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References

- 1 Gubler C, Bauerfeind P. Intestinal Behcet's disease diagnosed by capsule endoscopy. *Endoscopy* 2005;**37**:689.
- 2 Fritscher-Ravens A, Swain CP, von Herbay A. Refractory Whipple's disease anaemia: First lesson from Capsule endoscopy. *Endoscopy* 2004;**36**:659–62.
- 3 Swain P. Wireless capsule endoscopy and Crohn's disease. *Gut* 2005;**54**:323–6.
- 4 Albert JG, Martiny F, Krummnerl A, et al. Diagnosis of small bowel Crohn's disease: a prospective comparison of capsule endoscopy with magnetic resonance imaging and fluoroscopic enteroclysis. *Gut* 2005;**54**:1721–7.
- 5 Voderholzer WA, Beinhoezl J, Rogalla P, et al. Small bowel involvement in Crohn's disease: a prospective comparison of wireless capsule endoscopy and computed tomography enteroclysis. *Gut* 2005;**54**:369–73.
- 6 Fireman Z, Mahajna E, Briode E, et al. Diagnosing small bowel Crohn's disease with wireless capsule endoscopy. *Gut* 2003;**52**:390–2.
- 7 Vernia P, Marcheggiano A, Marinaro V, et al. Is Crohnite-Canada syndrome necessarily a late-onset disease? *Eur J Gastroenterol Hepatol* 2005;**17**:1139–41.
- 8 Samaha S, Arber N. Crohnite-Canada syndrome. *Digestion* 2005;**71**:199–200.

Immunonutrition enhances the expression and secretion of mouse intestinal defensins

The nutrient rich lumen of the small intestine provides a favourable environment for microbial cell growth. Secretion of defensins from Paneth cells constitutes a key source of antimicrobial peptide activity in the crypt lumen.¹ In addition, defensins also recruit the adaptive immune system.^{2,3} Chronic disease, major surgery, trauma, or malnutrition results in immune compromise and can

increase the risk of infection and sepsis.⁴ Several clinical studies have demonstrated that patients fed an immune enhancing formula had significantly fewer infectious complications, needed less antibiotics, and had a shorter hospital stay than patients fed standard diets.⁵ However, the mechanism by which these formulas bolster the immune system is unclear.

We hypothesised that a nutrient enriched diet bolsters the immune system by upregulating defensin expression in the jejunum and ileum, the two parts where cryptidins (mouse intestinal defensins) are highly expressed. To study the effect of an immune enhancing diet (Perative; Ross Products, Abbott Laboratories, USA) on intestinal defensin expression, we used mice fed either regular chow or Perative for a week. The volume of Perative (complete balanced nutrition containing partially hydrolysed peptide based high protein (sodium caseinate, lactalbumin, 66.7 g/l), arginine at 2.5% of total calories, dietary nucleotides, canola oil,

Figure 1 Expression levels of cryptidins 1–3 and cryptidin 4 after Perative supplementation. (A) Levels of cryptidins 1–3 in the jejunum and ileum of chow fed versus Perative fed mice. (B) Levels of cryptidin 4 in the jejunum and ileum of chow fed versus Perative fed mice. (C) Levels of cryptidins 1–3 and cryptidin 4 in primary Paneth cells treated with isoleucine (Ile), arginine (Arg), and Perative. In (A) and (B), total RNA was extracted and cryptidin levels analysed by Northern blot analysis and normalised to β -actin. In (C), cells were treated for six hours, total RNA was extracted, and cryptidin levels were analysed by quantitative real time polymerase chain reaction and normalised to *Gapdh*. Each bar represents the mean (SEM) of three independent experiments. CM, complete medium. ** $p < 0.005$ significantly different from control (Student's *t* test).

medium chain triglyceride oil, corn oil, soy lecithin, vitamins, minerals, fructooligosaccharides, lactose, gluten, and growth factor free) consumed by the mice was similar throughout the week (15 ml/day). Body weight did not differ between the Perative fed and chow fed groups. Northern blot analysis⁶ of total RNA extracted from the jejunum and ileum, using an oligo probe specific to cryptdin 1, cryptdin 2, and cryptdin 3 (>93% nucleotide identity), or cryptdin 4, revealed that levels of cryptdins 1–3 (fig 1A) and cryptdin 4 (fig 1B) increased by 1.6-fold (Student's *t* test, *p*<0.05).

