



Thresholds of allergenic proteins in foods

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Abstract

Threshold doses or Estimated Eliciting Doses (EEDs) represent an important new field of research in food allergy. Clinicians and regulators have embraced some toxicological concepts such as LOAEL and NOAEL and applied them to an area of significant clinical uncertainty and interest. The impact of intrinsic human factors (e.g., asthma and exercise) and extrinsic event factors (e.g., season, location and especially dose of allergen) on a future allergic reaction in the community needs to be considered carefully when interpreting results of clinical and research low-dose food challenges. The ongoing cooperation of food allergy research groups in medicine, food science and government will surely deliver results of the highest importance to the wider communities of allergology, food science and technology and the increasing number of allergic consumers.

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Introduction

Food allergy, as considered in this article, refers to immunologically-mediated reactions which require the presence of allergen specific IgE antibodies (Johansson et al., 2003). Food allergy affects up to 2% of the general population (Zuberbier et al., 2004). A double-blind, placebo-controlled food challenge (DBPCFC) is considered the gold standard diagnostic test (Sampson, 2004; Bindslev-Jensen et al., 2004).

From a toxicological point of view allergens need to be considered as risks that must be managed or eliminated. A lot of industrial and media attention has focussed on the risk to allergic subjects of accidental and hence, undeclared, inclusion of an allergen in a compound food. However it is a fact that most fatal outcomes arise from accidental ingestion

of large amounts of allergen, usually in its known form or in a food in which its presence could be suspected (Pumphrey, 2000; Bock et al., 2001).

The total elimination of risk is unlikely to be an achievable medical goal in the care of food allergic subjects, in societies where most food is produced and prepared on an industrial scale. Minimisation of risk may come at high costs to the subjects and their family, in the form of lifestyle and social restrictions (Primeau et al., 2000; Avery et al., 2003). From an industrial point of view such a goal of zero risk may place "...an unachievable burden on the food industry" (Taylor et al., 2002, 2004). Therefore, the cornerstone of clinical advice to most subjects with food allergy is to try to avoid the allergen, as best as possible, rather than make a judgement about how much of the allergen might be present. However, some people may know from their own experience that they can tolerate small amounts and may only need to avoid potential exposure to large amounts of allergen. Industry wants to know if there is an amount of protein that is unlikely to cause serious adverse effects in the majority of the population at risk.

In some cases, different forms of allergen have different levels of in vitro allergenicity as shown by studies of IgE

Abbreviations: ACE, Angiotensin converting enzyme; DBPCFC, Double-blind, placebo-controlled food challenge; EED, Estimated eliciting dose; IRBs, Institutional Review Boards; LOAEL, Lowest observed adverse event level; NOAEL, No observed adverse event level; OAS, Oral allergy syndrome; SPT, Skin prick test.

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binding. This is reflected in clinical practice by the fact that subjects who cannot eat unadulterated egg or raw egg often tolerate cooked egg (Sampson, 2004). Conversely, cooking can increase the allergenicity of a food (Maleki et al., 2000). Different forms of cooking may affect allergenicity of a single food in different ways, possibly by altering epitope availability or configuration (Beyer et al., 2001).

It is human nature to repeatedly assess risk ad hoc and all allergists are aware of patients who tailor their allergen avoidance to the level of risk they think a particular food represents. Many food allergic subjects feel powerless to do anything else as “may contain allergen” labels become more common, even appearing on packets of foods where there appears to be little obvious risk. It is clear that cooperative approaches are needed, involving all the stakeholders: allergic people, allergists, industry and government.

The concept of a threshold dose or Estimated Eliciting Dose (EED) has arisen from attempts by industry and clinicians to assess the lowest amount of a food allergen that subjects can or cannot tolerate (Taylor et al., 2002). The basic concepts are that if a Lowest Observed Adverse Effect Level and No Observed Adverse Effect Level (LOAEL and NOAEL, as defined) could be identified then industrial processes could be targeted to achieve these levels.

It is medically recognised that there are wide variations in the amount of a food allergen to which a population of food allergic people reacts. For instance, 25% of DBPCFCs with milk and 11% of DBPCFCs with egg performed in one centre elicited a reaction with the first dose of 100 mg (Sicherer et al., 2000). The remaining positive challenges were therefore in subjects who tolerated at least those amounts of milk and egg. Similarly, 5% of peanut allergic subjects reacted to a first dose of 1 mg (Rancé et al., 2002). In contrast, Wensing et al., found that none of the 26 adults in a DBPCFC study reacted to the first dose of peanut, 30 µg (Wensing et al., 2002).

