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Capsule Summary: A patient with alpha-gal allergy presented with anaphylaxis after receiving zoster vaccine. Subsequent testing of selected vaccines revealed the presence of alpha-gal allergen in MMR and zoster vaccines, which have in common a higher content of gelatin and content of bovine calf serum.

Key Words: galactose-alpha-1, 3-galactose, alpha-gal, anaphylaxis, vaccine, zoster, gelatin, MMR

To the Editor: In the Southeastern United States, galactose-alpha-1,3-galactose (alpha-gal) sensitivity has emerged as an etiology of red meat allergy that is causally linked to bites from the lone star tick. Alpha-gal sensitivity often presents with delayed anaphylaxis after consumption of red meat, with lesser degrees of reactivity to milk and gelatin. Gelatin and other non-primate mammalian derived products are common excipient ingredients in several vaccines, and it has been postulated that alpha-gal allergic patients might react to these vaccines.

A patient in our clinic with a documented history of red meat allergy since November 2008 required emergency department treatment and epinephrine administration upon receipt of live attenuated herpes zoster vaccine containing the Oka VZV strain in September 2014. Within minutes of vaccine administration in a local pharmacy she had a sensation of mental clouding progressing to lightheadedness, wheezing, and throat tightness and she self-administered 50 mg diphenhydramine five minutes after symptom onset. She sought emergency care 30 minutes after vaccine receipt at which point she was documented to be dyspneic, flushed, with facial, oral and uvular angioedema and bilateral conjunctival injections with stable vital signs and blood pressure of 149/83, without documented wheezing on pulmonary examination. She was placed on oxygen and
administered an additional 25mg of diphenhydramine, 8mg of intramuscular
dexamethasone, 20mg of famotidine, nebulized albuterol and 0.3mg of intramuscular
epinephrine for her respiratory distress, angioedema, and cutaneous signs. Her
symptoms resolved within 20-30 minutes and she was discharged uneventfully after 3
hours observation.

She originally presented to our clinic in 2009 at age 63 with a history of recurrent delayed
anaphylaxis, occurring 4-6 hours after eating, and was evaluated for food allergies. At
that time, laboratory evaluation in our clinic showed elevated blood specific IgE (sIgE) to
beef = 10.5 kU/L, pork = 10.4kU/L, and cow’s milk = 2.90kU/L (reference for all <0.35
kU/L). Other food IgEs were within normal limits, as was serum tryptase. She reported
that eating any and all mammalian meat would trigger her symptoms. She also reported
delayed abdominal symptoms, malaise, and diarrhea with consumption of dairy products.
She lived in a rural area, and frequently found lone star ticks embedded in her skin.

One month after her episode of anaphylaxis following vaccination in 2014, she was tested
for alpha-gal allergy, with galactose-alpha-1,3-galactose sIgE = 32.5 kU/L, beef sIgE =
23.1 kU/L, lamb/mutton sIgE = 12.2kU/L, and pork sIgE = 17.1 kU/L. She was
subsequently tested in 2015 for allergy to gelatin, with porcine gelatin sIgE = 1.84kU/L,
and bovine gelatin sIgE = 0.15kU/L (reference range for all sIgE tests <0.35kU/L).

We reviewed publicly available data from a searchable version of the Vaccine Adverse
Event Reporting System (VAERS) database using search terms of severe adverse events
occurring on the same day of vaccine administration of the Oka VZV strain. Out of 202 reported events, we encountered 14 cases of adverse reaction to zoster vaccine consistent with anaphylaxis. 5/14 (36%) of these potential cases of anaphylaxis had a known associated beef, pork, gelatin, or alpha-gal allergy, and 4 of those 5 cases were reported as taking place in the Southeast United States (Online Table).

