Antibody Production and Immunoassay Development

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We proposed to develop antibodies and immunoassays specific to the major peanut allergens. These antibodies are to be used in a variety of screening projects with multiple collaborators. The original goal of this project was to find naturally existing, engineered or processed peanuts or products thereof with reduced allergenic potency. The uses for the antibodies have expanded considerably over the years (see publications for examples). We are happy to report that we have successfully produced multiple antibodies, 7 of which have been used in screening a variety of samples with these antibodies for many years. An anti-Ara h 8 and the basic subunit of recombinant Ara h 3 were successful and we have optimized the conditions for the use of these antibody for immunoassays. Meanwhile, we previously reported identification of a region of the major allergen, Ara h 2 sequence (Ara h 2 peptide), responsible for cross-reactivity between peanuts and tree nuts (5). With GPC funds, we produced this fragment of Ara h 2, used it to develop an antibody which also reacts with specific allergenic proteins in tree nuts and may indeed be responsible of peanut and tree-nut cross-reactivity (i.e. allergies to multiple nuts). We have gained significant insight into why some tree nut allergic patients also react with peanut and vise versa. Ara h 3 is very abundant in peanut and may also contribute to cross reactivity among nuts and we plan to find the areas of this allergen responsible for cross-reactivity. Meanwhile, we have continued to screen peanut samples from all over the world using existing antibodies. The number of interested collaborators and companies increase every year. Of the most recent use of our antibodies was to 1) screen commercially produced peanut extracts used for skin prick tests and to determine the suitability of these extracts for immunotherapy and for improved diagnosis of peanut allergy (with less false positive and negative results, see publications 1& 2); 2) test processing treated samples for reduced allergenicity (see publications #6, 7, 8); 3) develop and validate methods to test for nuts on surfaces (see publication number 9). This year we are continuously using GPC funded antibodies to test peanut containing immunotherapeutic drugs and peanuts used in a novel probiotic for allergen levels (both confidential).

Accomplishments:

1. Many peanut samples either have been and are continuously being screened and results reported to collaborators for example peanuts from: TILLING and silencing projects by Dr. Ozias-Akins; differential processing, UC Davis (Dr. Teuber), North Carolina A&T State University (Dr. M. Ahmenda), LSU (Dr. S. Bahna), Univ. Nebraska (Dr. Taylor), Cal. State Univ. LA (Dr. Singh), and USDA Univ of Raleigh, NC (Dr. T. Sanders), peanut samples in the environment and on surfaces with King's College, UK (Dr. Gideon lack and Helen Brough), development of peanut vaccine n mouse model, McMaster's University, Canada (Dr. Manel Jordana) etc. Several publications have and will continue to arise from these studies (see publications at end of report) Dr. Koppelman from Holland is a new collaborator using antibodies, ARC (allergen research Corp), Mount Saini (Dr. XM Li), Dr. Shreffler, etc. Also, we continue to screen progeny of any crosses that were produced by Dr. Isleib.
2. Ara h 3, basic subunit (bArah 3) that was shown to be an important and abundant peanut allergen, an Ara h 2 fragment (important in cross-reactivity and diagnosis), and recombinant Ara h 8 were cloned into expression vectors, the proteins expressed, antibodies produced last year and immunoassays were developed and optimized this year. We continue to:

- improve current assays and implement new and more rapid immunoassays to accelerate the screening process
- purify recombinant and native Ara h 1, Ara h 2, acidic Ara h 3 (a,b Ara h 3), Ara h 6 and 8 for use as standards in all experiments. (GPC /USDA contribution)
- Produce, purify and continue to develop and optimize assays for antibodies against Ara h 1, bAra h 3, Ara h 6, 8, 2 (peptide) and screen variety of peanut samples from different collaborators and share these reagents worldwide.

