Diet, the intestinal microbiota and immune health in ageing

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Abstract:

Many countries are facing population ageing with those over 65 years of age likely to represent the largest population over the next 10-20 years. Living longer often comes with poor health and in particular a decline of immune function characterised by poor vaccine responses and increased risk of infection and certain cancers. Ageing and diet represent major intrinsic and extrinsic factors that influence the makeup and activity of resident intestinal microbes, the microbiota, the efficient functioning of which is essential for sustaining overall health and the effectiveness of the immune system. The provision of elderly specific dietary recommendations appears to be lacking but necessary since this population has an altered microbiota and immune response and may not respond in the same way as their healthy, younger counterparts. We have reviewed the evidence supporting a role for the diet and in particular dietary carbohydrate, protein and fat, in influencing the microbiota and its generation of key metabolites that impact on the efficient functioning of immune cells during ageing, and how dietary intervention might be of benefit in improving the intestinal health and immune status in the elderly.

Keywords

elderly, immunosenescence, short chain fatty acids, microbiota, fibre, protein, fat.

Introduction

The UK population is growing with increasingly more people reaching the eighth decade and beyond (Christensen et al., 2009). This is progressively becoming a problem since these extra years of life are often accompanied by failing health, with elevated incidences of age associated diseases such as type II diabetes, cardiovascular disease, cancer, and increased risk of infections causing influenza and pneumonia coupled with ineffective responses to vaccinations (Castle, 2000). This creates problems for social care, healthcare and the economy, with 72% of social care requests between 2014 and 2015 from people aged 65 years and older (hscic, 2015) implying that a large proportion of these elderly citizens are not in good health. Between 2001 and 2011 the proportion of care home residents aged over 85 years old increased in most regions in England and Wales with the highest increase, 13.2%, seen in the East of England (ONS, 2014).

There is uncertainty regarding the definition of ageing and aged and elderly populations, as ageing encompasses both biological and environmental factors in addition to chronological age. According to the United Nations report of World Ageing (DESA, 2002) older people are classed as being 60 years or older, with some studies classifying those aged over 85 years as the “oldest old” (Forsey et al., 2003; Wikby et al., 2002). In considering different life expectancies in different countries the classification of aged and elderly may differ widely across the globe (Reques Velasco, 2008; Wilson et al., 2011; Wilson, 2014). For the purpose of this review the terms “aged” and “elderly” refer to individuals aged 60 years and older, although some studies referenced use different definitions.

The age-related decline in overall health is accompanied by immunosenescence and the progressive decline in immune function, which has been associated with chronic low grade inflammation termed “inflammaging” (Franceschi et al., 2007). Consistent with this description is the increased serum levels of the pro-inflammatory cytokine IL-6 along with increased intestinal permeability in elderly people (Man et al., 2015). T cells are particularly affected by age, a key example being the reduction in naïve $CD8^+$ T cells and accumulation of oligoclonal memory $CD8^+$ T cells (Czesnikiewicz-Guzik et al., 2008; Khan et al., 2002). This reduction in naïve $CD8^+$ T cells has been shown to correlate with impaired T cell priming compared to middle aged subjects (Briceño et al., 2016). Declining T cell function during ageing is compounded by a progressive decline in naïve T cell production by the thymus. This process, termed as age-related thymic involution, starts from as early as the first year of human life with the loss of functional epithelium occurring at a rate of approximately 3% per year during adulthood (Aw et al., 2009; Taub and Longo, 2005). Ageing also impacts B cells although perhaps not in the same way or to the same extent as T cells (Scholz et al., 2013). Whereas mouse studies provide evidence of declining bone marrow haematopoiesis and B cell production during ageing, observations in humans suggest that B lymphopoiesis is maintained well into old age. More consistent are the findings that humoral immune responses in aged mice and humans are less robust and poorly protective compared with those in young adults, although there may be wide variation between individuals with environmental factors contributing to this variability. Intrinsic B cell defects and the decline in production of antibody to T cell-independent antigens in the elderly accounts for their poor response to pneumococcal vaccines and the reliance of herd immunity and vaccinating children to protect them against infection (Simonsen et al., 2011).

Ageing also impacts the motor and sensory function of the gastrointestinal (GI) tract with the elderly having increased susceptibility to GI-complications of comorbid illnesses (Rayner and Horowitz, 2013). Age-related changes in GI-tract function particularly affect the oesophagus and colon with reduced peristaltic pressure in the oesophagus leading to dysphagia and gastroesophageal reflux, as well as a reduction in motility in the colon, which can cause constipation (Grassi et al., 2011). These symptoms alone may impact on appetite or swallowing ability and thus affect the quantity and types of food eaten, in addition to the loss of dentition with age resulting in a reduced ability to chew food (Hickson, 2006). Ageing also impacts on resident intestinal microbes, the microbiota, with striking changes in its makeup and composition being described in elderly subjects (O'Toole and Brigidi, 2013). Utilizing fecal samples to interpret changes in populations within the GI-tract and in particular the colon, it has been shown that while the total number of bacteria remains fairly constant, the different genera making up the microbiota change with age (Woodmansey, 2007). Reductions in the proportion of *Bacteroides*, *Clostridia* and total lactobacilli populations paralleled with increases in fusobacteria, streptococci and staphylococci have been described in faecal samples from the elderly, compared to those from younger subjects (Woodmansey et al., 2004). Higher levels of bacterial groups from the *Clostridium* cluster XIVa have similarly been described in microbiota profiles of young and elderly subjects compared to centenarians, with Bacteroidetes having a lower relative contribution to the microbiota of elderly compared to young individuals (Biagi et al., 2010). The functional significance of these or any other changes in the relative abundance of specific bacterial populations is, in view of the inherent complexity of the microbiota, comprising more than 1,000 species (Tremaroli and Bäckhed, 2012), and the interdependency of different bacteria,