To further study the direct effect of Perative on Paneth cells, we isolated primary Paneth cells⁷ from mouse jejunum and tested the effect of different concentrations of Perative (0.0025%, 0.01%, 0.025%), isoleucine, and arginine (25, 100, 250 µg/ml) on cryptdin expression by real time polymerase chain reaction.⁶ Whereas different concentrations of arginine and isoleucine did not stimulate cryptdin expression (Student's *t* test, *p*>0.05), 0.025% Perative increased significantly expression of cryptdins 1–3 (Student's *t* test, *p*=0.003) (fig 1 C) and cryptdin 4 (Student's *t* test, *p*<0.0001) (fig 1C). The ability of similar concentrations of isoleucine and arginine to induce human β-defensin in HCT-116 cells⁸ stems, most probably, from the difference in the mechanism regulating expression of cryptdins (α-defensins) versus β-defensins. Different control of α- and β-defensin expression is further reiterated by the fact that spleens challenged with *Listeria monocytogenes*, lungs infected with influenza A, and kidneys challenged with *Candida albicans*, all β-defensin expressing tissues, revealed no differences in the cure rate when mice were fed Perative or other immune enhancing formulas.⁹

To further determine whether expression of cryptdin mRNA was concomitant with protein secretion in primary Paneth cells, we analysed the medium of primary Paneth cells stimulated with the aforementioned concentrations of isoleucine, arginine, and Perative. Cryptdins were isolated from the medium

using a method to denature, renature, and concentrate small cationic proteins¹⁰ followed by reverse phase high performance liquid chromatography.⁸ Protein data of the primary Paneth cell media correlated with that of RNA (that is, higher concentrations of secreted cryptdins were found in the 0.025% Perative treatment—data not shown). Furthermore, eluting fractions exhibited defensin characteristics, such as a size of 3 kDa on sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and activity against *Escherichia coli* DH5α (data not shown).

In summary, our results demonstrate that immune enhancing formulas, such as Perative, can upregulate cryptdin expression in Paneth cells. Further study is needed to delineate the molecular pathways by which nutrients lead to cryptdin upregulation. As defensins display antimicrobial activity as well as recruit the adaptive immunity by cytokine secretion and recruitment of lymphocytes,^{2,3} their secretion could be the first step in a cascade that leads to a general bolstering of the immune system.

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References

- 1 Bevins CL. The Paneth cell and the innate immune response. *Curr Opin Gastroenterol* 2004;**20**:572–80.
- 2 Yang D, Biragyn A, Kwak LW, et al. Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol* 2002;**23**:291–6.

- 3 Lin PW, Simon PO, Gewirtz AT, et al. Paneth cell cryptdins act in vitro as apical paracrine regulators of the innate inflammatory response. *J Biol Chem* 2004;**279**:19902–7.
- 4 Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992;**101**:1644–55.
- 5 Beale RJ, Bryg DJ, Bihari DJ. Immunonutrition in the critically ill: a systematic review of clinical outcome. *Crit Care Med* 1999;**27**:2799–805.
- 6 Froy O, Chapnik N, Miskin R. Mouse intestinal cryptdins exhibit circadian oscillation. *FASEB J* 2005;**19**:1920–2.
- 7 Weiser MM. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. *J Biol Chem* 1973;**248**:2536–41.
- 8 Sherman H, Chapnik N, Froy O. Albumin and amino acids upregulate the expression of human beta-defensin 1. *Mol Immunol* 2006 (in press) (Epub ahead of print, 29 Oct).
- 9 Alder JD, Meulbroek J, Jarvis K, et al. Enteral formula composition does not affect response to lethal infectious challenge in mice. *J Nutr* 1994;**124**:2156–62.
- 10 Froy O, Zilberberg N, Gordon D, et al. The putative bioactive surface of insect-selective scorpion excitatory neurotoxins. *J Biol Chem* 1999;**274**:5769–76.

