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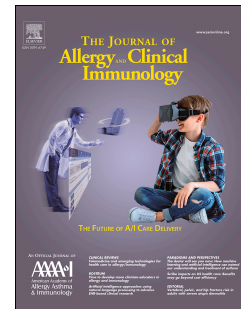
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Of Mice and Men, MMXXI: Anaphylaxis

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When Portier and Richet demonstrated in 1902 that repeated injections of a foreign substance could lead to increasingly severe reactions up to and including rapid death, they used a jellyfish toxin to immunize dogs and the Greek words ἀνύ-φύλαξις or non-protection. Thus there is no doubt that animal models can provide important information about these reactions and that they can be very severe. The authors of the article in the current issue of the journal present a detailed investigation of the factors influencing the severity of allergic reactions in two strains of mice. The data presented provides compelling evidence that the differences in reactions following oral exposure are not due to genetic differences in TLR4 or DOCK8, which had been proposed, and do not reflect differences in either IgE responses to peanut or in the microbiome between C3H/HeJ and C57BL/6 mice. On the other hand, they demonstrate significantly increased levels of gut absorption in the C3H/HeJ mice that correlate with their reactions to oral challenge (1).

Speaking as known agnostics about the use of mouse models to study allergic disease, this report creates an opportunity to consider what aspects of anaphylaxis in mice can provide useful or even important information about the phenomenon in humans. Several criticisms of animal models of anaphylaxis are well known. In mice, the primary marker used to assess reactions is a fall in body temperature, which has not been recognized as a useful marker of reactions in humans. In most mouse studies, all the animals are fully inbred and essentially identical genetically; thus demonstrating differences between two or more strains is not comparable to reporting results on 260 outbred *H. sapiens* patients (2). On the other hand, there are many questions that are difficult to approach in patients or normal subjects and some interesting experiments that are truly impossible. We would like to focus on two elements that are a challenge in our own current work. Firstly, it would be ill-advised to propose carrying out experimental studies which included immunizing naïve human individuals, if the sensitization could lead to a potentially dangerous outcome. Secondly, some reactions to food, even in subjects with high titer IgE take three or more hours to occur and nonetheless can be severe. Carrying out food challenges to investigate anaphylaxis especially when symptom onset can be delayed as much as 4 hours is a challenge for the investigators (3) (Fig 1).

Experiments in naïve mice have established that immunization through mildly abraded or tape stripped skin can induce serum IgE antibodies, leading to sensitization of an unexposed organ such as the lungs (4). This has provided extensive background for understanding the relevance of skin exposure to the onset of peanut allergy (5). In addition, the mice experiments have provided

a solid background for the investigation of tick bites as a cause of sensitization to cetuximab and red meat (6, 7). A recent series of experiments used tick saliva extracts to immunize alpha-gal knock out (AGKO) mice (7). Those studies produced high titers of IgE antibodies as well as marked increases in total IgE. Furthermore, the mice reacted rapidly to oral challenge with pork kidney allergens. Another problem is to understand why most clinical reactions to mammalian meat in individuals with IgE to galactose alpha-1,3-galactose are delayed (2). The current manuscript from Dr. Eisenbarth's group reports measurements of ovalbumin (OVA) protein entering the circulation within 30 minutes after gavage. In keeping with the stronger allergic reactions, they also document greater quantities of OVA in the circulation of C3H/HeJ mice than in other strains including C57BL/6 (1). Interestingly, the recent studies on challenges of alpha-gal sensitized mice using homogenized pork kidney reported a rapid fall in temperature in the AGKO mice which are on a C57BL/6 background (Fig 1) (7). The results of that study in a sense challenge the possibility that the C57BL/6 mice have a general difference which decreases gut absorption of foreign antigens. On the other hand, these observations in two different models of gut absorption using mice bring to light the difficulty of studying delayed absorption in humans or mouse studies. This is particularly relevant to food related exercise induced anaphylaxis (EIA) and delayed reactions to red meat.