difficult to discern. Of note, lower species diversity and a reduction in *Bifidobacteria* in faecal samples of elderly subjects (67-88y) (Hopkins et al., 2002) corresponds with altered functionality and the reduced ability of *Bifidobacteria* strains from elderly subjects (74-93y) to adhere to intestinal mucus (He et al., 2001; Ouwehand et al., 1999) compared to strains isolated from younger age groups.

Alterations in the intestinal microbiota have potential dietary implications in considering the essential role it plays in digestion and the efficient conversion of complex plant polysaccharides into short chain fatty acids (SCFAs) that provide a significant proportion of the hosts daily energy needs and regulate immune responses and inflammation (Marchesi et al., 2016). A loss of carbohydrate fermenting bacteria or reduction in dietary intake of plant based polysaccharides often seen in the elderly (Bartosch et al., 2004; Woodmansey et al., 2004) can lead to the microbiota switching to other substrates, notably protein or amino acids. The fermentation of amino acids, in addition to releasing beneficial SCFAs produces a range of potentially harmful compounds of which ammonia, phenols, p-cresol, certain amines and hydrogen sulphide can, in animal models, contribute to intestinal diseases such as colon cancer or inflammatory bowel disease (Windey et al., 2012). These findings highlight the need to carefully consider the dietary requirements of the elderly in terms of sustaining and promoting the health of the GI-tract and of its resident microbes. This review will discuss how the diet impacts on the immune system, focusing on the production of microbe-generated metabolites as a result of food processing, and how this knowledge may be of use in restoring gut health in the elderly.

The microbiota and its effect on the immune system

The colon is populated with in excess of 1×10^{14} bacterial cells and is dominated by the phyla Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria of which the Firmicutes and Bacteroidetes predominate (Maukonen and Saarela, 2015; Plé et al., 2015). The microbiota is highly variable between individuals although there is an identifiable core that is independent of gender, race and age comprising approximately 55 species that are common to >90% of individuals. This highly conserved core encodes gene products unique to bacterial genomes that are required for the degradation of complex plant-based carbohydrates, synthesis of SCFA and indispensable amino acids and vitamins, highlighting the interdependency and mutualistic relationship between the microbiota and its host (Arumugam et al., 2011; Qin et al., 2010; Turnbaugh and Gordon, 2009). Additional complexity is provided by the virome, which contains the most abundant and fastest mutating genetic elements (e.g. prophages, retroviruses) on earth (Minot et al., 2013; Reyes et al., 2010; Virgin, 2014), in addition to protozoa and fungi with much less being known of the role these non-bacterial constituents play in promoting GI-tract health, or disease. The stability of the intestinal microbiota is central to maintaining the integrity of the epithelial barrier and immune homeostasis. Disruptions to this balance and resulting dysbiosis are often associated with inflammation and compromised barrier function allowing microbiota constituents with the potential to cause significant disease (pathobionts) to translocate the epithelium and to spread and infect other distant sites of the body, with potentially catastrophic and fatal consequences (Plé et al., 2015). The importance of the intestinal microbiota for preserving health is further emphasised by sterile, germ-free, animals that suffer from nutritional deficiencies, poor growth, increased intestinal permeability, a functionally immature immune system and altered neurochemical production and brain development all of

which can be significantly overcome or reversed upon microbial colonization and conventionalization (Wostmann, 1981). The human intestinal microbiota is formed soon after birth and becomes fully established within the first decade of life at which time it resembles the complexity of the microbiota of adults (Palmer et al., 2007). The intestinal microbiota is established in parallel with the immune system, which is functionally immature in new-born infants that become immunocompetent and able to mount effective T and B cell responses by the time the microbiota is fully established (Adlerberth and Wold, 2009). An important factor in the efficient development of both the intestinal microbiota and immune system is early life nutrition and the positive impact breastfeeding and breast milk has in helping establish beneficial *Bifidobacteria*, which can also modulate the immune system (Fanaro et al., 2003; Solís et al., 2010). Breast-fed infants also exhibit reduced expression of genes encoding products that can prime mucosal inflammatory responses including natural killer (NK) cell lectin-like receptors (KLRF1), IL-1α and arachidonate 5-lipoxygenase (Schwartz et al., 2012). This highlights that the functioning and health of the GI-tract, the immune system and the microbiota are closely interrelated and that environmental factors, such as the diet, can have influential effects on this important relationship; discussed in more detail below.