Overall accomplishments to date:

- We have thus far purified Ara h 1, 2, 3 and 6 from peanuts and most recently recombinant Ara h 8, a cross-reactive-Ara h 2 peptide and Ara h 3 (acidic and basic subunit) and used these proteins to make 8 different antibodies. We have also made four more antibodies against raw and roasted peanut in both rabbit and chicken for a total of 12 very useful antibodies.
- Antibodies were purified and various immunoassays such as Western blot, Slot Blot, immunoprecipitation

and ELISA have been developed, optimized and used to screen literally 1000s of samples for all of these antibodies.

- The antibodies produced have been shared with many as discussed in proposal and above. The production of these antibodies has been much more impactful than originally imagined at the beginning of this project. The impact is apparent from the number of research projects in which these antibodies have been used and the resultant high quality publications.

Most recent publications:

1. Schmitt, D.A, Nesbit, J.B., Hurlburt, B.K., Cheng, H., Maleki, S.J. (2010) Processing Can Alter the Properties of Peanut Extract Preparations. J. Agric. Food Chem. J. Agric. Food Chem. 58 (2), 1138–1143.
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3. Maleki, S.J., Casillas, A.M., Kaza, U., Wilson, B.A., Nesbit, J.B., Reimoneqnue C, Cheng H., Bahna, S.L. (2010) Differences between Heat-Treated, Raw, and Commercial Peanut Extracts by Skin Testing and Immunoblotting. Ann Allergy Asthma Immunol. 105(6):451-7.
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5. Hurlburt, B.K., Schmitt, D.A., Isleib, T.G., Cheng, H., Garvey, C., Koenig R.L., Maleki S.J. (2011) Production of pure protein, antibodies and development of immunoassays to detect Ara h 3 levels in peanut varieties. International Journal of Food Science and Technology 46, 1477–1484.
6. Maleki, S.J., Teuber, S.T., Cheng, H., Chen, D., Comstock, S.S., Ruan, S., Schein, C.H. (2011) Computationally predicted IgE epitopes of walnut allergens contribute to cross-reactivity with peanuts. Allergy 66 (12): 1522-1529.
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9. Cabanillas, B., Maleki, S.J., Rodríguez, J., Burbano C., Muzquiz, M., Jiménez, MA, Pedrosa, M.M., Cuadrado, C., Crespo J.F. (2012) Heat and Pressure Treatments Effects on Peanut Allergenicity. Food Chemistry. 132:360–366.
10. Brough, H.A., Makinson, K., Penagos, M., Maleki, S.J., Stephens, A.C., Turcanu, V., Lack, G. Measuring peanut protein in settled dust and air: a validation study. submitted to Clin. Exp. Allergy, 2012
11. Maleki, S.J., Cheng, H., Schein, S.H. Cross-reactivity among peanuts and tree nuts. Proceedings of 41st Unites States Japan National Resource Panel. P. 1-2. 2012.
12. Nesbit J.B., Hurlburt B.K., Schein C. Wei, H., Maleki, S.J. Ara h 1 structure is retained after roasting and is important for enhanced binding to IgE. Molecular Nutrition and Food Research. Molecular Nutrition and Food Research. Molecular Nutrition and Food Research. 56(11):1739-47. 2012.
13. Maleki, S.J., Cheng, H., Schein, S.H. Cross-reactivity among peanuts and tree nuts. Proceedings of 41st Unites States Japan National Resource Panel. P. 1-2. 2012.
14. Bublin, M., Kostadinova, M., Radauer C., Hafner, C., Szépfalusi, Z., Varga E., Maleki S.J., Vogel, L., Vieths S., Hoffmann-Sommergruber K., Breiteneder H. IgE cross-reactivity between the major peanut allergen Ara h 2 and the non-homologous allergens Ara h 1 and Ara h 3. Journal of Allergy and Clinical Immunology 132(1):118-24. 2013.
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18. Dwovedo, S., Puppala, N., Maleki, S.J., Ozias Akins, P., Rodomiro, O. Allergen and toxin free and nutritionally dense peanuts. Plant Breeding Reviews. In Press 2013.
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