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Session 8: Probiotics in the defence and metabolic balance of the organism
Probiotics and the immune response to vaccines

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Probiotics are bacteria, but sometimes fungi, which when taken by the oral route may give some health benefits. The most compelling evidence for beneficial effects of probiotics is in the prevention and reduction in the duration of symptoms related to gut infectious disease. There is also evidence to show that some specific probiotics are beneficial in *Clostridium difficile* diarrhoea in the elderly. As further and better controlled clinical studies have appeared, some specific probiotics also appear to have beneficial effects in perhaps preventing and reducing the duration of symptoms due to acquired upper respiratory tract infections. In an attempt to explain these effects, attention has turned to the effects of some specific probiotics on the immune system. There is evidence that some specific probiotics can alter monocyte and natural killer cell function in the blood. Evidence is also accumulating that taking some specific probiotics can boost antibody responses to oral and systemically administered vaccines. The effect when shown is modest and is not always seen in different studies to all vaccines, but there is enough of a trend to make the area worthy of further investigation, particularly to tease out the mechanisms involved.

Probiotic: Vaccine: Antibody: Intestine

Probiotic drinks, capsules, sachets, etc. offer consumers a bewildering array of products with alleged health benefits. At the same time, although most probiotics are bacteria and belong to the Lactobacillus or Bifidobacterium families, a large number of individual strains, alone, in combination with other strains or with added prebiotics further confuses the picture. Taken together with the fact that different strains of probiotics may have markedly different functions, it is hardly surprising that the evidence-base to support health claims of probiotics is often contradictory and confusing.

Because probiotics are taken orally, the obvious place to look for health benefits is in the gut and it is in this area that the evidence-base is strongest. There are many studies in children and adults which clearly show that taking a probiotic gives some degree of protection against gut infections and that they also shorten the duration of symptoms[1–3]. Several mechanisms are probably involved. Probiotics may outcompete pathogens, produce molecules toxic to pathogens, or prevent binding to gut epithelial cells. They may enhance the gut barrier. Easily the most persuasive evidence for health benefits of some specific probiotics against gut infections comes from studies on antibiotic-associated diarrhoea in the elderly. Taking many of the commonly prescribed antibiotics, such as broad spectrum penicillins, clindamycin or cephalosporins, results in the antibiotics killing many of the bacteria normally present in the gut. In health, these commensal bacteria suppress the growth of *Clostridium difficile*; however, when the normal microbes are depleted, *C. difficile* multiplies and produces toxins that kill gut epithelial cells. This leads to a very serious condition termed pseudomembranous colitis with a high mortality. There are now many studies which show that taking a specific probiotic product

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Abbreviation: DTP, diphtheria, tetanus and pertussis; Hib, Hemophilus influenza type b; NK, natural killer.
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can reduce the incidence of antibiotic-associated diarrhoea[4,5]. In a recent study by Hickson et al.[6], the probiotic drink Actimel® reduced antibiotic-associated diarrhoea 3-fold and C. difficile diarrhoea specifically, to zero in the hospitilised elderly. The mechanism might be due to the probiotic preventing the growth of C. difficile in the gut.

Somewhat more surprisingly is the evidence-base which suggests that taking a probiotic orally can offer some protection against respiratory infections[7].This was first hinted at in a study by Turchet et al.[8] who gave autonomous elderly Actimel® and showed that while probiotic consumption did not reduce the incidence of infections (overwhelmingly respiratory), it reduced the duration of symptoms and temperature by about 20%. This result was confirmed by De Vrese et al.[9] who showed that taking probiotics (a mixture of Lactobacillus gasseri PA 16/8, Bifidobacterium longum SP 07/3 and Bifidobacterium bifidum MF20/5) did not reduce the incidence of common cold in healthy adults, but reduced the duration of symptoms by 2 d. In another intriguing study, probiotic treatment (HOAWARU Protect) over a 6-month period in children aged 3–5 years dramatically reduced the incidence and duration of fever, rhinorhoea and cough[10]. Finally, Guillemard et al. recently demonstrated that taking a probiotic drink (Actimel®) significantly reduced the duration of common infectious diseases in the elderly, particularly on upper respiratory tract infections[11]. Overall, therefore, these results indicate that while probiotics may not protect against the acquisition of an upper respiratory tract infection, they consistently appear to reduce the duration of symptoms.