Diet and the microbiota

A key example of the impact diet has on shaping the microbiota is the divergent dietary patterns of children from rural Africa and Europe, where Burkina Faso children consume up to 14.2 g fibre per day and display strikingly different faecal microbiota profiles compared to their European counterparts that consume 8.4 g dietary fibre (De Filippo et al., 2010). Burkina Faso

children were exclusively colonized by *Xylanibacter*, *Prevotella*, *Butyrivibrio* and *Treponema* species, consistent with their ability to utilize cellulose and xylose, present in plant fibres, for energy provision and generation of SCFAs. Negative correlations between faecal concentrations of SCFAs and intake of refined grains and added sugar and positive correlations between the intake of fruits and vegetables have also been reported (Yang and Rose, 2014). Additionally, increased intake of dietary fibre (40g) in healthy adults aged between 19 and 25 years old has been associated with overexpression of genes involved in methanogenesis and glycan and lipid metabolism, when compared to lower fibre intakes of 10g, consistent with increased fermentation as a result of higher levels of fermentable dietary fibre in the colon (Tap et al., 2015). A striking relationship between adherence to a Mediterranean (Med) diet and abundance of faecal SCFAs has also been reported, which strongly correlated with consumption of fruits, vegetables and fibre. This was accompanied with the prevalence of *Prevotella* in plant based diets with metagenomic analysis revealing a significant increase in the abundance of genes associated with polysaccharide degradation and SCFA metabolism (De Filippis et al., 2015). In an elderly (≥65y) population where subjects were fed low fat, high fibre diets, greater diversity in bacterial microbiota profiles were observed, while those with "moderate to high" levels of fat and "low" fibre intakes had the least diverse microbiota (Claesson et al., 2012). Collectively, these studies show that dietary fibre intake influences the diversity of the intestinal microbiota and the dominant species, suggesting that a richly diverse microbiota may be beneficial in, for example, protecting against and excluding enteric pathogens which cause intestinal diarrhea-associated diseases. Of note, despite poor sanitation and hygiene in Burkina Faso, species of diarrhea inducing *Enterobacteriaceae* were underrepresented (De Filippo et al., 2010).

Dietary fibre is not the only determinant of microbial species diversity as significant reductions in *Bacteroides* and *Bifidobacterium* species are apparent in both vegetarians and vegans. One comparative study showed a significant reduction of *Enterobacteriaceae* in vegans compared to omnivorous control subjects (Zimmer et al., 2012). However, this is inconsistent with earlier, smaller scale studies, reporting greater proportions of *Lachnospira* in vegetarians and vegans, compared to predominance of *Clostridium* in omnivores (Koeth et al., 2013). Differing dietary patterns can be seen to impact on the composition of the microbiota with regards to the production of specific metabolites. Consumption of protein rich animal foods and fat have been strongly associated with greater production of trimethylamine N-oxide (TMAO) from L-carnitine and phosphotidylcholine in these foods with *L. ruminococcus* abundance (De Filippis et al., 2015). High levels of TMAO in the blood are associated with increased risk of cardiovascular disease, which is thought to be a consequence of its negative impact on cholesterol metabolism in the intestine, liver and artery wall (Wilson et al., 2013). Of note, TMAO, has been reported as being undetectable in the plasma or urine of long term vegans after a meat challenge, implying that the required gut microbes to produce TMAO are absent or not present in sufficient numbers within the vegans' microbiota (Koeth et al., 2013). This suggests that long-established habitual dietary habits exert strong control over the makeup of the microbiota and that occasional shifts in dietary habits, such as high (meat) protein intake in vegans/vegetarians, may not lead to the production of harmful metabolites. The production of N-nitroso compounds from high meat consumption that have been associated with elevated risk of colorectal cancer which can be suppressed by including soy protein in high meat containing diets (Hughes et al., 2002),

indicating that it is important to consider the type of protein and the dietary interactions that occur and the whole diet and not just one component or nutrients in isolation.

The majority of dietary fats are absorbed in the small intestine (Scott et al., 2013) although some can reach the colon where they are metabolised by the microbiota as seen in studies assessing faecal recovery of radioactive carbon (^{14}C), after consumption of ^{14}C-labelled lipids (Gabert et al., 2011; Murphy et al., 1995; Thompson and Spiller, 1995). Conjugated linoleic acid (CLA) is naturally present in red meat and dairy products (O'Shea et al., 2004) and can also be produced by the human intestinal microbiota (Coakley et al., 2009; Coakley et al., 2003; Devillard et al., 2009; Gorissen et al., 2010; McIntosh et al., 2009). The variable production of positional and geometric isomers of CLA may impact on health in different ways with a comparison of two healthy individuals with different biohydrogenation characteristics finding that bacterial metabolism may influence the CLA isomers present. The subject having a microbiota dominated by Bacteroidetes had mostly rumenic acid in their faecal sample while the faecal samples of the other subject with a microbiota dominated by Firmicutes contained mostly *trans*-10,*cis*-12 linoleic acid (Devillard et al., 2009). This may have significant health implications since the *trans*-10,*cis*-12 isomer of linoleic acid can negatively effect blood lipid profiles (Tricon et al., 2004) and higher proportions of Firmicutes have been observed in high fat feeding studies (Ley et al., 2006; Turnbaugh et al., 2006). However, this is not wholly consistent with the findings of others (Daniel et al., 2014; Duncan et al., 2008) documenting the opposite effect, or no relationship at all.