We next proceeded to identification of five candidate vaccines that might contain alpha-gal antigen due to content of bovine or porcine derived products.2,3 (Table I)

We then evaluated if sera from alpha-gal allergic patients would interact with components of the candidate vaccines. To evaluate, we performed a direct biotinylation of each of the vaccines in full prescribed dose, after which protein concentration was determined and 5µg of biotinylated antigen was added to each streptavidin ImmunoCAP, in two identical trials. Forty microliters of undiluted serum from our index patient along with serum from three additional subjects with alpha-gal allergy was used in each sIgE assay to assess for IgE binding to the vaccines or gelatin (commercially available ImmunoCAP assay c74), similar to previously published methods.1,7 Serum from the same subjects was also pre-incubated with 50µL of bovine thyroglobulin (BT), a source of alpha-gal antigen, coupled to sepharose bead slurry to deplete alpha-gal sIgE. Assays for binding to biotinylated vaccines were then repeated in two trials to determine whether binding decreased following pre-incubation with bovine thyroglobulin, which suggests that any observed binding to vaccines was actually for alpha-gal. This was performed using previously published methods1.
The largest direct binding response that could be removed by the presence of bovine thyroglobulin was seen in the index patient to MMR and zoster vaccine (0.96-1.31 IU/ml, 
\textit{Table IIA}). There was also low positive binding (values were 0.27 - 0.45IU/ml) for MMR and zoster vaccine in sera from the subjects A and B that could be removed by the presence of bovine thyroglobulin, though sera from subject C did not demonstrate binding to any of the candidate vaccines. The direct binding "vaccine caps" method suggests the presence of an epitope in MMR and zoster vaccine that is recognized by alpha-gal IgE in sera from both the index patient and alpha-gal allergic subjects A & B. (\textit{Table IIA})

We next measured the baseline alpha-gal IgE titers in sera from our index patient and the same three additional subjects. To ascertain the presence of vaccine epitopes that would bind/remove alpha-gal specific IgE in excess of that expected for gelatin alone, we incubated sera samples from the index patient and the three alpha-gal positive subjects overnight, separately, with 100µg from each of the five vaccines, bovine gelatin, and porcine gelatin and re-measured alpha-gal IgE titers. (\textit{Table IIB}).

Incubation of the sera samples overnight showed partial depletion of the alpha-gal IgE response in sera from all four subjects when it was pre-incubated with zoster vaccine and MMR, greater than that for gelatin alone. There were also partial depletions observed in response to the yellow fever vaccine in subjects B and C. While we did note some expected variability in epitope binding to alpha-gal IgE, both MMR and zoster vaccines
consistently removed a portion of alpha-gal sIgE response upon re-assay. We did not
observe any evidence of epitope binding to alpha-gal IgE binding with either version of
TDaP vaccine.

To our knowledge, this is the first report of vaccine induced anaphylaxis associated with
alpha-gal allergy. We are somewhat limited in our claim of complete causality by the
presence of low level IgE antibodies to porcine gelatin in our patient. Nevertheless, the
presence of antigen binding directly to alpha-gal IgE found in patient sera and depletion
of alpha-gal sIgE in overnight incubation with both MMR and zoster vaccine would
suggest that either their increased gelatin content or some other shared element in the
manufacturing process of these two vaccines increases the likelihood of alpha-gal
contamination. Both MMR and zoster vaccine use bovine calf serum during their
production, and hypothetically additional alpha-gal antigen could be acquired at this step.
The lesser reactivity to yellow fever vaccine (which has a lower gelatin content), and
absent reactivity to two different TDaP vaccines, which contain other bovine derived
products but not gelatin, is also helpful, as patients with this allergy would be unlikely to
react to these vaccines. There are other vaccines that contain mammalian products, but
our findings would suggest that alpha-gal content is highest in MMR and zoster vaccine.

Alpha-gal allergy is an increasingly prevalent hypersensitivity syndrome in the Southeast
US, as well as other parts of the world. Clinicians who manage it should be made aware
of a risk of anaphylaxis to higher content gelatin containing vaccines such as MMR and
zoster vaccine, especially because of their parenteral delivery. While anaphylaxis from
zoster vaccine appears to be a low probability event,\textsuperscript{8} it has significant public health implications, and there is a need to determine on a population level how often patients who have anaphylaxis to higher gelatin content vaccines such as MMR and Zoster vaccine have an underlying alpha-gal allergy.

