Development of Enzyme Linked ImmunoSorbent Assays for Combined Detection of HMW Glutenins, Gliadins and Gluten Hydrolysates

Supervisor: Ciara O' Sullivan

Gluten sensitivity, manifesting as celiac disease (CD) affects possibly 1:100 people in Northern Europe and Northern America.[1,2] Celiac disease (CD) is an inflammatory disease of the upper small intestine and results from gluten ingestion in genetically susceptible individuals, and is the only life long nutrient-induced enteropathy.[3,4] The small bowel abnormalities are reversed on withdrawal of gluten from the diet. CD is a familial condition with around 10-15% of first degree relatives being similarly affected.[5]

Celiac disease, when untreated or poorly treated, that is with continued ingestion of gluten, leads to a large number of complications, [6,7] which can result in considerable morbidity and repeated hospital visits. In children symptoms such as growth retardation amongst others are observed[8,9] and patients with osteoporosis have a higher prevalance of CD ,[10] with bone mineral density in CD patients improves with a GFD.[11] Up to 50% of women with untreated celiac disease experience miscarriage and long term silent undiagnosed celiac disease can lead to infertility.[12] The condition is also strongly associated with other autoimmune conditions.[13, 14] Celiac disease sufferers who do not adhere to a strict gluten-free diet have an increased incidence of a fatal small intestinal lymphoma[15,16] and the standardised mortality rate is twice that of the general population, non-Hodgkin lymphoma being the main cause of death.[17]

Dieterich and colleagues identified tissue transglutaminase (tTG) as the autoantigen of celiac disease,[18] which was supported by a publication by Arentz-Hanson et al [19] and the sequence identified was further backed up by Anderson et al.[20] and it was later shown that a 19-mer peptide formed by the two overlapping peptides of Arentz-Hansen is indeed disease activating to the small intestines of celiac disease patients in vivo, causing the classic parameters of the condition.[21]

The development of legislation on levels of gluten permissible in foods labelled as gluten-free has been hampered by lack of a suitable assay system. The present standard has stood since 1981 (Codex Stan 118-1981) and is very non-specific.[22, 23] Codex Alimentarius have recently approved new standards for gluten free foods (July 2008). The new benchmark states that foods labelled "gluten-free" may not exceed a gluten content of 20 ppm, whilst those with a gluten content to a level between 20 ppm - 100 ppm may be called "low gluten" or "reduced gluten". A range of methods for the detection of gliadin have been reported, such as the use of gluten specific PCR,[24, 25] and SDS-PAGE in combination with immunoblotting, counter immuno-electrophoresis, or mass spectroscopy and MALDI-TOF analysis.[26] High-performance liquid chromatography and capillary electrophoresis have been widely used to analyse prolamins in food[27, 28], and gel permeation–high-performance liquid chromatography (GP-HPLC) has been described for the quantitative determination of gliadin. More recent reports have looked at the use of flow cytometry detecting as low as 10pg/mL levels of gliadin[29], as well as the first report of a biosensor for gliadin detection, which exploited the use of a recombinant glutamine-binding protein[30] The same group have gone on to report a fluorescence correlation spectroscopy assay, reporting a detection limit of 0.006ppm, which would require huge dilution of extracts from gluten free samples for detection.[31]

The most common method of measurement of gliadin is that of the enzyme linked immunosorbent assay (ELISA). Early assays used whole gliadin as the immunogen to produce polyclonal antisera and gave rise to antibodies that were insufficiently specific, for example, giving spurious cross-reactivities with non-toxic maize.[32] Later, advantage was taken of monoclonal antibody technology to produce more precisely targeted reagents.[33] The first toxic sequence to be identified in vivo was A-gliadin 31-49.[34] and a monoclonal antibody, has been raised against the celiac-toxic 19-mer A-gliadin peptide (LGQQQPFPPQQPYPQPQPF) and used for detection of gliadin and gluten hydrolysates.[35,36]

Description of Project

The project will focus on the development of five types of assays:

1. Sandwich assay for combined detection of HMW glutenins and gliadins.
2. Competition assay for combined detection of HMW glutenins and gliadins
3. Competition assay for detection of gliadin hydrolysates
4. Competition assay for detection of glutenins hydrolysates
5. Competition assay for combined detection of HMW glutenins and gliadins hydrolysates

Once the parameters for the development of each assay have been optimised using checkerboard assays, the assay will be further improved by looking at the effect of incubation times and temperatures, co-incubation of immunoreagents and standard curves will be constructed. Analytical parameters such as limit of detection, slope, reproducibility, stability of immunoreagents and compatibility with extraction reagents will be elucidated.

