Probiotics

Probiotics as mainstream allergy therapy?

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Commentary on the paper by Weston *et al* (see page 892)

Probiotics, live microorganisms which confer a health benefit on the host when administered in adequate amounts,[1] continue their impressive march from the fringes of scientific propriety to potential mainstream therapy. Claims for their efficacy, from the time of Metchnikoff and Nissle onwards, have sometimes appeared unfeasibly broad, with suggested benefits ranging from increased longevity to prevention of cancer and immunomodulation.[1] Remarkably, the earliest patient known to have shown resolution of eczema with probiotics was Adolf Hitler, whose symptoms of irritable bowel syndrome also abated when treated with Mutaflor (*Escherichia coli* Nissle 1917) by the extravagantly quackish Theodor Morell, after dysbiosis was diagnosed by Nissle.[2] With over 1¼ million internet pages, many commercially driven, probiotics remain big hitters in the alternative medicine arena. In contrast to many inhabitants of the fringe scene, they do however have a record of properly conducted trials confirming efficacy in specific areas – notably infectious diarrhoea and urogenital infection – and a burgeoning portfolio of basic scientific studies, which have led to the remarkably rapid introduction into human trials of transgenic probiotics, engineered to pump out immunosuppressive cytokines.[3–5] No other therapeutic modality spans the divide between internet voodoo and cutting edge high tech in this way.

One very topical question is whether probiotics may have a role in the prevention or treatment of allergies, by providing appropriate exposures to the nascent immune system for generation of tolerance.[6] A possible benefit in primary prevention of allergy was suggested by a study of perinatal administration of *Lactobacillus* GG (LGG) to at risk infants, showing reduction of eczema to age 4, but without effect upon overall atopy or IgE responses.[7] Less well characterised reduction of allergies to age 20 was reported in a Czech cohort given perinatal *E coli*.[8] Further studies are needed to determine whether perinatal probiotics may help infants at genetic risk of allergy.

So far, the data for probiotic treatment of established allergy stack up best for eczema, and those temperamentally disposed to meta-analysis will now be sharpening their pencils. The randomised controlled trial reported here by Weston and colleagues, using *Lactobacillus fermentum* PCC, shows that probiotics may indeed provide benefit in infant eczema,[9] and augments similar recent findings from three other groups. In infants with mild eczema, LGG and *Bifidobacterium lactis* Bb12 both induced striking resolution of disease with reduction in inflammatory markers.[10] Subsequent studies have shown more modest benefit, possibly limited to those with IgE mediated allergy. In a study of older children (1–13 years old), combination *L rhamnosus* and *L reuteri* strains improved both symptoms and extent of eczema in comparison to placebo, reducing serum eosinophil cationic protein without effects upon stimulated cytokine production.[11] The effects were more pronounced in children with IgE mediated allergy (with positive skin prick or specific IgE). Further analysis identified significant reduction in both intestinal symptoms and paracellular intestinal permeability, suggesting augmentation of the intestinal barrier as one mechanism of action.[12] A further large scale study using LGG in comparison to a mixture of four organisms (LGG plus *L rhamnosus*, *B breve*, and *Propionibacterium freudenreichii* strains) found benefit only with the single organism, again limited to those with IgE mediated allergy.[13] Study of the mechanism identified modest reduction in intestinal TNF-α and increase in faecal IgA in those with IgE mediated allergy.[14] The study by Weston and colleagues is notable for the severity of eczema in comparison to some previous studies, and 75% of the children included had IgE mediated allergy.[9] Subgroup analysis would certainly be interesting, to determine whether children without IgE mediated allergy consistently fail to respond.

So is the mechanism of action of probiotics in eczema simple augmentation of the epithelial barrier? Maybe so, but that would suggest that all probiotics with this effect should act pretty equally, and that does not seem to be the case so far. What is now becoming clear from basic studies, notably from Jeffrey Gordon's group, is the immense and individually specific influence members of the flora exercise upon gene expression within the host, including, but not limited to, remodelling of villous structure, global energy balance, induction of immune tolerance, and priming of the hypothalamo-pituitary axis.[15] The widespread change in initial colonisation patterns of infants during the last half century may thus have been of much greater importance than previously recognised.[6] Certainly, there is evidence that allergic infants have different flora from non-allergic infants, often pre-dating the manifestation of allergy and thus potentially causative.[16 17] Abnormal initial colonisation patterns, as found following caesarean section, show some normalisation with age, but abnormalities remain at 7 years and are probably lifelong.[18]

