

A novel combined *in-vitro* and *in-vivo* model to predict the allergenicity of food proteins

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BACKGROUND

There is a growing need for the development of approaches suitable for the characterization of the allergenic potential of novel and modified food proteins. The current methodology is effective at identifying proteins that are likely to cross-react with known allergens, however to date, there are no validated *in vitro* and *in vivo* models for allergenicity testing of novel proteins. In addition, most assays investigated show false positive results for non-allergens. For this purpose, an *in vitro* DC-T cell assay and an *in vivo* mouse model were used to test distinctions between food proteins known to cause food allergy (Ara h1, β -Lactoglobulin) and food proteins rarely associated with food allergy (soy lipoxigenase, gelatin).

METHODS

DC-T cell assay: C3H/HeOuj mice were immunized with 100 μ g protein and alum. After 29 days, the CD4+ T cell pool containing protein specific T cells was isolated using magnetic beads. Bone marrow was cultured for 6 days with GM-CSF, after which the bone marrow derived dendritic cells were pulsed with 50 μ g/ml or 100 μ g/ml protein. The CD4+ T cells were incubated for 72 hrs with protein-pulsed dendritic cells. Hereafter IL-5, IL-10, IL-13 and IFN γ production was analyzed.

Mouse model: C3H/HeOuj mice (n=6/group) were sensitized by intra-gastric administration of 2 mg protein with 10 μ g cholera toxin on days 0 and 7. On day 16, mice were challenged intra-gastrically with 50 mg protein and blood samples were taken after 30 min. Serum was analyzed for protein-specific immunoglobulins and mouse mast cell protease-1 (mMCP-1) release.

RESULTS

All proteins induced protein-specific IgG1 upon i.p. immunization with alum (data not shown). Ara h1 and β -lactoglobulin-pulsed but not soy lipoxigenase and gelatin-pulsed DC induced the production of IL-5, IL-10 (data not shown) and IL-13 from T cells. This Th2 type profile selectively induced by β -lactoglobulin and Ara h1 *in vitro* was further confirmed *in vivo*. This was shown by induction of mMCP-1 release and protein-specific IgG1 and IgE by β -lactoglobulin and Ara h1 but not by soy lipoxigenase and gelatin.

DISCUSSION

- The sensitivity and specificity of both test systems need to be further tested by extending the test panel of proteins
- Soy lipoxigenase might be debatable as representative non/low allergenic protein since it is present in trace amounts in the feed of the breeder

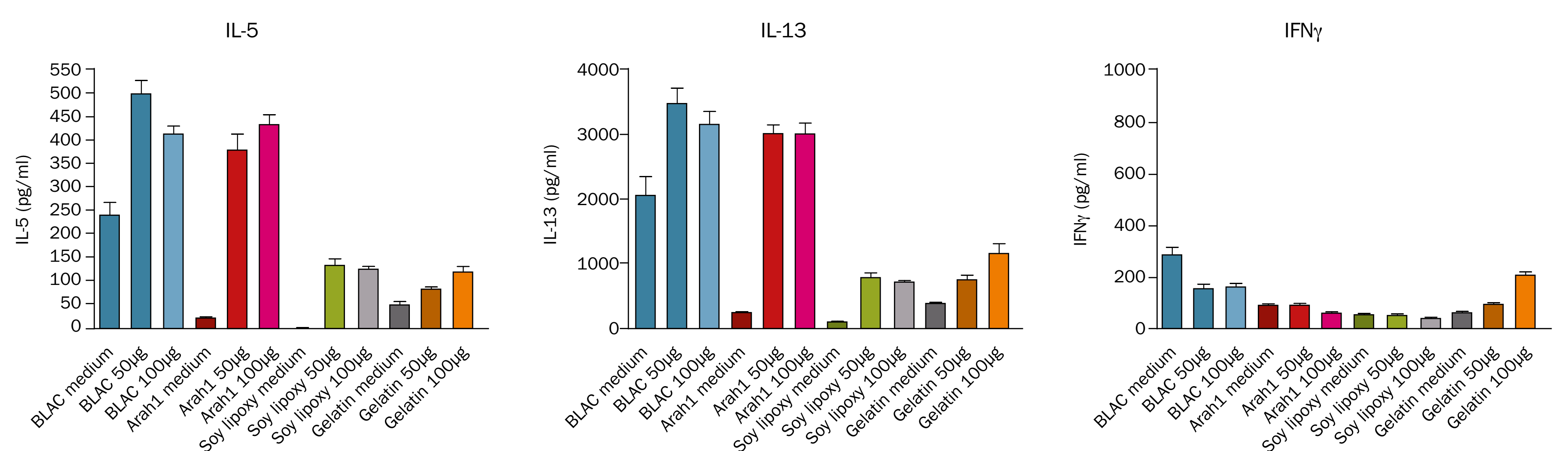


Figure 1. Cytokines produced in DC-T cell culture. DC were stimulated with medium, 50 or 100 μ g protein. CD4+ T cells were added for 72 hours. Cell supernatants were analysed using ELISA.