An allergic reaction can be considered as an interaction of host factors and event factors (Table 1 and Fig. 1).

Table 1
Interpretation of low dose challenge studies to establish estimated eliciting doses (EEDs)

Factor	Solution/interpretation ^a
Inter individual variation	Test enough subjects (29)
No reaction to first dose (will whole challenge be negative?)	Threshold/EED not exceeded ? NOAEL established Ensure study population likely to react (recent convincing reaction, adequately high SPT/specific IgE)
Reaction to first dose	Threshold/EED exceeded ? LOAEL established Need to use lower doses to establish NOAEL and to confirm this LOAEL

^a See Refs. Taylor et al., 2002, 2004.

Host factors

Clinical history

Community reactions, i.e., those experienced outside the setting of a formal challenge can be difficult to assess retrospectively. Children cannot usually recall events occurring under the age of 3 years. With appropriate supervision, many years may pass before another exposure to the allergen happens (in the case of some foods reactivity may have disappeared long before medical re-evaluation). Older children, adolescents and adults may remember the details of recent reactions well, but often rely on parental reports of reactions that happened earlier in childhood. Wheezing and pallor are medically well-recognised signs of severe or anaphylactic reactions to allergens but parents may not appreciate their significance, especially during the first reaction their child suffers or if their child already has asthma. Conversely, parents may over-report symptoms after reflecting upon them after the event. As medical practice is moving away from routine diagnostic challenges (Bindslev-Jensen et al., 2004; Sampson, 2004), the reliability of the clinical history is becoming even more important.

Volunteer self-selection

Volunteers for research studies are special people and subjects who volunteer for a research-motivated food challenge must by definition be only a subset of the population at risk. Patients having clinically motivated challenges will have been selected for challenge on the basis of their clinical history and results of IgE assays (3). The criteria for inclusion in challenge-based research studies may specifically exclude those with a previous life-threatening reaction. Even if such a reaction is not identified some Institutional Review Boards (IRBs) may exclude subjects whose specific IgE levels predict reactivity (Taylor et al., 2002). To date, only one study has found any association between clinical severity and EED (Wensing et al., 2002). Interestingly, this group has since reported that within individuals EEDs do not vary much over time (van der Zee et al., 2003). This implies that variation in reactions in the community may be due to changes in dose, though it remains likely that other factors are active too.

Health status

Most volunteers for medical studies are in good general health. The presence of hypertension and ischaemic heart disease may render subjects less resistant to the vasodilatation that is so characteristic of an allergic reaction. Such subjects are usually excluded from challenge studies. The use of some medications may also make a volunteer ineligible for challenge studies. Good examples of these are

Reaction severity = (Host factors X Event factors) X ?

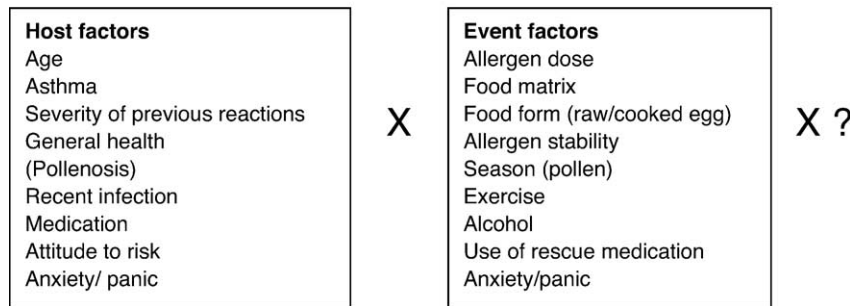


Fig. 1. Severity of an allergic reaction is a product of host factors, event factors and unpredictable/unidentifiable factors.

Angiotensin Converting Enzyme (ACE) inhibitors, Beta blockers and non-steroidal anti-inflammatory medicines.

Asthma

Asthma appears critical in fatal and severe reactions to peanut. It has been shown in several small case series that asthma is common in subjects who die of food allergic reactions (Bock et al., 2001; Sampson et al., 1992). It also appears that food allergy is much more common (odds ratio of >5) in severe asthmatics than in people with mild-moderate asthma (Roberts et al., 2003). Subjects who volunteer for food challenge studies will be excluded, for their own safety, if their asthma is not mild or is not managed optimally (Bock et al., 1988, Sicherer, 1999).

