

### Use of hyperlac in coeliac disease and its effect on disaccharidase deficiency

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**Introduction** Study aimed at determining the effect of Hyperlac (a colostrum and hyperimmune milk-based product containing growth factors) on histology, villous length and disaccharidase enzyme levels in the small bowel of patients with coeliac disease (CD).

**Methods** Nine patients with CD (5M, 4F; aged 18–75) received omeprazole 20 mg mane and Hyperlac 20 g bd orally for 8 weeks. Gastroscopy was performed pre- and post-treatment to obtain biopsies to compare the classic histological features of CD including the extent of villous atrophy, crypt cell hyperplasia and inflammation of the lamina propria. Villous length and levels of disaccharidases (sucrase, lactase, maltase) were also measured. Gluten-free diet was maintained for the duration of study. HREC approval was obtained for this open pilot study.

**Results** Classic histological characteristics did not change over treatment period. Some recovery of villous length was seen in 5/9 patients ( $P < 0.039$ ). Pre- and post-treatment mean villous length  $\pm$  SD was  $255 \pm 52 \mu\text{m}$  (95% CI: 215–282  $\mu\text{m}$ ) and  $309 \pm 36 \mu\text{m}$  (95% CI: 295–337  $\mu\text{m}$ ), respectively. Disaccharidase levels returned to normal in all patients with baseline levels below normal range with the exception of lactase in 2 patients. Sucrase and Maltase levels increased in all patients. At baseline, 3/9 (33%) patients had below normal range sucrase levels (median 3.2 U/g) and 3/9 (33%) patients had below normal range maltase levels (median 12.6 U/g). Of these, all levels returned to normal range post-treatment,  $p < 0.0039$  (median 6.3 U/g) and  $p < 0.0039$  (median 28.3 U/g), respectively. Lactase levels increased in 8/9 (89%) patients. At baseline, 7/9 (78%) patients had below normal range lactose levels (median 0.4 U/g; range 0.1–1.5). Of these, 5/7 (71%) increased to normal range post-treatment (median 1.3 U/g) ( $P < 0.0078$ ). Of the 7 patients with baseline coeliac symptoms, 4 (57%) had symptoms improve or resolve with treatment. Five out of 9 patients achieved weight gain  $\geq 1.5$  kg over the treatment period. No treatment-related adverse events were reported during the 8-week period.

**Conclusions** 1: Hyperlac, with a gluten-free diet, has the potential to improve some clinical aspects of CD. 2. In patients with disaccharidase deficiencies significant measurable improvements can be achieved with minimal side-effects. 3. Randomised, double blind, placebo-controlled studies with larger patient populations are now required to confirm the data.

### Wheat gluten T cell epitopes in hla-dq2 coeliac disease: A comprehensive bioinformatic and functional screen *in vivo*

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Coeliac disease (CD) is a genetically determined immune enteropathy driven by dietary gluten. Previously we screened a wheat gliadin peptide library and found 'six-families' of structurally related sequences that activated T cells. The aim of this study was to screen all wheat gluten (240 gliadin and glutenin proteins) to define the complete hierarchy and the molecular structure of 'optimal' epitopes in HLA-DQ2 CD.

**Methods** Bioinformatic tools were developed that allowed a 2152 20mer peptide library to be generated from all available GenBank entry listings for wheat (*T. aestivum*) gliadins (53 alpha/beta, 53 gamma and 2 omega gliadins) and glutenins (77 LMW and 55 HMW glutenins) covering all 13 209 12mer peptides. These 20mers were synthesised as a Pepset peptide library (Mimotopes). 46 HLA-DQ2 + CD subjects on a gluten-free diet consumed wheat bread (300 g/day) for three days. Peripheral blood mononuclear cells collected six days after commencing gluten challenge were incubated overnight in interferon-gamma ELISpot assays with individual peptides at 50  $\mu\text{g}/\text{mL}$ . A bioinformatic algorithm (the 'EM algorithm') was used to define and quantify interferon-gamma responses to sequences within the peptide library.

**Results** All data relating to individual peptide responses in coeliac subjects was pooled and analysed by the EM algorithm. A series of 9mer sequences were identified and ordered according to the intensity of gamma-interferon responses and the proportion of individuals responding. By reviewing the 110 most 'active' 9mer sequences, the 'list' of 9mer motifs could be condensed to 41 9mers, many of which overlapped. In selected cases, high-grade peptides were synthesised and confirmed the bioactivity of peptides identified by the EM algorithm. The hierarchy of epitopes is highly conserved between individuals. Novel glutenin epitopes were defined but are seldom  $> 50\%$  as potent as the dominant gluten epitope (A-gliadin 57–73).

**Conclusion** The molecular identity of gluten peptides activating T cells will allow design of novel diagnostics, therapeutics, nontoxic grains and improve food testing. All wheat gliadins and glutenins contain 'toxic sequences'. Genetic modification rather than selective breeding is likely to be the only approach to 'nontoxic' wheat, rye and barley.