For the assay for detection of hydrolysates, a procedure for enzymatic hydrolysis will be developed using a modified pepsin/trypsin digestion protocol using consitions compatible with ELISA detection.

The developed assays will be validated with a range of commercially available gluten free and gluten containing foodstuffs.

It is anticipated that at least two publications will result from this work, and the student would gain experience in the development of ELISAs as well as the optimisation of enzymatic hydrolysis, both of which are widely used in the clinical and food control industries.

Bibliography

[1] Lee, S. K.; Green, P. H. R. *Curr. Opin. Rheumatol*. **2006**, *18*, 101.
[2] Dube, C.; Rostom, A.; Sy, R.; Cranney, A.; Salojee, N.; Garritty, C.; Sampson, M.; Zhang, L.; Yazdi, F.; Mamaladze, V.; Pan, I.; MacNeil, J.; Mack, D.; Patel, D.; Moher, D. *Gastroenterol*. **2005**, *128*, S57.
[3] Maki M.; Collin, P. *Lancet* **1997**, *349*, 1755
[4] Turski, A.; Georgetti, G.; Brandimarte, G.; Rubino, E.; Lombardi, D. *Hepatogastroenterology* **2001**, *48*, 462
[5] Marsh, M. N. *Gastroenterol.* **1992**, *102*, 330.
[6] Fasano A.; Catassi, C. *Gastroenterol.* **2001**, *120*, 636.
[7] Green, P. H. R. *Gastroenterol*. **2005**, *128,* S74.
[8] Farrell, R. J.; Kelly, C. P. *N. Engl. J. Med*. **2002**, *346*, 180.
[9] Catassi, C.; Fabiani E.; *Baillieres Clin. Gastroenterol*. **1997**, *11*, 485.
[10] Stenson, W.; Newberry, R.; Lorenz, R.; Baldus, C.; Civitelli, *R. Arch. Intern. Med*. **2005**, *28*, 393.
[11] Mustalahti, K.; Collin, P.; Sievanen, H.; Salmi, J.; Maki, M. *Lancet,* **1999**, 28, 744.
[12] Rostami, K.; Steegers, E. A. P.; Wong, W. Y.; Braat, D. D.; Steegers-Theunissen, R. P. M. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2001**, *96*, 146.
[13] Ventura A.; Magazzu G.; Greco L. *Gastroenterol.*, **1999**, *117*, 297.
[14] Corrao, G.; Corazza, G. R.; Bagnardi, V.; Brusco, G.; Ciacci, C.; Cottone, M.; Sattegna Guidetti, C.; Usai, P.; Cesari, P.; Pelli, M.; Loperfido, S.; Volta, U.; Calabro, A.; Certo, M. *Lancet*, **2001**, *4*, 356.
[15] Cooper, B.; Holmes, G.; Cooke, W. *Digestion*. **1982**, *23*, 89.
[16] West, J.; Logan, R.; Smith, C.; Hubbard, R.; Card, T. *Br. Med. J.* **2004**, *25*, 716.
[17] Jacobson, D. L.; Gange, S. J.; Rose, N. R.; Graham, N. M. *Clin. Immunol. Immunopathol.* **1997**, *84*, 223.
[18] Dieterich, W.; Ehnis, T.; Bauer, M.; Donner, P.; Volta, U.; Riecken, E. O.; Schuppan, D. *Nat. Med.* **1997, *3*,** 797.
[19] Arentz-Hansen, H.; Körner, R.; Molberg, O.; Quarsten, H.; Vader, W.; Kooy, Y. M.; Lundin, K. E.; Koning, F.; Roepstorff, P.; Sollid, L. M.; McAdam, S. N. *J. Exp. Med*. **2000**, *191*, 603.
[20] Anderson, R. P.; Degano, P.; Godkin, A. J.; Jewell, D. P.; Hill, A. V. S. *Nat. Med.* **2000**, *6*, 337.
[21] Fraser, J. S.; Engel, W.; Ellis, H. J.; Moodie, S. J.; Pollock, E. L.; Wieser, H.; Ciclitira, P. J. *Gut*, **2003**, *52*, 1698.
[22] Skerritt, J. H.; Hill, A. S. *Cereal Chem.* **1992**, *69*, 110.
[23] Codex Alimentarius Commission. Standard for Gluten-Free Foods. FAO/WHO 1981, 118.
[24] Allmann, M.; Candrian, U.; Hofelein, C.; Luthy, J. *Z. Lebensm. Unters. Forsch.* **1993***, 196*, 248.
[25] Köppel, E.; Stadler, M.; Luthy, J.; Hubner, P.; *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 399.
[26] Méndez, E.; Valdés, I.; Camafeita, E. *Methods Mol. Biol.* **2000,** *146*, 355.
[27] Nicolas, Y.; Martinant, J.-P.; Denery-Papini, S.; Popineau, Y. *J. Sci. Food Agric.* **1998***, 77*, 96.
[28] Wieser, H.; Seilmeier, W.; Belitz, H.-D. *J. Cer. Sci*. **1994**, *19*, 149.
[29] Capparrelli, R.; Ventimaglia, I.; Longobardo, L.; Iannelli, D. *Cytometry A* **2006**, *63A*, 108
[30] De Stefano, L.; Rossi, M.; Staiano, M.; Mamone, G.; Parracino, A.; Rotirotti, L.; Rendina, I.; Rossi, M.; D'Auria, S. *J. Proteome Res*. **2006**, *5*, 1241.
[31] Varriale, A.; Rossi, M.; Staiano, M.; Terpetschnig, E.; Barbieri, B.; Rossi, M.; D'Auria, S. *Anal. Chem*. **2007**, *79*, 4687.