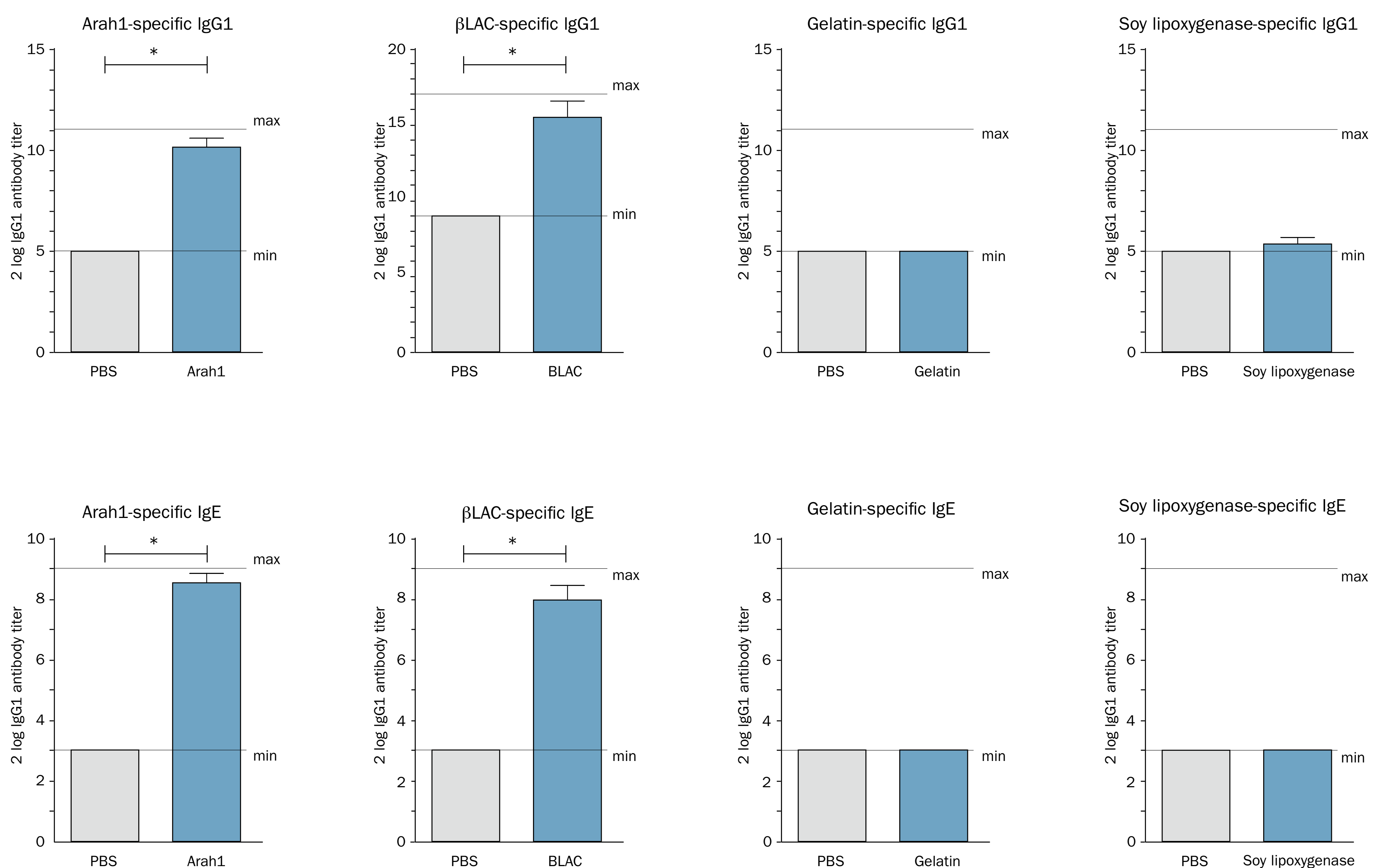


Figure 2. Protein-specific antibody responses in serum (2 log antibody titer) after oral sensitisation with 2 mg proteins and cholera toxin as adjuvant.