However, these data do present a conundrum, namely how taking a probiotic orally, which does not colonise the gut, or invade the mucosa, results in a beneficial effect at a distant mucosal site. One potential solution to this problem is to postulate that probiotics affect host immune defences.

Do probiotics taken orally affect systemic leucocyte activity?

Several studies have addressed innate immune responses through the analysis of leucocytes in volunteers fed probiotics. After feeding B. bifidum or Lactobacillus acidophilus to healthy adult volunteers, Schiffrin et al. observed an increase in the phagocytic capacity of blood monocytes[12]. Similarly, after feeding Bifidobacterium lactis to healthy elderly volunteers, Gill et al. showed increases in the phagocytic capacity of blood monocytes and neutrophils[13]. Finally, after feeding healthy volunteers Actimel®, Parra et al. demonstrated an increase in oxidative burst by blood phagocytes[14].

Natural killer (NK) cell activity is also modulated by probiotic intake. Three separate studies have shown increases in the NK activity after taking probiotics (B. lactis in one study and Yakult in the two others); with the intriguing results that effects are maximal in individuals with low NK activity[13,15,16].

The commonality between these two sets of studies is that both monocyte function and NK activity are enhanced

by pro-inflammatory cytokines, and data have been produced which suggest that feeding probiotics to children induces a small increase in blood C-reactive protein, another marker of increased systemic cytokine production[17].

In totality, therefore, these results suggest that taking a probiotic can affect the immune function of cells in the blood.

Effects of probiotic intake on the immune response to vaccines

The immune system is enormously resilient and full of redundancy, so it is not possible, for example, to determine if increases in the blood NK activity or monocyte function are biomarkers of increased immune function which would have a clinical relevance, such as less cancer or fewer infections. The best way to measure the function of the immune system is to ask it to do something, for example, respond to an infection. It is, however, not acceptable to experimentally give real infections to people to test the immune system. But there is a well-accepted surrogate of the response to infection, namely the immune response to vaccines against the common infectious diseases. Moreover, vaccine efficacy is measured by the ability to induce serum antibody responses and the level of specific serum antibody elicited by a vaccine usually correlates with protection. Consequently, studying vaccine-induced antibody responses in individuals receiving a probiotic is a gold-standard way to determine if probiotics can modulate immune responses.

In 1995, it was first shown that giving infants a single dose of Lactobacillus casei GG just before the oral administration of a rotavirus vaccine followed by 5 d of probiotic or placebo treatment, resulted in higher serum IgM, IgG and IgA anti-viral titres on day 8[18]. P values, however, did not achieve statistical significance. A very similar type of study was reported by De Vrese et al. who gave young adult volunteers an oral polio vaccine. Probiotic or placebo treatment started a week before vaccination and continued for another month and two different probiotics were used, Lactobacillus rhamnosus GG or L. acidophilus CRL431. Serum antibody responses were measured up to 7 weeks later and it was clear that the probiotics induced significantly higher levels of neutralising antibodies and higher levels of anti-polio serum IgG and IgA[19].