A new silybin-vitamin E-phospholipid complex improves insulin resistance and liver damage in patients with non-alcoholic fatty liver disease: preliminary observations

Non-alcoholic fatty liver disease (NAFLD) may occur as an expression of a metabolic syndrome or in association with hepatitis C virus (HCV) chronic infection. The contemporaneous presence of NAFLD in this later group of patients may negatively affect the progression of fibrosis and the response to antiviral treatment.^{1,2} It has been suggested

Table 1 Results in the two groups; group A had primitive non-alcoholic fatty liver disease and group B had hepatitis C virus (HCV) related chronic hepatitis C in combination with NAFLD, all HCV genotype 1b, and were non-responders to previous antiviral treatment

	Treated			Not treated		
	Basal	6 months	12 months	Basal	6 months	12 months
Group A						
ALT (nv <40 U/l)	79 (51)	40 (15)**	59 (5)**	54.2 (20)	55.2 (24)	45.2 (34)
γGT (nv <50 U/l)	75 (112)	59 (100)**	60 (33)**	64 (20)	72 (24)	64 (35)
Insulinaemia (nv 5–25 µU/ml)	41.5 (34)	29.6 (26.4)*	30.6 (15.4)*	48.3 (17)	45.2 (12)	40.2 (11)
HOMA	12.3 (6.4)	6.2 (3.9)**	6.4 (3.2)**	13 (6)	11 (4)	11 (5)
Hyaluronic acid (nv <120 ng/ml)	383 (627)	176 (184)*	331.1 (293.2)	422 (504)	517 (283)	498 (145)
MMP-2 (nv 117–410 ng/ml)	151 (132)	141 (84)*	158.2 (165.5)	160 (183)	173 (102)	183 (97)
TGF-β (nv 34.7–63.9 ng/ml)	45.3 (17.3)	32.9 (24.3)*	42.9 (22)	41.2 (22)	43.3 (27.3)	44.7 (31)
US score (median (range))	2 (2–3)	1 (1–2)**	1 (1–2)**	2 (2–3)	2 (2–3)	2 (2–3)
Group B						
ALT (U/l)	69 (28)	45 (18)**	62 (16)	47.8 (30)	50.3 (32)	60.3 (33)
γGT (U/l)	118 (70)	56 (20)**	83 (24)	115 (60)	100 (53)	150 (13)
Insulinaemia (µU/ml)	36.2 (1.5)	27.4 (1.5)*	28.9 (6.5)*	35.4 (3)	34.2 (5.7)	38.2 (4.7)
HOMA	8.4 (7.3)	6 (4.8)*	6.2 (5.1)*	9.8 (5.6)	8.4 (5.1)	8.2 (4.8)
Hyaluronic acid (ng/ml)	1295 (259)	625 (122)*	586.7 (496.2)*	1180 (703)	1372 (806)	1332 (452)
MMP-2 (ng/ml)	292 (201)	137 (53)*	196.6 (94.2)*	280 (300)	311 (278)	299 (123)
TGF-β (ng/ml)	54.1 (21.7)	27 (12.2)*	21.2 (17.4)*	53.3 (18.3)	45.2 (28.5)	49.6 (30)
US score (median (range))	2 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)

p*<0.05, *p*<0.01 versus basal values.

Values are reported as the mean (SD).

ALT, alanine aminotransferase; γGT, gamma-glutamyl-transpeptidase; MMP-2, metalloproteinase 2; TGF-β, transforming growth factor β; US, ultrasonography; nv, normal value.