[32] Troncone, R.; Vitale, M.; Donatiello, A.; Farris, E.; Rossi, G.; Auricchio, S. *J Immunol. Meth*. **1986**, *92*, 21.
[33] Freedman, A. R.; Galfre, G.; Gal, E.; Ellis, H. J.; Ciclitira, P. J. *J. Immunol. Meth*. **1987**, *98*, 123
[34] Sturgess, R. P.; Day, P.; Ellis, H. J.; Lundin, K.; Gjertsen, H.; Kontakou, M.; Ciclitira, P. J. *Lancet* **1994**, *343*, 756.
[35] Ellis, H. J.; Rosen-Bronson, S.; O'Reilly, N.; Ciclitira, P. J. *Gut* **1998**, *43*, 190.
[36] Bermudo-Redondo, M. C.; Griffen, P. B.; Garzon Ranaz, M.; Ellis, H. J.; Ciclitira, P. J.; O'Sullivan, C. K. *Anal. Chim. Acta* **2005**, *551*,105.
[37] Skerritt, J.; Hill, A. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 257.
[38] Wieser, H.; Seilmeier, W.; Belitz, H.-D. *J. Cer. Sci*. **1994**, *19*, 149.
[39] Valdes, I.; Garcia, E.; Llorente, M.; Mendez, E. *Eur. J. Gastroenterol. Hepatol*. **2003**, *15*, 465.
[40] Warsinke, A. *Adv. Biochem. Eng. Biotechnol*. **2008**, *109*, 155.
[41] Fragoso A.; Laboria, N.; Latta, D.; O'Sullivan, C. K. *Anal. Chem*. **2008**, *80*, 2556.
[42] van Eckert, R.; Berghofer, E.; Ciclitira, P. J.; Chirdo, F.; Denery-Papini, S.; Ellis, H. J.; Ferranti, P.; Goodwin, P.; Immer, U.; Mamone, G.; Méndez, E.; Mothes, T.; Novalin, S.; Osman, A.; Rumbo, M.; Stern, M.; Thorell, L.; Whim, A.; Wieser H. *J. Cer. Sci.* **2006**, *43*, 331.
[43] Ciclitira, P. J.; Dewar, D. H.; Suligoj, T.; O'Sullivan, C. K.; Ellis, H. J. *Proceedings of the 12th International Celiac Symposium*, New York 2007. (2008, in press).
[44] Ciclitira, P. J.; Ellis, H. J.; Evans, D. J.; Lennox, E. S. *Brit. J. Nutr.* **1985**, *53*, 39.
[45] Pänke, O.; Balkenhohl, T.; Kafka, J.; Schäfer, D.; Lisdat, F. *Adv. Biochem. Eng. Biotechnol*. **2008**, *109*, 195.