Other allergic conditions

Some subjects have pollen-related oral allergy syndrome (OAS) and may only react to the relevant foods in their relevant pollen season, when their already active rhinoconjunctivitis and asthma may increase their symptoms. Similarly, OAS-related fruits only cause problems if eaten fresh, as oxidation and cooking may destroy the labile allergens implicated.

Event factors

Dose of allergen

The key event factor is dose of allergen. It is widely considered that a dose–severity relationship exists and that subjects who react to very low doses are those most at risk of a severe outcome: a bigger dose would cause a worse reaction. A dose–response curve of this nature has never been established. A hypothetical model is shown in Fig. 2.

In formal challenges, subjects do not proceed after a reaction has been elicited (this dose is the EED) and the higher areas of the curve are not explored. In real life, it is common for people to taste tiny amounts of food to see

if they will react. If they do not experience symptoms then they proceed with the meal. This implies that at the lower end of the curve, such a dose–severity relationship may exist.

However, a counter argument exists in that low dose reactors may have better early warnings that allow them to successfully identify the food hazard and stop eating. People without this low dose reactivity may therefore consume large amounts of an allergen before detecting it.

It will never be ethically permissible to examine the dose–response curve completely in a human individual in vivo. It is also unlikely that EEDs will ever be accurately established from clinical histories and post hoc estimation of exposure because so many other variables are in play. Therefore, all must recognise that even if EEDs are established in formal studies, they may not translate to concrete protection for all vulnerable subjects in the community.

Delivery of allergen

The delivery of allergen in different matrices may profoundly affect the recognition of allergen. Experimentally, chocolate specifically impairs protein detection in vitro

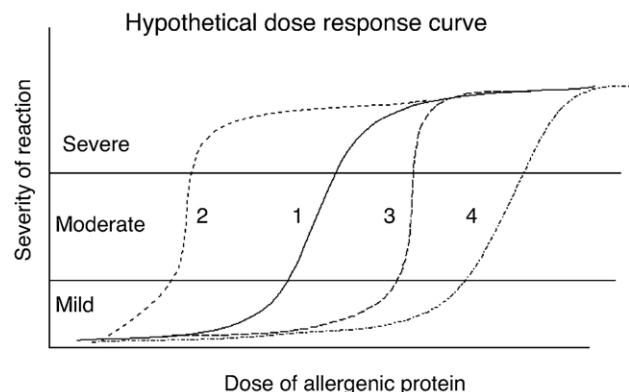


Fig. 2. A hypothetical doseresponse curve showing possible doseresponse curves under different. 1 "Normal" dose response curve. 2 Curve shifted to left, slope changed: ?unstable asthma/alcohol/exercise. 3 Curve shifted to right, slope changed: ?food matrix/antihistamine medication. 4 Curve shifted to right, same slope: ?currently unidentified factors.

(Pomes et al., 2004). Clinically, this may account, in part, for the relatively increased frequency of fatal outcome after compound foods compared to reactions after exposure to unadulterated allergen (Bock et al., 2001; Furlong et al., 2001). We have recently confirmed this matrix effect in vivo (Grimshaw et al., 2003). The fat content of a food has been shown both to delay the onset of taste perception and to reduce its intensity, particularly when the flavour molecule is lipophilic (Brauss et al., 1999). This is thought to be because lipophilic flavours are released more slowly from oils than from water (Plug and Haring, 1994).

Adjuvants to an allergic reaction

Most severe allergic reactions occur outside the home, often to food prepared by a person with little awareness of the individual's allergies. Nonetheless, on many occasions the allergic subject has contributed to his or her own downfall (literally) by failing to communicate adequately with the food provider (Furlong et al., 2001). In addition, alcohol consumption is a feature of eating outside the home and it can be inferred from case series that alcohol has impaired the judgement of the food allergic person (Yunginger et al., 1988).

Usefulness of EEDs as a read-out for therapeutic interventions

For any individual, allergic reactions to foods are usually sporadic and irregular, but inevitable events. It is not therefore fruitful to measure the success of any therapy as a reduction in number of accidental exposures and reactions. Current optimal medical management is crude, relying on minimisation of exposure and empowerment of patients to self-treat with rescue medication. There is a new era of therapy of food allergy imminent (Sampson, 2004) and EEDs clearly have a key role in measuring success of treatment. EEDs have already been used as such in the first report of anti-IgE treatment of peanut allergy (Leung et al., 2003) and in a study of changes in cross-reactivity to apple after immunotherapy for birch pollen allergy (Bolhaar et al., 2004). When trials of food allergen specific immunotherapy take place, challenge-based assessment of subjects using EEDs will have a key role.

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