While it is not yet fully understood how the microbiota interacts with and modulates the immune system, direct activation of immune cells by microbial metabolites is a major focus of current research activity.

Dietary metabolite impact on the immune system and the influence of the microbiota

There are three possible metabolic pathways that can produce bacterial metabolites that influence the immune system with evidence for their effect in healthy adults and in elderly populations coming from various lines of investigation. The first involves the production of SCFA by bacterial fermentation of complex plant based polysaccharides, such as non-starch polysaccharides, resistant starch and oligosaccharides, in addition to gases such as hydrogen, carbon dioxide and methane (Scott et al., 2013) (Figure 1A). These products of bacterial fermentation are thought to promote the establishment of T_{reg} cells that are important in establishing and maintaining immune tolerance, as well as providing a source of energy for host cells. Although it is difficult to attribute the production of beneficial metabolites such as SCFA to particular members of the intestinal microbiota, in the colon members of the Clostridia genera that are particularly abundant in the colonic mucosa have been shown to be required for the generation of regulatory T (T_{reg}) cells (Atarashi et al., 2013; Atarashi et al., 2011). Also, segmented filamentous bacteria which are prominent in the small intestine have been shown to bind IECs and provide them with SCFAs, including butyrate (Hamer et al., 2008) that provides up to 85% of colonic epithelial cell energy requirements (Cultrone et al., 2015). Although these findings provide documentary evidence of the effect of dietary fibre derived metabolites on T_{reg} cell induction in animal models (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013),

extrapolating this to humans is not straightforward as the anatomy and physiology of the mouse and human GI-tract is not identical (Nguyen et al., 2015). It is important therefore to validate the effects and outcomes seen in mice in human intervention trials, which currently are very limited.

Some *ex vivo* studies using cultured human cells have shown that provision of SCFAs, and butyrate, have immunosuppressive effects on antigen presenting cells and in particular, dendritic cells (DC) (Berndt et al., 2012; Bohmig et al., 1997; Liu et al., 2012). Other *in vitro* model studies suggest that while SCFA's are important, the plant fibres themselves may have a direct effect on immune cells, and DCs, in reducing pro-inflammatory cytokine production and DC maturation (Bermudez-Brito et al., 2015). These findings must however be interpreted with caution since *in vitro* models cannot directly represent the *in vivo* situation since other essential components of the intestinal mucosal barrier including mucus and the microbiota are absent.

An *in vivo* study investigating the impact of probiotics and prebiotics (galacto-oligosaccharides, GOS) during pregnancy and after birth have shown that the treated infants displayed a reduction in IgE-associated eczema and atopic eczema (Kukkonen et al., 2007). These findings were interpreted as a positive impact of the biotic therapy on the development of these diseases via effects on the microbiota, since the GI-tract of the supplemented infants were colonised with higher levels of the beneficial *Lactobacilli* and *Bifidobacteria*. However, as this study focused primarily on the probiotics provided to the infants, as opposed to co-administered GOS, the observed effects may have been due to a synergistic effect of both the probiotics and prebiotic (synbiotics). Similarly, provision of xylo-oligosaccharides and inulin have been shown to increase faecal secretory IgA content and increased levels of anti-inflammatory IL-13, while reducing pro-inflammatory IL-1β in whole blood samples incubated with bacterial

lipopolysaccharide (LPS) (Lecerf et al., 2012). These effects were associated with increased production of total SCFA and reduced concentrations of p-cresol and acetate, implying that the observed beneficial immunomodulatory effects were a direct result of these bacterial metabolites.

The second metabolic pathway involves protein based indigestible matter that is fermented by the resident bacteria to produce phenols, ammonia, nitrates and branched chain fatty acids (BCFAs), as well as SCFA and gases (Figure 1B). Some of these metabolites can have detrimental effects on health in promoting colonic inflammation and dysbiosis (Cultrone et al., 2015). Interpreting the effects of these metabolites on health is therefore complicated and compounded by contrasting effects of the same or different metabolites in different groups of individuals. No difference in immune parameters or immune cell numbers were found when comparing consumption of a vegetarian versus meat-rich western diet in male athletes (Richter et al., 1991). However, supplementing the diet of endurance athletes or adults carrying out intensive exercise with amino acids has been shown to improve immune responses by preventing the immune suppressive effects of elevated IL-1β and IFN-γ, decreased IL-10 (Kraemer et al., 2014), reduced lymphocyte counts, increased levels of acute phase response proteins (C-reactive protein, CRP), and increased neutrophil counts (Murakami et al., 2009) observed in placebo groups. Since endurance athletes display compromised immunity and increased susceptibility to infections similar to the immune status of elderly individuals, this data may be relevant to an ageing population. Significantly increased oxidative burst activity of monocytes and lymphocytes was observed from *ex vivo* p-cresyl sulphate (pCS) administration to whole blood samples, while *p*-cresol significantly inhibited these effects (Schepers et al., 2007) implying that *p*-cresol suppresses monocyte and lymphocyte activation, while its metabolite pCS may have a

