

Clinical Communications

α -Gal on Crotalidae-polyvalent Fab antivenom (CroFab): Investigating the relevance to immediate hypersensitivity reactions

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Clinical Implications

- Crotalidae-polyvalent immune Fab [ovine] (FabAV) is a sheep-derived antivenom that contains galactose- α -1,3-galactose. The high frequency of immediate hypersensitivity reactions to FabAV may be partly explained by IgE to galactose- α -1,3-galactose, especially in areas where mammalian meat allergy is common.

Crotalidae-polyvalent immune Fab [ovine] (FabAV) is a polyvalent Fab fragment antivenom that is generated by papain digestion of Crotalidae-specific antibodies derived from hyper-immunized sheep. FabAV is approved for administration in patients being treated for snake envenomation of the Crotalidae subfamily, which includes rattlesnakes, copperheads, and cottonmouths/water moccasins.^{1,2} The frequency of acute adverse reactions is lower with FabAV than with whole immunoglobulin IgG antivenom products, but nonetheless immediate reactions (ie, occurring within 3 hours) do occur and can be severe.²⁻⁴ The package insert provides a warning for patients allergic to papain, chymopapain, papaya extracts, and/or bromelain, but the cause(s)

the syndrome of delayed anaphylaxis to mammalian meat,⁵ was present in multiple mammal-derived antivenom products.⁶ The authors postulated that α -Gal could be a possible target in IgE-mediated reactions to antivenom; however, there were no clinical cases reported, nor was Crotalidae-polyvalent immune Fab/FabAV among the products studied in that report. In 2017, Rizer et al⁷ reported an anaphylactic reaction to FabAV in a patient who was found to be sensitized to α -Gal and suggested that IgE to α -Gal may have been relevant to that reaction. Of note, α -Gal sensitization is caused by tick bites and in the United States IgE to α -Gal has a regional distribution that is most pronounced in southeastern States.⁸ Interestingly, a retrospective study of the Arizona Poison and Drug Information Center database, which services an area where neither ticks nor α -Gal cases are common, demonstrated a 1.4% rate of acute allergic reactions to FabAV.³ Because this reaction frequency is below the rate reported in other studies, it implies that there may be regional variability in reaction frequency. Thus, we hypothesized that α -Gal is present on FabAV and that IgE to α -Gal could be relevant to immediate hypersensitivity reactions to FabAV, particularly in snake bite cases managed at our hospital and other hospitals in the Southeast.

To systematically examine the frequency of immediate hypersensitivity reactions in patients receiving FabAV at the University of Virginia Health System, an institutional review board-approved retrospective analysis of all patients administered FabAV at the University of Virginia from 2011 to 2020 was conducted. In total, 72 patients received FabAV for snake bites, of which 4 experienced acute allergic reactions (5.6%) requiring treatment with epinephrine, methylprednisolone, and/or diphenhydramine (Table I). All 4 reactions involved urticaria, with 3 of these cases also involving angioedema and 1 described as anaphylaxis. Information about IgE to α -Gal was available for only 2 of the 72 subjects.

TABLE I. Characteristics of 72 patients treated with FabAV for snake envenomation

Characteristic, allergic symptoms, and α -Gal IgE status	Subjects with acute reaction	Subjects without acute reaction
N	4	68
Age (y), median (range)	21 (5-61)	36 (2-85)
Sex: female, n (%)	2 (50)	30 (44)
Vials of FabAV administered, median (range)	3 (2-8)	4 (2-16)
Hives, n (%)	4 (100)	0 (0)
Angioedema, n (%)	3 (75)	0 (0)
Anaphylaxis, n (%)	1 (25)	0 (0)
α -Gal IgE test conducted, n (%)	1 (25)	1 (1.5)
α -Gal IgE level (IU/mL)	4.5	<0.1

for the immediate reactions to FabAV is not well understood. In 2012, a meta-analysis of 11 unique studies reported an incidence rate of immediate hypersensitivity reactions to FabAV at 8%, but the range varied by study (0%-22%).⁴ A more recent nationwide registry of 373 patients found an incidence rate of 2.7%.²

In 2016, a group in Germany reported that the oligosaccharide galactose- α -1, 3-galactose (α -Gal), the relevant allergen in

We next carried out studies to determine whether α -Gal was detectable on FabAV (for methods, see this article's Online Repository at www.jaci-inpractice.org). Western blot analysis was conducted using a mouse antibody (clone M86) specific for α -Gal. The signal on FabAV was less pronounced than seen with cetuximab, a mAb known to carry α -Gal,⁵ but nonetheless α -Gal was present on FabAV (Figure 1, A). Next, we prepared