In both the above cases, oral vaccines were used, but more recent studies have determined if probiotics can affect parenteral vaccines. Kukkonen et al. carried out a complex study where a combination of four different bacteria (L. rhamnosus GG, L. rhamnosus LC705, Bifidobacterium breve Bbi99 and Propionibacterium freudenreicherii ssp. shermanii) and a prebiotic were given to mothers pre-term and their children for 6 months after birth[20]. Children received routine diphtheria, tetanus and pertussis (DTP) vaccines at 3, 4 and 5 months and the Hemophilus influenza type b (Hib) vaccine at 4 months and serum antibodies were measured. No effect was seen with DTP, but significantly higher protective Hib titres were seen in children receiving the complex mixture. In a similar, but simpler study along the same lines, West et al.
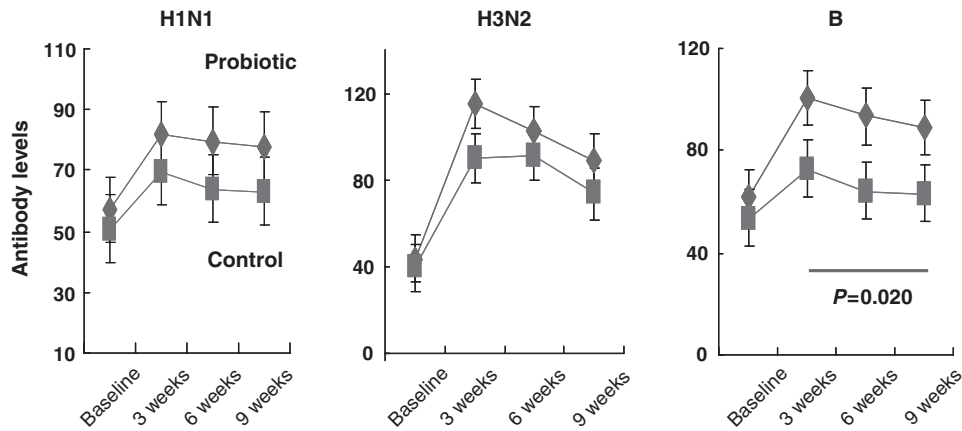
Fig. 1. Taking the probiotic drink Actimel® boosts serum antibody response to influenza vaccination in the elderly, particularly against the B strain. Reproduced from Boge *et al.*[23], with permission of the publishers.

gave infants weaned at between 4 months and 13 months, *Lactobacillus paracasei* F19 for 4 months and measured serum antibody responses to DTP and Hib. Overall infants receiving probiotics had marginally higher levels of antibody to tetanus and diphtheria, but the effects were small and rarely significant[21].

In conclusion, the studies described give limited support for the idea that taking a probiotic can boost vaccine responses, but the effect, especially in children, seems to be modest and inconsistent.

Probiotic effects on influenza vaccination

Adults in the western world rarely receive vaccinations, except in the case of foreign travel, or in a hospital where Hepatitis B vaccination is compulsory. However, for several years, most countries in the developed world have adopted annual influenza vaccination in the elderly and at risk groups (e.g. asthmatics). The elderly are particularly at risk from influenza infection, because their immune system is less efficient than younger individuals because of immunosenescence. Vaccination gives the best protection against influenza, but in the case of flu vaccine, whereas nearly all young people seroconvert after vaccination, in the elderly, even in the autonomous and healthy, seroconversion rates are much lower. Flu vaccination is therefore an excellent tool to study the positive effects of probiotics on immune responses, because it is given as routine clinical practice in the elderly who make suboptimal responses. It also has the advantage that measurement of antibody levels is standardised (haemagglutinin inhibition), antibody levels broadly correlate with clinical protection[22], seroprotection is defined as having a titre >40 and seroconversion is recognised as a 4-fold increase in antibody titre.

Studies have therefore been carried out to determine if taking a probiotic, in this case, Actimel®, can boost antibody responses in the elderly to flu vaccine[23].

In the first pilot study that took place in the winter of 2005–2006, 86 elderly individuals were randomised to receive Actimel® or control for 4 weeks, were vaccinated,

and then received Actimel® or control for a further 7 weeks. In 2005–2006, the two A strains in the vaccine were for H1N1, A/NewCaledonia/20/99, and for H3N2 was A/California/7/2004; the B strains were B/Shanghai/361/2002. Antibody titres, seroprotection rate and seroconversion rate were higher at 3 weeks in the elderly who received Actimel® compared to control; however, in no case did the increase achieve statistical significance.