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References:


Table I: Reported Gelatin or Mammal Derived Product Content of Selected Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Reported Gelatin Content of Vaccine</th>
<th>Reported Type of Gelatin or Animal Derived Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoster (Merck)</td>
<td>15,580 µg per 0.65 mL dose</td>
<td>Porcine Gelatin, Bovine Calf Serum</td>
</tr>
<tr>
<td>Measles, Mumps and Rubella (MMR) (Merck)</td>
<td>14,500 µg per 0.5 mL dose</td>
<td>Bovine Gelatin, Bovine Calf Serum</td>
</tr>
<tr>
<td>Yellow Fever (Sanofi Pasteur)</td>
<td>7,500 µg per 0.5 mL dose</td>
<td>Gelatin, type not reported</td>
</tr>
<tr>
<td>Tetanus, Diptheria and acellular Pertussis (TDaP) (GSK)</td>
<td>None</td>
<td>Bovine Casein, Bovine Extract</td>
</tr>
<tr>
<td>Tetanus, Diptheria and acellular Pertussis (TDaP) (Sanofi Pasteur)</td>
<td>None</td>
<td>Bovine Casamino Acids</td>
</tr>
</tbody>
</table>
Table II: Serologic Assays for Alpha-gal in Selected Vaccines

IIA: IgE binding (kU/ml) to biotinylated vaccines assayed with alpha-gal positive sera from three subjects, with and without bovine thyroglobulin (BT) to deplete alpha gal IgE

<table>
<thead>
<tr>
<th></th>
<th>TdaP (Sanofi)</th>
<th>TdaP (GSK)</th>
<th>MMR</th>
<th>Yellow Fever</th>
<th>Zoster</th>
<th>Gelatin Immunocap c74</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial</strong> 1</td>
<td>Index Patient</td>
<td>0.11 &lt;0.1</td>
<td>&lt;0.1</td>
<td>1.31</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>w/ BT Beads</td>
<td></td>
<td>0.11 &lt;0.1</td>
<td>&lt;0.1</td>
<td>0.12</td>
<td>0.13</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>Subject A</td>
<td>0.10</td>
<td>0.27</td>
<td>0.37</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>w/ BT Beads</td>
<td></td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.39</td>
<td>0.40</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>Subject B</td>
<td>1.28</td>
<td>0.27</td>
<td>0.37</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>w/ BT Beads</td>
<td></td>
<td>1.06</td>
<td>0.27</td>
<td>0.39</td>
<td>0.40</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>Subject C</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.37</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>w/ BT Beads</td>
<td></td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.39</td>
<td>0.40</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

IIB: Serum alpha-gal IgE (kU/ml) levels at baseline and after overnight incubation with vaccines and gelatins

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>TdaP (Sanofi)</th>
<th>TdaP (GSK)</th>
<th>MMR</th>
<th>Yellow Fever</th>
<th>Zoster</th>
<th>Porcine Gelatin</th>
<th>Bovine Gelatin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index Patient</strong></td>
<td>58.3</td>
<td>55.9</td>
<td>57.8</td>
<td>31.2</td>
<td>56.9</td>
<td>33.1</td>
<td>54.7</td>
<td>56.6</td>
</tr>
<tr>
<td>Subject</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>----------</td>
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<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Subject A</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.96</td>
<td>&gt;100</td>
<td>91.4</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Subject B</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>71.5</td>
<td>87.6</td>
<td>83.3</td>
<td>94.6</td>
<td>97.3</td>
</tr>
<tr>
<td>Subject C</td>
<td>84.4</td>
<td>72.6</td>
<td>86.9</td>
<td>60.7</td>
<td>75.7</td>
<td>56</td>
<td>82.8</td>
<td>81</td>
</tr>
</tbody>
</table>

Table IIA: BT= Bovine Thyroglobulin. All values are in units of kU/mL.