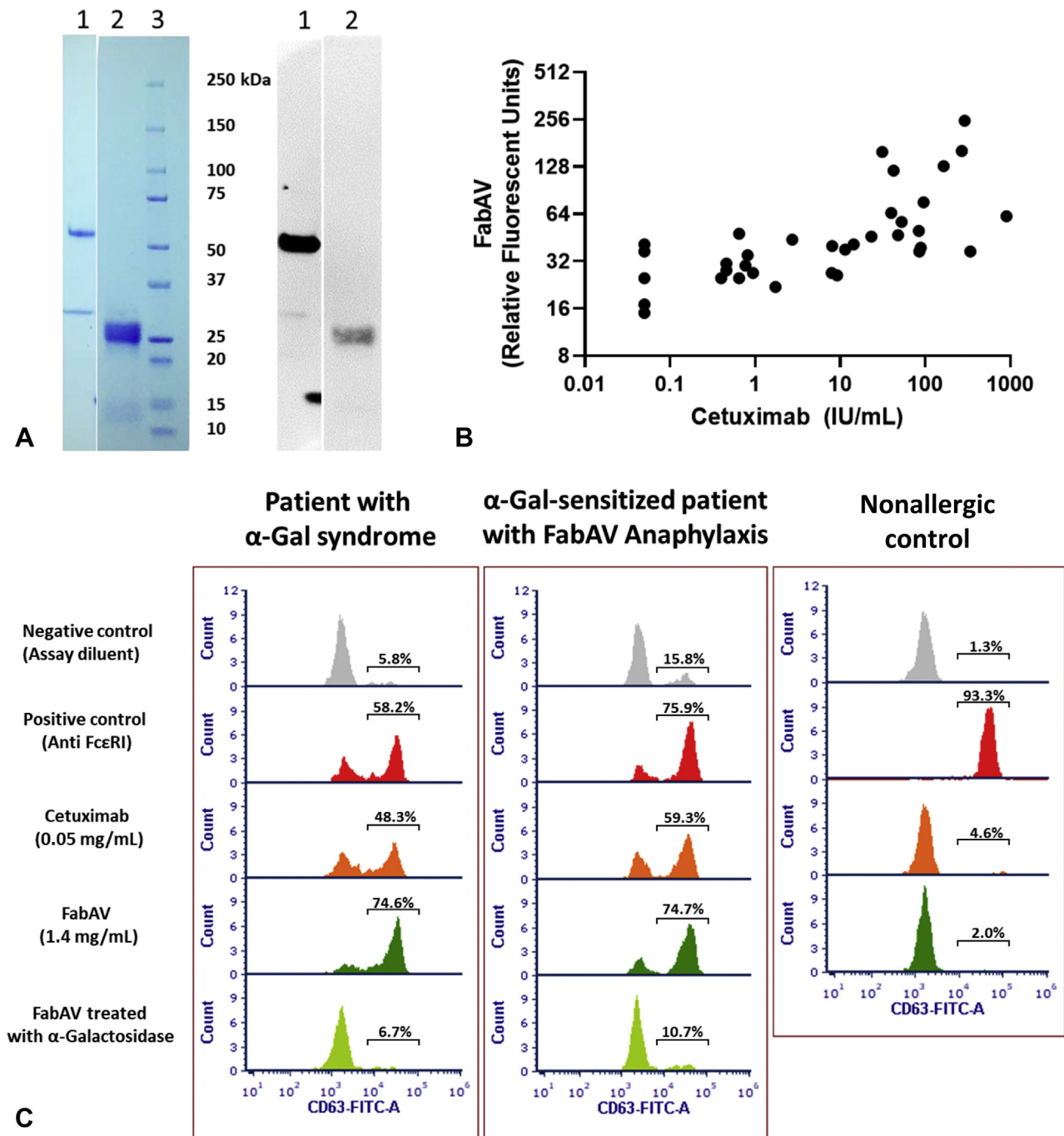


FIGURE 1. (A) SDS-PAGE and Western blot of (1) cetuximab (0.5 μ g/lane), (2) FabAV (2 μ g/lane), and (3) reference ladder. (B) IgE to FabAV (relative fluorescence units) in relation to IgE to cetuximab (ie, the α -Gal IgE assay). (C) Basophil activation test results from a subject with α -Gal syndrome (α -Gal sIgE = 5.6 IU/mL), from a subject who was α -Gal–sensitized and had FabAV-related anaphylaxis (α -Gal sIgE = 1.4 IU/mL*), and from a nonallergic control (α -Gal sIgE < 0.1 IU/mL). *The α -Gal sIgE level of this patient at the time of FabAV-related anaphylaxis was 4.5 IU/mL (see Table I).

ImmunoCAPs using FabAV on the solid phase of the assay using the biotin-streptavidin technique. IgE activity to the FabAV ImmunoCAP was determined in sera from 30 subjects with IgE to α -Gal greater than 0.35 IU/mL and 5 subjects with IgE to α -Gal less than 0.1 IU/mL who were enrolled in a previously reported institutional review board–approved study.⁹ Among

the 30 subjects with IgE to α -Gal, 8 of the sera had detectable activity to FabAV (cut-off 0.1 IU/mL), but all these were low level at less than 1 IU/mL. Using relative fluorescence units as a read-out, subjects with α -Gal sIgE levels higher than approximately 30 IU/mL showed evidence of binding to FabAV, but not subjects who had low-level or undetectable IgE to α -Gal

