than 0.2 in the antipegloticase ELISA (see

text for details). *Blue and red bars* are, respectively, the A405 obtained in the absence and presence of competing antigen (10 kDa PEG-diol). The *darker blue and red bars* with sample IDs indicate the 3 patients who experienced SARs to pegnivacogin.

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TABLE I

Subjects experiencing SARs in RADAR

Patient ID	Onset (min)	Symptoms	Treatment	Resolution (h)	Allergy history
602-004	5	GI, D, P, H	IVF, H1, IVV, S	6	2 mo prior diffuse urticaria to unknown agent
406-003	25	D	S, H1, H2	0.4	Contrast dye, hay fever
418-008	5	P, D, H	S, H1, H2, IVF, IVV, I, Inh	1144	Recent allergy to β -blockers and steroids

All 3 patients were female; 602-004 was treated at a site in Poland and the other 2 patients were treated in Germany. For additional information regarding these patients, see this article's REGI-CLIN211a section in the Online Repository at www.jacionline.org. *Note:* As substantially more information is available on these 3 subjects than for other trial participants, inferences regarding the possible role of sex, geography, or allergic history are cautioned against.

D, Dermal; *GI*, gastrointestinal; *H*, hypotension; *H1*, H1 blocker; *H2*, H2 blocker; *I*, intubation; *Inh*, inhalers; *IVV*, intravenous vasopressors; *IVF*, intravenous fluid resuscitation; *P*, pulmonary; *S*, steroids.