pro-inflammatory effect. This is further implied since reduction in p-cresol levels was associated with increased faecal secretory IgA and increased levels of IL-13 but reduced IL-1β in LPS-stimulated whole blood samples (Lecerf et al., 2012). Supplementing amino acid consumption with carbohydrates such as resistant starch in human intervention studies has shown reductions in the production of protein metabolites including phenols (Birkett et al., 1996). In contrast, another study has shown that N-nitroso compounds and ammonia were unaffected although this study was underpowered and there was substantial variability between subjects (Silvester et al., 1997). On balance therefore, if excess fermentable carbohydrate or resistant starch is present in addition to any residual protein reaching the colon, its higher availability may enable bacterial metabolism of SCFAs for example, while the protein may provide an energy source for the colonic bacteria.

The third pathway involves fatty acids reaching the colon, typically of polyunsaturated fatty acids (PUFA) origin, which are metabolised by the microbiota to produce CLA isomers (Figure 1C). The potential for the colon to contribute to fatty acid absorption has not been studied in detail to date. *Bifidobacterium* species can metabolise linoleic acid and α-linolenic acid to produce CLA and conjugated linolenic acid (CLnA) (Coakley et al., 2003; Gorissen et al., 2010) although their overall effect on the mucosal and systemic immune system is less clear. Of the limited number of human studies carried out to date most find no differences in immune parameters after CLA supplementation (Albers et al., 2003; Kelley et al., 2001; Kelley et al., 2000; Tricon et al., 2004). However, in one study supplementation of CLA to human volunteers was found to increase plasma IgA, as well as IgM and reduced IgE, in addition to reducing IFN-γ, with the reduction still apparent after the washout period (Song et al., 2005). Another study

using a higher dose of 3g per day found a significant increase in mitogen-induced lymphocyte proliferation in subjects supplemented with 80:20 *cis-9,trans-11: trans-10,cis-12* (Nugent et al., 2005). In contrast, Albers et al. (2003) found no significant differences when comparing sunflower oil to a 50:50 or 80:20 mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 linoleic acid. Although, comparison of the reference oil to 50:50 CLA supplementation, and 50:50 to 80:20 CLA, revealed that twice as many subjects produced sufficient hepatitis B-specific antibodies (≥10 IU/l) in their serum to be considered protective against hepatitis B infection, this did not reach statistical significance. In a cross over study to determine whether these two main isomers have differing effects subjects were given capsules containing varying doses of each isomer with no differences in immune function being evident, although *trans*-10,*cis*-12 showed detrimental effects on blood lipids (Tricon et al., 2004). Isomers aside, a dose dependent reduction in mitogen induced T cell activation was also observed, along with significant dose effects on TNF-α, IL-1β, IFN-γ, IL-10 and IL-5.

The consumption of high levels of both *n-3* and *n-6* PUFAs has also been be associated with reductions in secreted TNF receptors (sTNFR1 and 2) in plasma but this effect is only apparent with high intakes of *n-3* PUFAs. Without *n-3* PUFAs *n-6* PUFAs appear to be detrimental and elevate levels of sTNFR1 and 2 (Pischon et al., 2003), particularly in men. While provision of *n-3* PUFA has also been demonstrated to have no effect on immune parameters of healthy adults (Kew et al., 2003), in infants it has been associated with a reduction in the incidence of allergies (Dunstan et al., 2003).

Dietary intervention to improve gut and immune health in the elderly

Carbohydrate

The beneficial immunoregulatory effects of increased colonic T_{reg} cell numbers and proliferation, as well as elevated anti-inflammatory cytokines observed in animal studies upon bacterial fermentation of dietary fibre to SCFAs (Smith et al., 2013) together with the elevated production of beneficial SCFAs in adult human studies after increased fibre intake could be targeted in elderly human subjects by the consumption of adequate amounts of appropriate foods. Increased fruit and vegetable intake significantly increases production of antibodies specific for pneumococcal capsular polysaccharide, and the number of participants achieving a 4-fold increase in their antibody response to Pneumovax II was significantly increased when consuming 5 portions per day (Gibson et al., 2012). The consumption of fructo-oligosaccharides by elderly subjects was shown to produce a reduction in IL-6 mRNA expression and in phagocytic activity of granulocytes and monocytes (Guigoz et al., 2002). Administration of beta-galacto-oligosaccharides to elderly subjects similarly increased mononuclear cell phagocytosis in addition to NK cell activity and production of IL-10, along with reduction of IL-6, IL-1β and TNF-α (Vulevic et al., 2008). Consumption of prebiotic supplements (70% raftilose, 30% raftiline) by elderly subjects however had no effect on response to influenza A vaccination (Bunout et al., 2002). On balance therefore the source, choice and amount of dietary fibre in the diet needs to be carefully considered and "matched" with the microbiota profile and immune status (health) of the person consuming them, which as discussed above, changes with age.

