In vitro tests and experimental animal models for investigation of the allergenic potential of biotechnology-derived proteins

Attila Bacsi, PhD

**Institute of Immunology,
Medical and Health Science Center,
University of Debrecen,
Hungary**

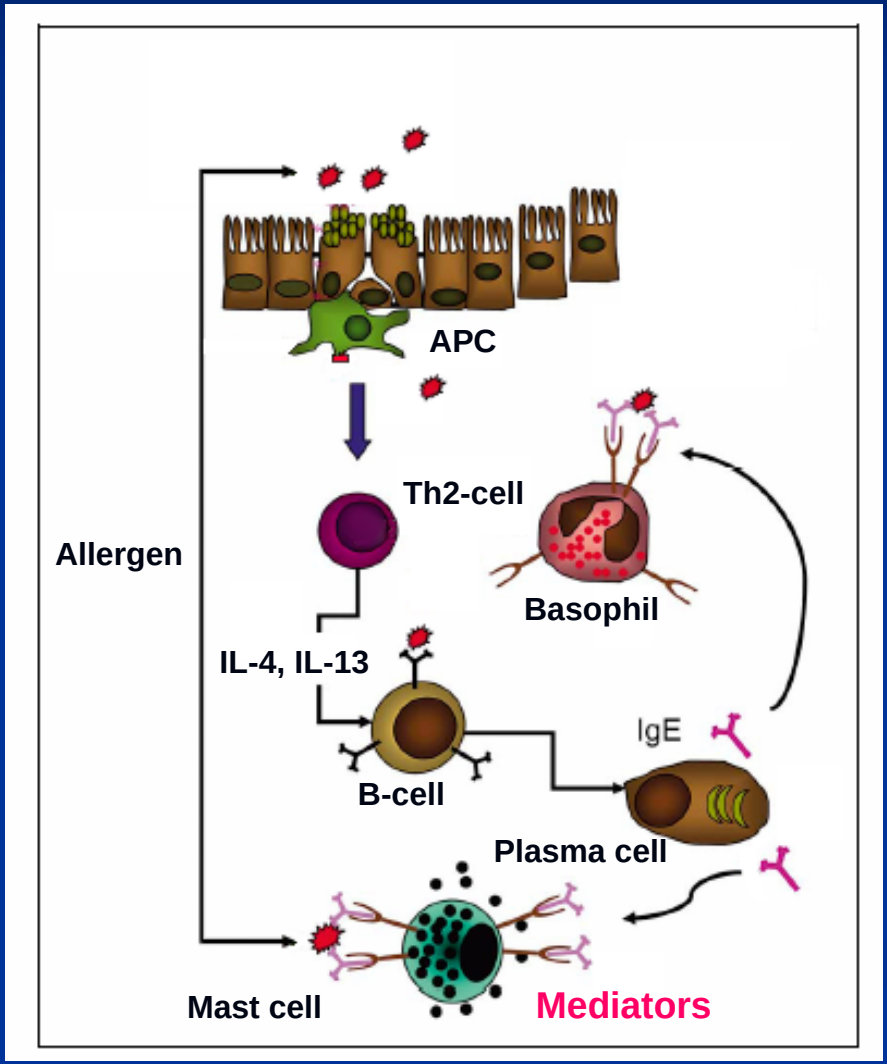
- **Biotechnology-derived therapeutic proteins may induce an immune response, especially when administered as multiple doses over prolonged periods.**

- **The clinical manifestations of antibodies directed against a given protein may include neutralization of the natural counterpart, loss of efficacy and general immune system effects (including allergy and anaphylaxis).**

Severe anaphylactic reaction after repeated intermittent exposure to lepirudin.
Veach et al. 2007. *Pharmacotherapy*. 27(5):760-5.

The Cologne stroke experience: safety and outcome in 450 patients treated with intravenous thrombolysis.
Sobesky et al. 2007. *Cerebrovasc Dis.*;24(1):56-65.