Table IIB: All values in units of kU/mL. Baseline values are the patient’s serum alpha-gal IgE values prior to overnight incubation.
Online Supplement Table I: Reported cases* of anaphylaxis associated with the live attenuated Oka strain zoster vaccine in patients with coexisting beef, pork, gelatin, or alpha-gal allergy

<table>
<thead>
<tr>
<th>Case (Year)</th>
<th>Reaction</th>
<th>Associated Allergy</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>76 Year Old Female (2007)</td>
<td>20 minutes after vaccine receipt at a pharmacy developed anaphylaxis, admitted for overnight hospital stay. Description of the event is limited. Diagnosed as anaphylaxis by treating physician.</td>
<td>Positive specific IgE to Beef, pork, bovine gelatin, porcine gelatin, titters not reported. Reported negative alpha gal sIgE.</td>
<td>Maryland</td>
</tr>
<tr>
<td>70 Year Old Female (2008)</td>
<td>Developed life threatening hypotension, shortness of breath, angioedema shortly after vaccine receipt, with overnight hospital stay, treatment not reported. Diagnosed as anaphylaxis by treating physician.</td>
<td>Clinical report of preexisting beef, pork, dairy allergy. sIgE testing and titters not reported. Alpha-gal status and sIgE testing not reported.</td>
<td>Arkansas</td>
</tr>
<tr>
<td>70 Year Old Female (2009)</td>
<td>Patient developed immediate severe allergic reaction upon receipt of vaccine in a pharmacy. Was taken to the emergency room, received epinephrine for treatment of hypotension. Diagnosed as anaphylaxis by treating physician.</td>
<td>Report of positive testing to gelatin, not reported if testing was skin or sIgE testing. Beef, pork, alpha-gal allergy status, sIgE testing and titters not reported.</td>
<td>Location not reported</td>
</tr>
<tr>
<td>61 Year Old Male (2013)</td>
<td>Patient developed disseminated rash shortly after vaccine receipt at a pharmacy. Returned to pharmacy and was given diphenhydramine 25mg. After 10 minutes of observation, patient developed dizziness and syncope. IM epinephrine was administered, and patient was transferred by EMS to hospital. Vital signs not reported.</td>
<td>Clinical report of preexisting beef and pork allergy. Results of sIgE testing and titters not reported.</td>
<td>Virginia</td>
</tr>
<tr>
<td>63 Year Old</td>
<td>5 minutes after vaccine receipt, patient developed generalized</td>
<td>Beef, pork, alpha-gal allergy reported.</td>
<td>Maryland</td>
</tr>
</tbody>
</table>
Male (2015)

itching followed by generalized hives and throat discomfort, resolution with diphenhydramine in the emergency department. Diagnosed as anaphylaxis by treating physician.

Reported sIgE porcine gelatin=0.58 kU/L, bovine gelatin <0.10 kU/L, alpha-gal=3.70 kU/L

*Case Definition:

1) a provider diagnosis of anaphylaxis or 2) use of published criteria for defining cases of vaccine-associated anaphylaxis when provider diagnosis was not reported.\(^1\)

**Supplemental References:**

A previous report indicates an increased likelihood of gelatin hypersensitivity when alpha-gal allergy is present.\(^2\) Parenteral route of administration was also reported to increase the likelihood of allergic response, and alpha-gal was detected in gelatin colloids.\(^2\) In studies of Japanese Encephalitis Virus Vaccine, reductions in gelatin content reduced the likelihood of anaphylaxis.\(^3\) In reviewing the original randomized controlled trial of zoster vaccine, there was one episode of an anaphylactic event in a patient who received the gelatin placebo.\(^4\)

An important question is whether there is a reactivity threshold that can be tested to predetermine the likelihood of anaphylaxis to zoster vaccine in patients with alpha-gal allergy. Our patient had high titer alpha-gal IgE antibody and anaphylaxis. In contrast, administration of zoster vaccine without any adverse reaction has been reported in an alpha-gal allergic patient with a lower titer of alpha-gal IgE (alpha-gal sIgE = 6.0 kU/L).\(^5\)