Protein

Studies conducted in healthy adults suggest that protein intake changes the microbiota composition and that its consumption should be carefully monitored in order to be beneficial and not detrimental to health, noting that dietary protein intake and amino acids are important energy substrates for immune cells (Ruth and Field, 2013). When addressing an elderly population, recommendations for protein intake should be considered carefully since the US RDA for adults is 0.8 g protein per kg weight regardless of age, though the elderly may benefit from a higher protein intake (Campbell et al., 2001; Wolfe et al., 2008). This is reinforced by the observation that elderly women consuming half of their daily protein requirement for 9 weeks had a 50% reduction in their delayed type hypersensitivity (DTH) response compared to their baseline measure while a protein intake of 2.94 g protein per kg body cell mass per day, which is the recommended daily amount (RDA), achieved a 47% increase in the DTH reaction to dermally applied antigens. This suggests that achieving the RDA for protein is a minimum requirement for effective cell-mediated immune responses (Castaneda et al., 1995). This is of particular interest as interventional or retrospective studies of elderly populations in Australia (Zhu et al., 2010) and the UK (Bates et al., 2014) imply that adults aged over 65 years had a lower meat intake than that of younger adults (Bates et al., 2014) and that with age people consume lower amounts of protein, when in fact their requirements need to be greater.

To date there is little research into the effect of protein consumption on the immune response in an elderly population. However, a combination of resistance training and increased red meat consumption by elderly women has been shown to reduce serum levels of pro-inflammatory IL-6 by 16% (Daly et al., 2014). Since elderly people may have a greater protein requirement in order to counterbalance muscle protein breakdown and achieve an overall positive protein balance

(Daly et al., 2015), the current recommendations to reduce intake of red meat may be misguided for elderly populations.

The source of protein may also be relevant to immune health. Whey protein in comparison to soy milk protein has been shown to increase the antibody response to pneumococcal vaccines, and in particular to the four pneumococcal serotypes which cause the most severe infections in adults (Freeman et al., 2010). However, the protein intake between the intervention and control group in this study were significantly different when the supplement was excluded, which may impact on these findings. This is significant as soy milk given to a group of postmenopausal women produced an increase in B cell numbers (Ryan-Borchers et al., 2006), which is central to considering vaccine antibody responses.

Fat

When studying the effect of PUFA consideration must be given to the specific population under investigation and generalization of health benefits cannot be made, since an elderly population may react differently. This is apparent when considering the effect of eicosapentanoic acid (EPA) containing supplements in old and young men which showed that older men had reduced neutrophil respiratory burst which was further reduced with increasing doses of EPA (Rees et al., 2005). Other observations also suggest a detrimental effect of fish oils on the aged immune system, even at very low doses (Meydani et al., 1991; Bechoua et al., 2003). It should be noted, however, that studies reporting the use of very low doses, from 600 mg fish oil to 2.4 g *n-3* PUFA per day, translate to a greater consumption of fish than the current UK recommendation of 2 portions per week (Bates et al., 2012). Thus, the doses used in the studies referred to exceed

UK recommendations and are probably unachievable through dietary intake when converted to actual fish intake questioning the biological relevance of these findings. Other findings imply a gender difference in immune effects. Observational data on the consumption of α-linolenic acid and linoleic acid by semi-quantitative food frequency questionnaires revealed no effect on the risk of developing pneumonia (Merchant et al., 2005), while the observational data from a group of women found the opposite (Alperovich et al., 2007).

High intakes of fish, 4 times per week, had no effects on serum cytokine levels in a group of healthy older Australians (Grieger et al., 2014), suggesting that the lowering effect of PUFA on inflammatory cytokines may only be apparent when baseline inflammatory levels are high to start with. Also, a multi ethnic study found increased consumption of *n-3* PUFA and all other non-fried fish was inversely associated with IL-6, Matrix Metalloprotease Protein-3 and CRP serum levels (He et al., 2009). A reduction in lymphocyte proliferation with increased intake of γ-linoleic acid and EPA and DHA, when administered as capsules to older healthy subjects (Thies et al., 2001) suggests that these PUFA may contribute to a reduction in inflammation in these subjects. However, these effects were only observed when EPA was combined with DHA, not with DHA alone, implying that EPA mediates the observed anti-inflammatory effects. Provision of γ-linolenic and α-linoleic acids from a non-fish source, blackcurrant seed oil, has demonstrated an immune enhancing effect via greater induration of DTH responses to tetanus toxoid in a group of elderly subjects as well as a significant reduction in prostaglandin E_2 (PGE_2) serum levels (Wu et al., 1999). This is interesting since it has been shown that PGE_2 synthesis is regulated by CLAs as CLA administration to cultured human macrophages significantly reduced PGE_2 production (Stachowska et al., 2009). Therefore, CLA, the metabolite produced by the

intestinal microbiota from consumption of PUFAs, may reduce PGE_2 concentrations and help to reduce inflammation.

The EU-sponsored ELDERMET and Nu-AGE studies are currently underway investigating the age-associated changes in microbiota diversity of elderly individuals, as well as changes in immune parameters as a result of alterations in dietary intake, respectively. They should provide valuable data and may assist in understanding how dietary intake might influence outcomes of the ageing process and whether parameters of immune function can be improved in elderly subjects.