In 2006–2007, the study was repeated, but this time with 222 elderly volunteers. The vaccine strains had changed somewhat from 2005 to 2006. A/New Caledonia 20/99 was still present but the other A strain was A/Wisconsin/67/2005 and the B strain was B/Malaysia/2506/2004. The protocol was very similar except that product was consumed for 9 weeks post-vaccination. Antibody titres were higher against all three strains at 3, 6 and 9 weeks post-vaccination in elderly receiving Actimel® compared to control, which only reached statistical significance for the B strain (Fig. 1). Furthermore for the B strain, seroconversion was significantly higher in the Actimel® group at all time points, even 5 months after vaccination. Elderly individuals taking Actimel® had higher rates of seroconversion to the H3N2 strain and B strain than the elderly taking the control drink. Taken together, these results suggest that taking a probiotic can boost antibody responses but the effects are quite small and larger studies with a higher power need to be done.

Putative mechanisms by which probiotics may increase antibody titres to parenteral vaccines

Influenza vaccines are given intramuscularly in the upper arm and the immune response generated mainly in the draining axillary lymph nodes. Some communication must therefore exist between probiotic bacteria in the gut and the immune response at a distant site to explain the weak adjuvant effect seen in the clinical studies described earlier. At the moment, however, it is only possible to speculate. A general feature of adjuvants, which boost immune responses, is that they are inflammatory and elicit a danger response in the host. It has already been suggested
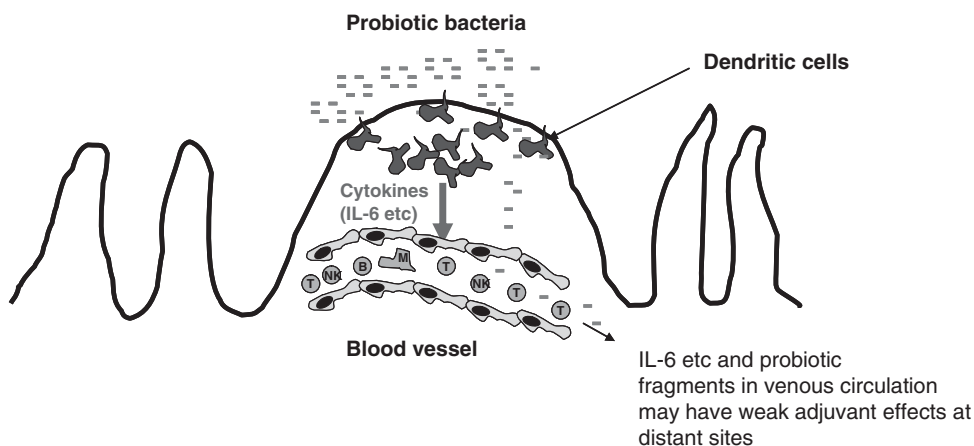
Fig. 2. Putative mechanisms by which probiotics could enhance systemic immune responses. By activating dendritic cells in Peyer's patches, probiotic bacteria may cause cytokine release into the circulation. Alternatively, fragments of probiotic may enter the bloodstream and have a direct but weak adjuvant effect at a distant lymph node.

that probiotics can elicit a response similar to low grade inflammation in children with raised serum C-reactive protein[17]. It is also very well known that C-reactive protein synthesis by liver cells occurs in response to IL-6[24], and finally, one of the best known effects of IL-6 is as a B-cell differentiation factor[25]. So it may be that when taking probiotics, pulses of IL-6 may be generated in the gut, which affect B cells in the axillary lymph nodes (Fig. 2). There is some other evidence to support this idea. First, many probiotics induce pro-inflammatory cytokine responses in dendritic cells *in vitro*, including an IL-6 response[26–28]. Administration of *Lactobacillus plantarum* into the human upper bowel also elicits a transient inflammatory response in the tissue[29].