In patients with food dependent EIA, the response depends on exposure to a specific allergen followed by exercise up to 3 hours later (Fig 1) (8). The interesting point here is that the most common allergen source is wheat and the relevant protein component has been shown to be omega-5-gliadin or Tri a 19. Furthermore there is a well-known form of this condition where sensitization to the relevant protein occurs from a face cream containing wheat applied to the skin (9). There is an interesting model of exercise in a mouse strain where there is an associated increase in absorption of a relevant allergen (10). However, it is difficult to work out how it would be possible to explain the relevance of a specific wheat allergen.

A significant problem with animal models of allergic disease arises because the quantities of allergen are higher than natural exposure of patients experiencing symptoms. Clearly, there is a very wide range of sensitivity among allergic individuals such that some peanut allergic individuals react to quantities of the allergen that are difficult to measure. With delayed reactions to red meat, the patients may eat or be challenged orally with large quantities of the antigen in the form of beef, or pork kidney. However, the quantity of the allergen that is present in the

76 circulation at the time of reactions may be only a tiny fraction of the quantity eaten. In one series
77 of experiments, in the present publication, the authors report that almost 50% of the mice either
78 died or had a symptom score of 4 and were sacrificed (1). Clearly that number implies a high
79 dose since the mortality rate in naturally occurring episodes of anaphylaxis in allergic patients is
80 generally considered to be <1%. So the question has to be would it be possible to study delayed
81 in time effects using lower doses of allergens in the challenges.

82 We recognize that the current manuscript provides important information about the basis for the
83 severity of anaphylaxis to peanut in two strains of mice. However, in humans there is a high
84 degree of inter- and intra-personal variation in the “threshold doses” of allergen needed to elicit a
85 reaction and in the severity of symptoms observed. That is, in human subjects allergic reactions
86 to a known allergen exposure are highly variable. There are several well-recognized factors that
87 can influence anaphylactic responses, including alcohol, aspirin, NSAIDs or exercise. However,
88 in addition, there must be other factors that make human responses difficult to predict. Thus,
89 there are major differences in both the timing and the severity of human allergic reactions to food
90 and it is always important to keep in mind the problems that exist in investigating the
91 mechanisms of anaphylaxis either in patients or in sensitized mice.

References

1. Gertie JA, Zhang B, Liu EG, Hoyt LR, Yin X, Xu L, et al. Oral anaphylaxis to peanut in a mouse model is associated with gut permeability but not with Tlr4 or Dock8 mutations. *J Allergy Clin Immunol*. 2021.
2. Wilson JM, Schuyler AJ, Workman L, Gupta M, James HR, Posthumus J, et al. Investigation into the alpha-Gal Syndrome: Characteristics of 261 Children and Adults Reporting Red Meat Allergy. *J Allergy Clin Immunol Pract*. 2019;7(7):2348-58 e4.
3. Commins SP, James HR, Stevens W, Pochan SL, Land MH, King C, et al. Delayed clinical and ex vivo response to mammalian meat in patients with IgE to galactose-alpha-1,3-galactose. *J Allergy Clin Immunol*. 2014;134(1):108-15.
4. Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest*. 1998;101(8):1614-22.
5. Brough HA, Nadeau KC, Sindher SB, Alkotob SS, Chan S, Bahnson HT, et al. Epicutaneous sensitization in the development of food allergy: What is the evidence and how can this be prevented? *Allergy*. 2020;75(9):2185-205.
6. Chandrasekhar JL, Cox KM, Loo WM, Qiao H, Tung KS, Erickson LD. Cutaneous Exposure to Clinically Relevant Lone Star Ticks Promotes IgE Production and Hypersensitivity through CD4+ T Cell–and MyD88-Dependent Pathways in Mice. *The Journal of Immunology*. 2019;203(4):813-24.
7. Choudhary SK, Karim S, Iweala OI, Choudhary S, Crispell G, Sharma SR, et al. Tick salivary gland extract induces alpha-gal syndrome in alpha-gal deficient mice. *Immun Inflamm Dis*. 2021;9(3):984-90.
8. Christensen MJ, Eller E, Mortz CG, Brockow K, Bindslev-Jensen C. Wheat-dependent cofactor-augmented anaphylaxis: a prospective study of exercise, aspirin, and alcohol efficacy as cofactors. *The Journal of Allergy and Clinical Immunology: In Practice*. 2019;7(1):114-21.
9. Yokooji T, Kurihara S, Murakami T, Chinuki Y, Takahashi H, Morita E, et al. Characterization of causative allergens for wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat proteins in facial soap. *Allergol Int*. 2013;62(4):435-45.
10. Yano H, Kato Y, Matsuda T. Acute exercise induces gastrointestinal leakage of allergen in lysozyme-sensitized mice. *Eur J Appl Physiol*. 2002;87(4):358-64.