(Figure 1, B). The correlation between IgE to FabAV and IgE to cetuximab was strong (Spearman coefficient, $r = 0.71$; $P < 0.001$). Finally, basophil activation tests were conducted using peripheral blood from subjects with and without α -Gal syndrome (for methods, see this article's Online Repository). In accordance with the physiologic concentration that could be expected with intravenous infusion of 4 to 16 g of FabAV, these assays were performed at 1.4 mg/mL FabAV. Basophils from 2 α -Gal-sensitized subjects, but not a nonsensitized control, demonstrated basophil activation (CD63 upregulation) upon incubation with FabAV (Figure 1, C). Of note, one of these subjects was the same patient who experienced anaphylaxis when administered FabAV in the report of Rizer et al.⁷ Importantly, this *in vitro* effect was lost when FabAV was pretreated with an α -galactosidase enzyme that removes the terminal galactose of the α -Gal sugar.

Taken together, the data indicate that α -Gal is present on FabAV and has functional activity in the basophil activation test. The levels of α -Gal are clearly lower on a molar basis in FabAV than in cetuximab, but on the other hand patients are administered large amounts of antivenom intravenously. A limitation of the current investigation is that information regarding allergic reactions to FabAV was acquired from emergency provider documentation and most patients did not have information about α -Gal IgE available. In keeping with the indications for administering FabAV, many patients presented to the emergency department with systemic manifestations (eg, flushing, nausea, and vomiting) related to the toxic envenomation. Thus, some patients may have received prophylactic intravenous diphenhydramine and/or methylprednisolone treatment before FabAV administration as part of standard management.

In summary, our study demonstrates that α -Gal antigen is present on FabAV and suggests that IgE to α -Gal may contribute to immediate hypersensitivity reactions. Although unproven, this would provide a simple explanation for the finding that reaction rates observed in our hospital (5.6%) were higher than rates reported from Arizona (1.4%), an area where lone star ticks are not present and α -Gal sensitization is rare. All providers who administer FabAV need to be vigilant for hypersensitivity reactions, but our data suggest that this may be particularly true for subjects with histories of mammalian meat allergy and/or recurrent tick bites.

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METHODS

FabAV ImmunoCAP preparation

FabAV ImmunoCAPs were generated using the biotin-streptavidin technique. In brief, 1 mL of 2 mg/mL FabAV solution was reacted with 120 μ L of 2 mg/mL Enzotin solution for 4 hours. The biotinylated FabAV solution was purified in a 10,000 MWCO dialysis chamber and then 20 μ g biotinylated FabAV was conjugated to streptavidin ImmunoCAPs. Analysis was conducted using a Thermo Fisher/Phadia ImmunoCAP 250 instrument with sera from subjects from a previously described case-control study of red meat allergy. Statistical analysis was conducted using GraphPad Prism 8.0 (GraphPad Software Inc, San Diego, Calif).

Basophil activation test

The Flow CAST (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) was used for direct quantitative measurement of basophil activation. Briefly, blood was drawn into a K-EDTA tube. To 50 μ L of blood, 100 μ L of stimulation buffer containing IL-3, calcium, and heparin was added and 50 μ L of 1 of the following was subsequently added to each tube: (1) stimulation buffer (diluent) as negative control and to evaluate the background signal, (2) anti-Fc ϵ RI mAb as positive control, (3) cetuximab (final concentration of 50 μ g/mL), (4) FabAV (final concentration of 1.4 mg/mL), and (5) FabAV treated with

α -Galactosidase (final concentration of 1.4 mg/mL). Staining reagent (anti-CCR3-phycoerythrin mAbs and anti-CD63-fluorescein isothiocyanate) was added and tubes were incubated at 37°C for 15 minutes. After lysis and washing steps, cells were analyzed using Attune NxT Acoustic Focusing Cytometer (Thermo Fisher Scientific, Waltham, Mass). Alpha-galactosidase from green coffee bean (Sigma, St. Louis, Mo), which cleaves terminal α -linked galactose residues, was used to treat the FabAV; 0.1 unit of enzyme was used to treat 1 mg protein at room temperature overnight.

SDS-PAGE and Western blot

SDS-PAGE under reducing conditions was performed to evaluate the approximate molecular weight and purity of FabAV and cetuximab. Subsequently, cetuximab (0.5 μ g) and FabAV (2 μ g) were loaded onto a 4% to 20% Mini-PROTEAN Precast Gel and run at 120 V for 80 minutes using a Mini-PROTEAN Tetra Cell (Bio-Rad Laboratories, Inc, Hercules, Calif). Protein bands were visualized using the Coomassie blue staining reagent. For Western blot analysis, the protein bands were transferred onto a nitrocellulose membrane (10 V for 30 minutes) using a Trans-Blot SD Semi-Dry cell (Bio-Rad). The membrane was blocked using 1% HSA. Anti- α -Gal IgG HRP conjugate (Absolute Antibody, Oxford, UK) was used to probe for α -Gal. The images were captured using ChemiDoc XRS system (Bio-Rad). Precision Plus Protein WesternC was used as the molecular weight standard (Bio-Rad).