It is not known which component of the probiotic bacteria may induce local inflammation, or indeed at what site. Indeed it is possible that fragments of probiotic bacteria may enter the blood stream and lodge in the distant lymph nodes to promote a local adjuvant effect (Fig. 2). The small bowel contains many tens of thousands of isolated lymphoid follicles and up to 300 large Peyer's patches[30]. The epithelium overlying the gut lymphoid tissue is specialised to take up bacteria; so, it is likely that when a bolus of probiotic enters the small bowel, where there are few indigenous microbes, they will be taken up by M cells and delivered to the network of dendritic cells in the dome region[31], which respond via pattern recognition receptors and secrete cytokines into the blood. This may also explain the effects of probiotics on monocytes and NK cell function, since these cells are largely resident in the blood and when trafficking through the abundant gut-associated lymphoid tissue, they may be exposed to low doses of cytokines which boost their function.

Why might probiotics shorten the duration of symptoms to common infectious disease in the respiratory tract?

In common infectious diseases, symptoms are due to the direct effects of the pathogen in the tissue and the host response. Symptoms ameliorate when pathogen numbers are reduced by intervention, e.g. an antiviral or antibiotic, or protective immunity. The consistent beneficial effects of probiotics in shortening the duration of symptoms is probably due to their ability to accelerate protective immunity by a day or two resulting in earlier pathogen clearance. Therefore, we would propose that probiotics have a weak adjuvant effect, mediated perhaps by bacterial fragments or cytokines. This can be tested in the model of influenza vaccination in the elderly, since one would expect higher levels of serum antibodies at 7 d post-vaccination in the elderly taking Actimel® compared to placebo.

References

1. Solis B, Samartin S, Gómez S *et al.* (2002) Probiotics as a help in children suffering from malnutrition and diarrhoea. *Eur J Clin Nutr* **56**, Suppl. 3, S57–S59.
2. Hatakka K & Saxelin M (2008) Probiotics in intestinal and non-intestinal infectious diseases – clinical evidence. *Curr Pharm Des* **14**, 1351–1367.
3. Guandalini S (2008) Probiotics for children with diarrhea: an update. *J Clin Gastroenterol* **42**, Suppl. 2, S53–S57.
4. Parkes GC, Sanderson JD & Whelan K (2009) The mechanisms and efficacy of probiotics in the prevention of clostridium difficile-associated diarrhoea. *Lancet Infect Dis* **9**, 237–244.
5. Prantera C & Scribano ML (2009) Antibiotics and probiotics in inflammatory bowel disease: why, when, and how. *Curr Opin Gastroenterol* **25**, 329–333.
6. Hickson M, D'Souza AL, Muthu N *et al.* (2007) Use of probiotic lactobacillus preparation to prevent diarrhoea

associated with antibiotics: randomised double blind placebo controlled trial. *BMJ* **335**, 80; Epublication 29 June 2007.

7. Vouloumanou EK, Makris GC, Karageorgopoulos DE *et al.* (2009) Probiotics for the prevention of respiratory tract infections: a systematic review. *Int J Antimicrob Agents* **34**, 197.E1–10.

8. Turchet P, Laurenzano M, Auboiron S *et al.* (2003) Effects of fermented milk containing the probiotic *Lactobacillus casei* DN-114 001 on winter infections in free-living elderly subjects: a randomised, controlled pilot study. *J Nutr Health Aging* **7**, 75–77.

9. De Vrese M, Winkler P, Rautenberg P *et al.* (2006) Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. *Vaccine* **24**, 6670–6674.

10. Layer JG, Li S, Mubasher EM *et al.* (2009) Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics* **124**, E172–E179.