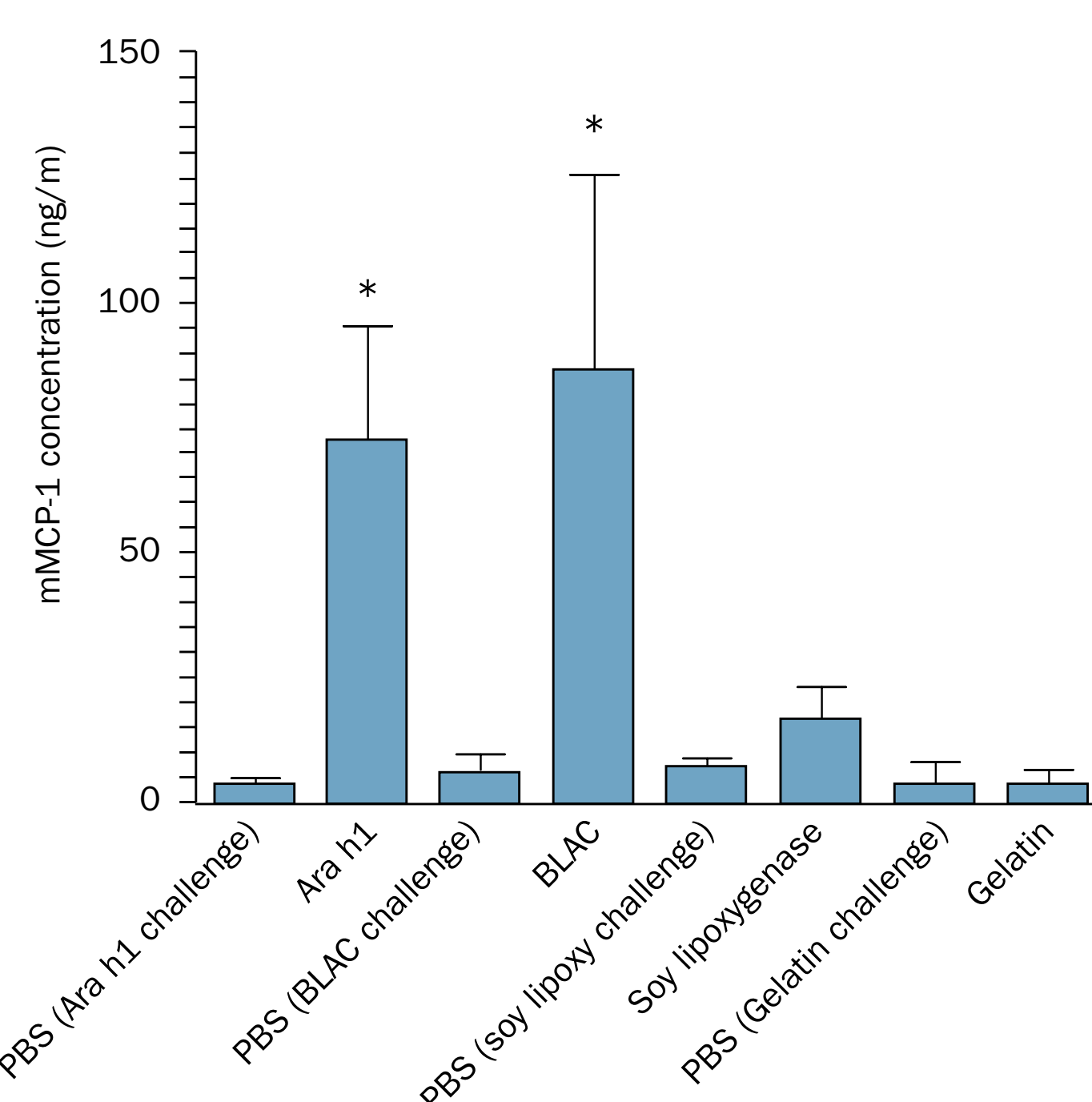


Figure 3. Mast cell degranulation measured as serum mMCP-1 after oral whey challenge. Mice were orally sensitised with protein (2 mg) using cholera toxin as an adjuvant. Mice were challenged orally and blood was taken after 30 minutes for mMCP-1 analyses (* p <0.05 compared to non-sensitised mice)

CONCLUSION

Both the DC-T cell assay and the mouse model were able to distinguish known allergens from low/non-allergens. This approach may prove to be promising method to identify new proteins that have not been experienced previously in the diet. This will be the focus of future research.

In relation to this presentation, there are no conflicts of interest

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