Conclusions

More studies need to be carried out in ageing models to understand how bacterial metabolites important for human health are produced in response to diet and the mechanisms by which they act on the immune system during ageing. Such studies are made more difficult and complicated by confounding factors attributable to the ageing process and changes in eating habits, nutritional requirements, GI-tract physiology and the microbiota that must be taken into consideration when designing such studies and interpreting the data obtained.

Additionally, very few studies have investigated the effects of changing the whole diet on the immune system. Some studies have shown the effect of the Med diet on the immune system, with decreases in plasma levels of hallmark indicators of inflammation including CRP, IL-6, IL-8 and TNF- α (Dedoussis et al., 2008; Mena et al., 2009), some of which are positively associated with red meat intake. These findings along with those of others (Camargo et al., 2012) imply that the Med diet has an anti-inflammatory effect, which could be a result of an increase in

SCFAs, from the elevated intake of fruit and vegetables and whole grains, increasing proliferation of T_{reg} cells. Additionally, fat intake and its source is important with the Med diet fat being derived from oily fish and olive oil which promotes EPA and DHA synthesis along with production of CLA isomers that may inhibit secretion of pro-inflammatory eicosanoids and lipid mediators.

There is increasing evidence of a link between dietary intake and changes in the intestinal microbiota metabolism, in the form of metabolites, and that these metabolites can have effects on the mucosal and, or systemic immune system. The clearest and most recognised findings to date comes from animal models and the induction and expansion of regulatory T cells as a result of bacterial fermentation of indigestible carbohydrates such as NSP. There are currently insufficient human intervention trials to corroborate these findings. Other dietary effects on the immune system are less clear, with implied positive effects of protein derived metabolites such as sulphur amines and BCFA from bacterial fermentation on immune function. The effects of conjugated linoleic acid isomers on the immune system are also less established but the data obtained to date is promising. More research is clearly required to establish and prove these effects in both models of ageing and in large-scale human intervention studies. This is especially important since the majority of the data available thus far relies on associations and/or is obtained from low powered intervention studies due to small subject numbers. However, it may be both timely and appropriate to suggest that the elderly tailor their dietary intake to increase consumption of dietary fibre and manage protein intake so that recommended intakes are, as a minimum, achieved and that the sources of fat intake be considered so as not to include potentially deleterious quantities.

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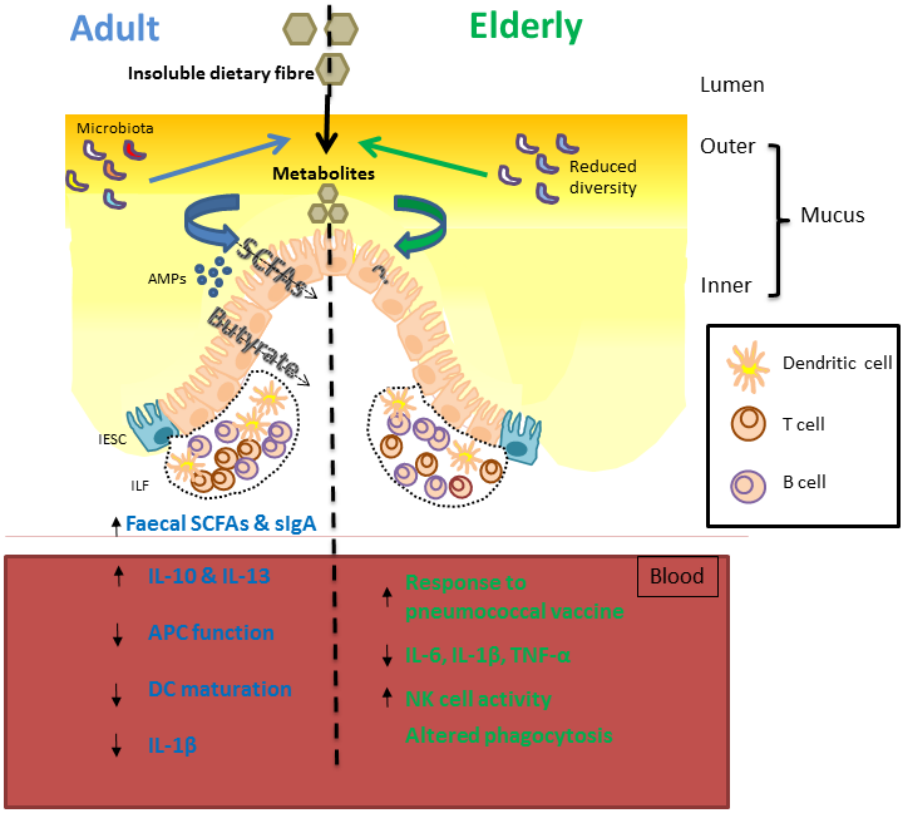