Schematic presentation of the mechanisms in type I, IgE-mediated hypersensitivity reactions



Clinical symptoms:

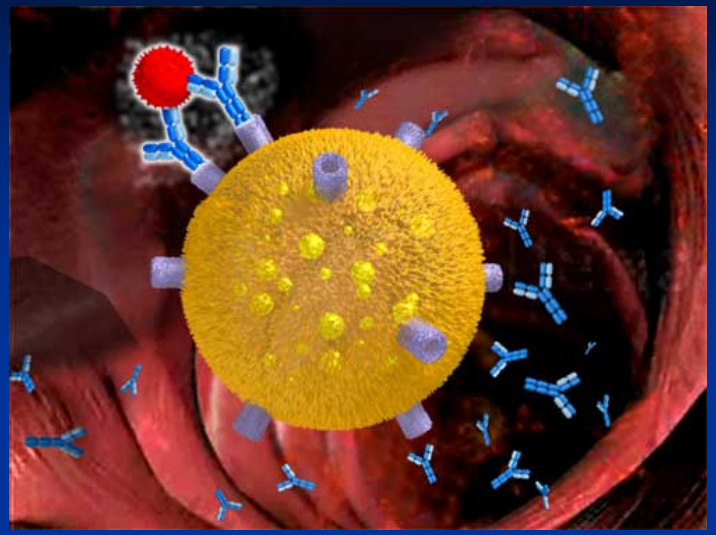
Rhinitis, Bronchospasm, Asthma

Urticaria, Rash, Edema, Eczema

Nausea, Vomiting, Diarrhea

Anaphylactic shock

- Because the allergic responses require complex interactions between the protein and the immune system, they are notoriously difficult to predict.



- Allergens contain B-cell epitopes to which IgE can bind, and T-cell epitopes capable of inducing a type 2 T-lymphocyte response. However, the presence of appropriate epitopes alone is not sufficient to endow a protein with allergenic potential.

- Many factors can contribute to the overall allergenicity of a given protein, such as the glycosylation status, resistance to proteolysis, permeability across a mucosal epithelium, and enzymatic activity.

Immunoresponses to recombinant self-proteins I.

- A variety of human proteins, such as anticoagulants, cytokines, growth factors, interferons, enzymes, peptide hormones and monoclonal antibodies, have been produced as recombinant proteins expressed in bacteria, yeast, insect and mammalian cells, and used for therapeutic purposes.

- Primarily, self-proteins do not induce immune responses and thus they are non-immunogenic. However, several post-translational modifications of proteins occur in the host cell biosynthesis system, and these alterations could be a cause of allergenicity of self-proteins.

- Recombinant proteins with Asn-X-Thr/Ser motif often undergo *N*-glycosylation. The *N*-linked sugar chains in plant and insect cells, but not in mammalian cells, often contain β1-2 linked xylose and/or α1-3 linked fucose residues.

Immunoresponses to recombinant self-proteins II.

- These xylose/fucose-containing sugar chains can be bound by IgE from individuals allergic to tree and grass pollen, fruits and vegetables. Therefore, recombinant glycoproteins, even self-proteins, produced in plant or insect expression system, are potentially allergenic. (Matsuda et al. 2006. *J Biosci Bioeng 101:203-211)*

- Some enzymatic and chemical modifications, furthermore conformational alterations including the aggregation of the proteins can trigger the production of antibodies specific for the recombinant self-proteins.

- Human antibodies may become immunogenic in the human immune system when they are monoclonal. The frequent administration of such a monoclonal antibody may result in unusually high exposure of an epitope in the variable region to the immune system, and accordingly induce the response of anti-idiotypic antibodies.

Prediction of allergenicity I.

The allergenic potential of a novel non-self protein, regardless of whether it is natural or recombinant, could be assessed by comparison of several properties of the given protein with those of known allergic proteins. Such properties include sequence similarity to known allergic proteins, the reactivity with IgE antibodies in the serum originated from patients allergic to known allergen with structural similarity to the given protein and digestibility (in the case of orally administered protein)

Sequence homology to known allergens

To assess the potential allergenicity of the novel proteins in genetically engineered foods, two criteria have been suggested by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) expert consultation: short (6-8) contiguous sequences of identical amino acids, or 35% identity over a sliding window of 80 amino acids.

Prediction of allergenicity II.

Specific serum screening

Serum screening is useful when either (1) the gene is derived from a known allergenic source or (2) the search for sequence homology identifies a possible match with a known allergenic source. Typically, the novel protein is bound to a solid phase and incubated with blood serum from individuals known to have allergic reactivity to the relevant allergenic source to determine if the allergen-specific IgE in the serum reacts with epitopes on the novel protein. The availability of sera from well-characterized patients is an important and sometimes challenging issue.