127 **Figure 1.** Sensitization and challenges related to anaphylaxis in mice and human

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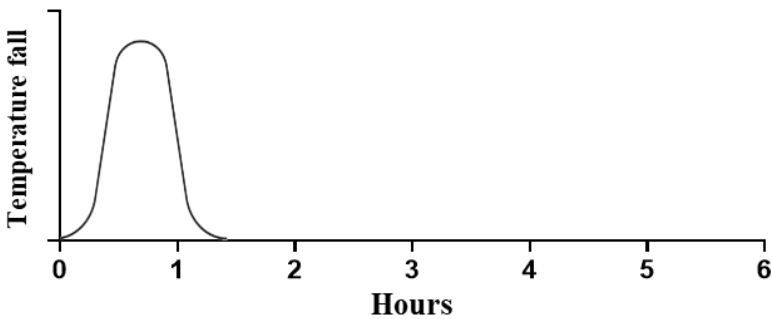
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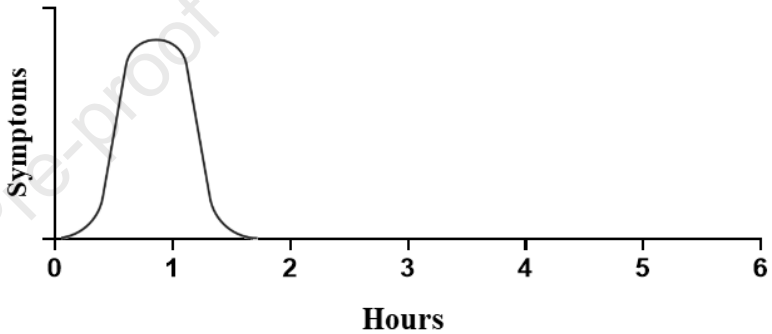
Figure 1.

% Response to oral exposure

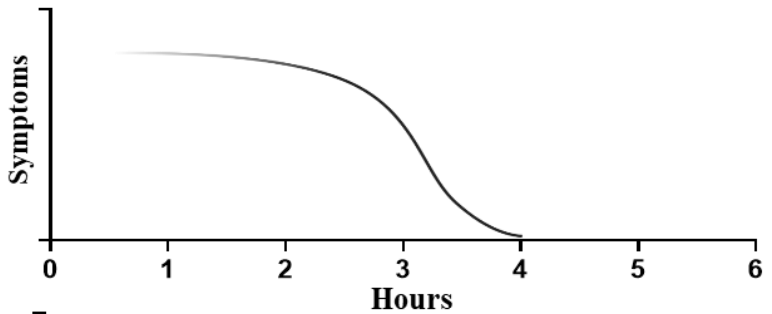
Mouse	Sensitization
<u>Immediate</u>	
Ovalbumin – C3H/HeJ	Intraperitoneal
Peanut – C3H/HeJ	injection or
Alpha-Gal – C57BL/6	oral



Human	Sensitization
I. <u>Immediate</u>	
Peanut (Ara h 2)	Skin or oral
Venom	Skin



II. <u>Food-related</u>	
<u>exercise induced</u>	
Wheat (Tri a 19 or omega-5-gliadin)	Skin or oral
Shrimp	Skin



III. <u>Delayed (in patients</u>	
<u>with alpha-gal syndrome)</u>	
Alpha-gal	Tick bites