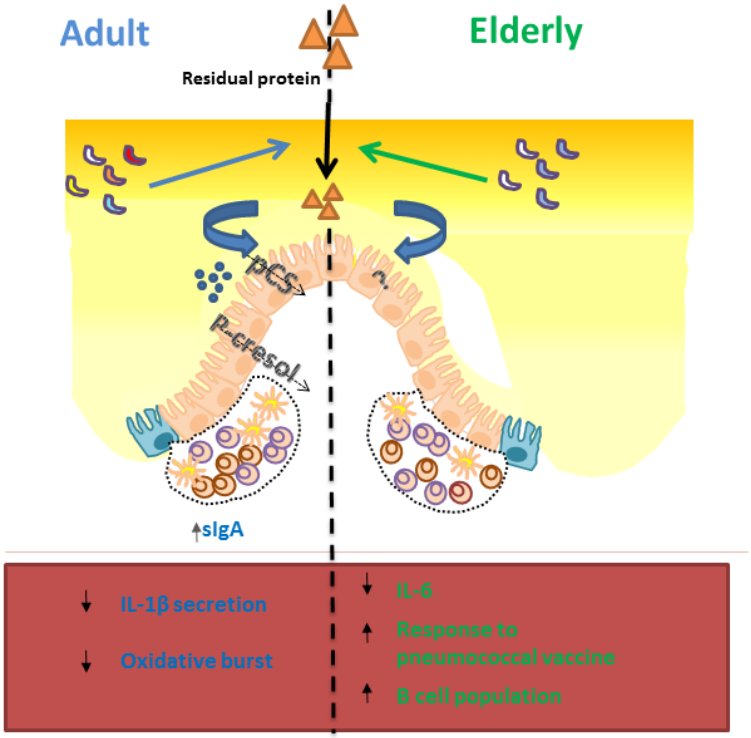

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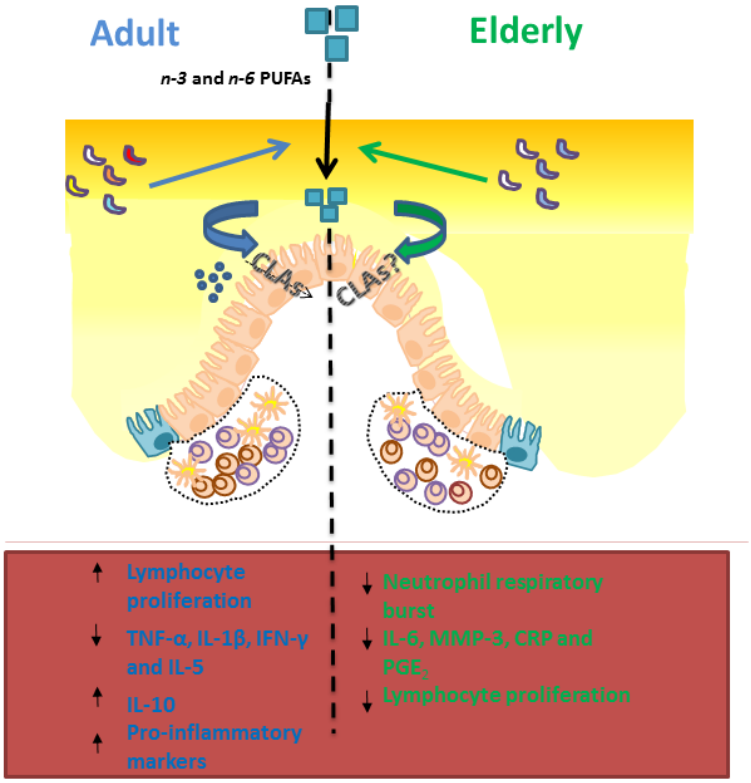

C

Figure 1. Pictorial representation of key differences in the gastrointestinal environment of adults and elderly individuals in their response to the consumption of different dietary components. **A**) Insoluble fibre reaching the colon is metabolised by the microbiota to produce short chain fatty acids (SCFAs) such as butyrate, which can impact on local immune cells to alter cytokine secretion and T cell priming by dendritic cells (DCs). In the elderly, consumption of fibre has been shown to have beneficial effects although SCFA interaction with cells of the immune system can only be implied. **B**) Consumption of protein can lead to the production of p-cresyl sulphate (pCS) and p-cresol as a result of microbial metabolism; p-cresol appears to suppress pro-inflammatory effects, while pCS has been shown to induce pro-inflammatory effects.

Evidence from the elderly shows that increased protein intake may improve immune response to vaccination and reduce production of pro-inflammatory mediators, however, it is unknown whether these effects are as a result of protein derived bacterial metabolites. **C**) Consumption of *n*-3 and *n*-6 PUFAs or CLA has been shown to reduce the secretion of pro-inflammatory cytokines and increase the secretion of anti-inflammatory IL-10, in addition to increasing lymphocyte proliferation. In an elderly population it is not known whether conjugated linoleic acids (CLAs) are involved although since CLAs regulate synthesis of prostaglandin E2 (PGE_2) they may be involved. Lymphocyte proliferation and neutrophil respiratory burst are affected by *n*-3 and *n*-6 PUFA consumption, implying that quantities of fatty acid intake in the elderly should be managed so as not to impair immune function. Abbreviations: AMPs, antimicrobial proteins; IESCs, intestinal epithelial stem cells; ILF, isolated lymphoid follicle.