11. Guillemard E, Tondu F, Lacoin F *et al.* (2009) Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114 001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial. *Br J Nutr* **103**, 58–68.

12. Schiffrin EJ, Rochat F, Link-Amster H *et al.* (1995) Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *J Dairy Sci* **78**, 491–497.

13. Gill HS, Rutherfurd KJ, Cross ML *et al.* (2001) Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. *Am J Clin Nutr* **74**, 833–839.

14. Parra D, Martinez de Morentin B, Cobo JM *et al.* (2004) Monocyte function in healthy middle-aged people receiving fermented milk containing *Lactobacillus casei*. *J Nutr Health Aging* **8**, 208–211.

15. Nagao F, Nakayama M, Muto T *et al.* (2000) Effects of a fermented milk drink containing *Lactobacillus casei* strain Shirota on the immune system in healthy human subjects. *Biosci Biotechnol Biochem* **64**, 2706–2708.

16. Morimoto K, Takeshita T, Nanno M *et al.* (2005) Modulation of natural killer cell activity by supplementation of fermented milk containing *Lactobacillus casei* in habitual smokers. *Prev Med* **40**, 589–594.

17. Marschan E, Kuitunen M, Kukkonen K *et al.* (2008) Probiotics in infancy induce protective immune profiles that are characteristic for chronic low-grade inflammation. *Clin Exp Allergy* **38**, 611–618.

18. Isolauri E, Joensuu J, Suomalainen H *et al.* (1995) Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. *Vaccine* **13**, 310–312.

19. De Vrese M, Rautenberg P, Laue C *et al.* (2005) Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination. *Eur J Nutr* **44**, 406–413.

20. Kukkonen K, Nieminen T, Poussa T *et al.* (2006) Effect of probiotics on vaccine antibody responses in infancy – a randomized placebo-controlled double-blind trial. *Pediatr Allergy Immunol* **17**, 416–421.

21. West CE, Gothefors L, Granström M *et al.* (2008) Effects of feeding probiotics during weaning on infections and antibody responses to diphtheria, tetanus and Hib vaccines. *Pediatr Allergy Immunol* **19**, 53–60.

22. Nauta JJ, Beyer WE & Osterhaus AD (2009) On the relationship between mean antibody level, seroprotection and clinical protection from influenza. *Biologicals* **37**, 216–221.

23. Boge T, Rémigy M, Vaudaine S *et al.* (2009) A probiotic fermented dairy drink improves antibody response to influenza vaccination in the elderly in two randomised controlled trials. *Vaccine* **27**, 5677–5684.

24. Mortensen RF (2001) C-reactive protein, inflammation, and innate immunity. *Immunol Res* **24**, 163–176.

25. Akira S, Hirano T, Taga T *et al.* (1990) Biology of multifunctional cytokines:IL 6 and related molecules (IL1 and TNF). *FASEB J* **4**, 2860–2867.

26. Karlsson H, Larsson P, Wold AE *et al.* (2004) Pattern of cytokine responses to gram-positive and gram-negative commensal bacteria is profoundly changed when monocytes differentiate into dendritic cells. *Infect Immun* **72**, 2671–2678.

27. Mohamadzadeh M, Olson S, Kalina WV *et al.* (2005) Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. *Proc Natl Acad Sci USA* **102**, 2880–2885.

28. Hart AL, Lammers K, Brigidi P *et al.* (2004) Modulation of human dendritric cell phenotype and function by probiotic bacteria. *Gut* **53**, 1602–1609.

29. Van Baarlen P, Troost FJ, Van Hemert S *et al.* (2009) Differential NF-kappaB pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci USA* **106**, 2371–2376.

30. MacDonald TT (2008) The gut is still the biggest lymphoid organ in the body. *Mucosal Immunol* **1**, 246–247.

31. Jameson B, Baribaud F, Pöhlmann S *et al.* (2002) Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in human and rhesus macaques. *J Virol* **76**, 1866–1875.