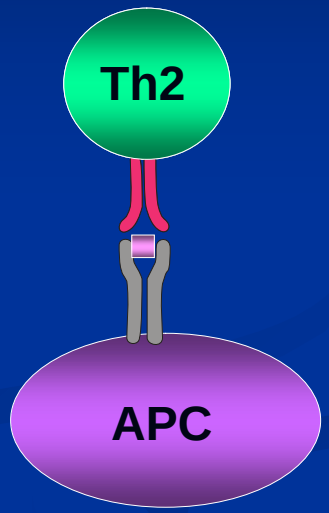
Resistance to pepsin

The comparative resistance to pepsin proteolysis is likely a reasonable comparative measure in the allergenicity assessment. Novel proteins resistant to pepsin are more likely to become allergenic than proteins that are rapidly hydrolyzed by pepsin. However, the conditions of the pepsin resistance assay are critical in this evaluation.

Human dendritic cell–based method to identify CD4+ T-cell epitopes in potential protein allergens

(Stickler et al. 2003. *Environ. Health Perspect* 111:251-254)

- This assay was developed to predict functional T-cell epitopes in a population of individuals who have not been previously exposed to the questionable protein.



- Synthetic peptides were constructed to describe the sequence of the protein of interest. Peptides were synthesized as 15-mers that overlapped by 12 amino acids.

- Dendritic cells were differentiated *in vitro* from blood monocytes. Purified CD4+ T cells from the same donor were cocultured with dendritic cells and 5 µg/mL of peptide for 5 days.

- After 5 days, T-cell proliferation was assessed by tritiated thymidine incorporation.

Assessment of allergenicity in animal models I.

BALB/c mouse model

(Kimber et al. 2003. *Toxicology Letters* 140-141:297-302)

- **Mice are exposed by intraperitoneal injection to the test protein in phosphate-buffered saline on days 0 and 7.**

- **At various periods thereafter (14, 28 and 42 days) sera are collected and specific IgE antibody responses are measured using homologous passive cutaneous anaphylaxis (PCA) assay, and IgG responses by enzyme-linked immunosorbent assays (ELISA).**

- **Cytokine expression profiling: Fourteen days after the initiation of exposure, draining lymph nodes are excised and a single cell suspension of lymph node cells (LNC) is prepared. LNC are cultured in the presence of the test protein for 120 h. Supernatants are harvested and the concentrations of interferon γ (IFN-γ), IL-12, IL-4, IL-5, IL-10 and IL-13 are measured by ELISA.**

Assessment of allergenicity in animal models II.

Brown Norway rat model

(Knippels and Penninks 2003. *Environ. Health Perspect* 111:233-238)

- **Young Brown Norway rats are exposed to test protein by daily gavage dosing during 42 days without the use of an adjuvant.**

- **Serum antibodies (IgG, IgE) specific for the tested protein are measured by ELISA. To confirm the presence or absence of anaphylactic antibodies, rat sera are also tested by PCA assay.**

- **This model allows the study of some clinical symptoms of food allergy. Upon an oral challenge with allergenic protein gut permeability is increased and in some animals breathing frequency and systolic blood pressure are temporarily decreased.**

Assessment of allergenicity in animal models III.

BALB/c mouse model for
protein respiratory sensitizers

(Ward and Selgrade 2007. *Methods* 41:80-90)

- **Mice are exposed by intraperitoneal injection to the test protein in the presence of an adjuvant (aluminum-based). Two weeks following systemic sensitization, the animals are challenged by the respiratory route and appropriate endpoints assessed.**

- **Assessment methods:**
  - **Airway reactivity assessment**
  - **Determination of total cell count and differential cell counts in BAL fluids**
  - **Immunological assessment (detection and quantification of IgE, IgG1, IL-4, IL-5, IL-10, IL-13, TNFα and eotaxin, RANTES, IL-8 by ELISA or multiplex assays /Luminex/)**
  - **Lung histopathology**

Assessment of allergenicity in animal models IV.

Swine model and the atopic dog model

(Helm et al. 2003. *Environ. Health Perspect* 111:239-244)

- **The advantage of both models lies in their propensity to develop clinical symptoms of food allergy, primarily gastrointestinal and dermatologic reactions, after the sensitized animal is challenged with food antigens. In contrast, clinical responses to food allergens in rodent models are not as reminiscent of human responses.**

Limitations of animal models

- Each animal model has proven highly reactive for some specific allergens, but they have either not been tested with a variety of allergenic and non-allergenic proteins, or they have been shown to react with non-allergenic proteins.

- Factors that appear to influence the outcome include: genetic differences across the human population and across animal species, possible prior dietary exposure to test proteins or highly similar proteins, the presence of adjuvants, routes and doses of sensitization, challenge procedures, native state of the test materials and prior or concurrent viral infection.
(Goodman et al. 2005. *Int Arch Allergy Immunol.* 137:153-66)