Specific input from the flora, relevant to the pathogenesis of eczema, may be mediated in several ways in addition to regulation of permeability. Firstly, and particularly in infants wearing nappies, gut bacteria will contribute significantly to the cutaneous flora. The pro-inflammatory role of cutaneous staphylococci and their toxins in the pathogenesis of eczema is well established,[19] suggesting that the addition of organisms that competitively inhibit staphylococcal persistence within the gut may be of benefit in established infant eczema. Secondly, probiotics may stimulate mucosal IgA production, which regulates antigen entry, and can render food antigen components less immunogenic.[20] Thirdly, gut bacteria are important in inducing regulatory T cell (T_{REG}) production, and blockade of interaction between gut bacteria and Toll receptors on innate immune cells impairs mucosal tolerance.[6 21] Although many allergic phenomena are driven by type 2 T helper cell (T_H2) responses, their initiation or termination is effectively determined by T_{REG} populations, including TGF-β producing type 3 T helper cells (T_H3), IL-10 producing TR1 cells, and CD4+CD25+ cells.[21] Gut derived T_{REG} cells play an important role in prevention of systemic sensitisation, and sensitised infants and children show defective induction of these populations[22] with delayed immune maturation.[23] Importantly, tolerance is regained when an adequate T_{REG} response occurs.[24] We do not yet know which organism(s) are most potent in inducing T_{REG} responses, but it is likely that these

would have greater efficacy. Use of single lactobacilli may be providing some proof of principle, but is unlikely to be the end of the story.

Fourthly, probiotics might influence lymphocyte tropism. Could lymphocytes become activated within the intestine, and then drive inflammation within the skin? Maybe. Homing of lymphocytes to the intestine is mediated by expression of β7 integrins: interaction between lymphocyte α4β7 integrin and endothelial MAdCAM-1 is required to enter Peyer's patches, while intraepithelial lymphocytes require αEβ7 to bind to epithelial E-cadherin.[25] By contrast, for homing to normal skin, lymphocytes must express cutaneous leukocyte antigen (CLA), which binds to E-selectin.[25] This specificity is lost in established eczema, and large numbers of $\alpha E\beta7^+$ cells, potentially of gut origin, are found within inflamed skin.[26] This might explain a role for luminal antigens in ongoing disease, but what about its initiation? What is notable about CLA expression is that it requires a glycosylation process, dependent upon addition of a single fucose molecule to P-selectin glycoprotein-1, a molecule expressed on all peripheral T cells, by fucosyltransferase VII (FucTVII).[27] At birth, there is virtually no CLA expression upon peripheral lymphocytes, while β7 integrin expression is widespread.[28] Skin tropism is therefore acquired post-natally and is dependent on factors that upregulate lymphocyte FucTVII gene expression. Such newly addressed lymphocytes are predominantly T_H2 phenotype,[25] which might explain why eczema is more common in early life. One property used by gut bacteria to establish a niche is their ability to regulate host fucosyl transferase gene expression, which alters the composition of the glycocalyx.[15] It is not yet known whether FucTVII, critical also for biosynthesis of sialyl Lewis-x, can be induced in this way, but it is notable that sialyl Lewis-x is expressed by *Helicobacter pylori* and other gut organisms, and its upregulation can block host type 1 T helper cell (T_H1) responses.[29 30] Thus it remains possible, although clearly speculative, that overgrowth of bacteria capable of regulating FucTVII expression may promote a skin-trophic T_H2 phenotype amongst mucosally activated lymphocytes. Conversely, dominance of probiotic organisms that do not modulate FucTVII may reduce mucosal conversion of lymphocytes to this phenotype and thus inhibit development of eczema.

Finally, an unexpected link has emerged from multiple gene-array studies. The small proline rich protein SPPR2a, important in epithelial differentiation and permeability, is strikingly induced in mucosal allergy by IL-13.[31] SPPR2a expression is also notably modulated by the gut flora, being upregulated 280-fold by *Bacteroides thetaiotamicron* while unaffected by *E coli* or *Bifidobacterium infantis*.[32] Different bacteria may thus have very different effects upon this axis of the allergic response.

All this is very theoretical, and maybe over the top for a therapy whose potency could hardly so far be described as rocket fuel. However, despite knowing so little about mechanism, there is consistency of effect in a common and therapeutically challenging childhood disorder. Adverse effects are so far uncommon, to the extent that milk manufacturers are marketing probiotic enriched formulae. But much more basic work is needed on all aspects of mechanism if this is to mature into a fully fledged therapy, and that clearly mandates more focus on the gut in children with eczema. Do we know which probiotic or combination we should be using? Is this a one size fits all treatment? I don't think so.

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