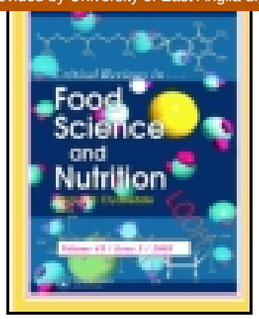

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Diet, the intestinal microbiota, and immune health in aging

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ABSTRACT

Many countries are facing aging populations, 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 in the immune function characterized by poor vaccine responses and increased risk of infection and certain cancers. Aging 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 is necessary since this population has an altered microbiota and immune response and may not respond in the same way as their healthy and younger counterparts. We have reviewed the evidence supporting the role of diet and, in particular, dietary carbohydrate, protein, and fat in influencing the microbiota and its generation of key metabolites that influence the efficient functioning of immune cells during aging, 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; fiber; protein; fat

Introduction

The UK population is growing with increasingly more people reaching the eighth decade of age 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 of social care, healthcare, and the economy, with 72% of social care requests received from people aged 65 years and older between 2014 and 2015 (Health & Social Care Information Centre, ASCST, 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 of 13.2% seen in the East of England (ONS, OFNS, 2014).

There is uncertainty regarding the definitions of aging and aged and elderly populations, as aging encompasses both biological and environmental factors in addition to chronological age. According to the United Nations report of World Ageing (DESA, UN, 2002), older people are classed as being 60 years or older, with some studies classifying those aged over 85 years as the "oldest old" (Wikby et al., 2002; Forsey et al., 2003). 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 have used 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 as "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 the accumulation of oligoclonal memory $CD8^+$ T cells (Khan et al., 2002; Czesnikiewicz-Guzik et al., 2008). This reduction in naïve $CD8^+$ T cells has been shown to correlate with impaired T cell priming when compared to middle aged subjects (Briceño et al., 2016). Declining T cell functioning during aging 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 (Taub and Longo, 2005; Aw et al., 2009). Aging also affects B cells, although, perhaps, not in the same way, or to the same extent as T cells (Scholz et al., 2013). Whereas studies in mice provide evidence of declining bone marrow haematopoiesis and B cell production during aging, observations in humans suggest that B lymphopoiesis is maintained well into old age. More consistent are the

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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).

Aging also affects the motor and sensory functions 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 reduction in the motility of the colon, which can cause constipation (Grassi et al., 2011). These symptoms alone may influence 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). Aging also affects 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 fecal samples from the elderly compared with 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 with that of centenarians, with bacteroidetes having a lower relative contribution to the microbiota of elderly compared with young individuals (Biagi et al., 2010). The functional significance of these or any other changes in the relative abundance of specific bacterial populations, in view of the inherent complexity of the microbiota, comprising more than 1000 species (Tremaroli and Bäckhed, 2012), and the interdependency of different bacteria, is difficult to discern. Of note, lower species diversity and a reduction in *Bifidobacteria* in fecal samples of elderly subjects (aged 67–88 years) (Hopkins et al., 2002) corresponds with altered functionality and the reduced ability of Bifidobacteria strains from elderly subjects (aged 74–93 years) to adhere to intestinal mucus (Ouwehand et al., 1999; He et al., 2001) 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 host's 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 sulfide 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 consider carefully 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 affects the immune system, focusing on the production of microbe-generated metabolites because 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 more than 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, and synthesis of SCFA and indispensable amino acids and vitamins, highlighting the interdependency and mutualistic relationship between the microbiota and its host (Turnbaugh and Gordon, 2009; Qin et al., 2010; Arumugam et al., 2011). Additional complexity is provided by the virome, which contains the most abundant and fastest mutating genetic elements (e.g. prophages and retroviruses) on earth (Reyes et al., 2010; Minot et al., 2013; Virgin, 2014), in addition to protozoa and fungi with much less being known of the role that these non-bacterial constituents play in promoting GI tract health, or disease. The stability of intestinal microbiota is central to maintaining the integrity of 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 intestinal microbiota for preserving health is further emphasized by sterile and 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 by 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 newborn 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 the immune system is early life nutrition, and the positive effect that 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). Breastfed 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 diet, can have influential effects on this important relationship, which is discussed in more detail below.

Diet and the microbiota

A key example of the effect that 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 fiber per day and display strikingly different fecal microbiota profiles compared with their European counterparts that consume 8.4-g dietary fiber (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 fibers, for provision of energy and generation of SCFAs. Negative correlations between fecal 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). In addition, increased intake of dietary fiber (40 g) in healthy adults aged between 19 and 25 years has been associated with overexpression of genes involved in methanogenesis and glycan and lipid metabolism, when compared with lower fiber intake of 10 g, consistent with increased fermentation as a result of higher levels of fermentable dietary fiber in the colon (Tap et al., 2015). A striking relationship between adherence to a Mediterranean (Med) diet and abundance of fecal SCFAs has also been reported, which strongly correlated with consumption of fruits, vegetables, and fiber. 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 population (aged $\geq$65 years), where subjects were fed with low fat and high fiber diet, greater diversity in bacterial microbiota profiles were observed, while those with "moderate to high" levels of fat and "low" fiber intakes had the least diverse microbiota (Claesson et al., 2012). Collectively, these studies show that intake of dietary fiber influences the diversity of intestinal microbiota and the dominant species, suggesting that a richly diverse microbiota may be beneficial in, for example, protecting against and excluding enteric pathogens that cause intestinal diarrhea-associated diseases. Of note, in spite of poor sanitation and hygiene in Burkina Faso, species of diarrhea inducing *Enterobacteriaceae* were underrepresented (De Filippo et al., 2010).

Dietary fiber is not the only determinant of microbial species diversity, as significant reduction in *Bacteroides* and *Bifidobacterium* species are apparent in both vegetarians and vegans. One comparative study showed a significant reduction in *Enterobacteriaceae* in vegans compared with 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 with predominance of *Clostridium* in omnivores (Koeth et al., 2013). Differing dietary patterns can be seen to affect the composition of the microbiota with regard to the production of specific metabolites. Consumption of protein-rich animal foods and fat has 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 blood are associated with increased risk of cardiovascular disease, which is thought to be a consequence of its negative effect 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 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 has 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), indicates 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 these are metabolized by the microbiota as seen in studies assessing fecal recovery of radioactive carbon (^{14}C) after consumption of ^{14}C-labeled lipids (Murphy et al., 1995; Thompson and Spiller, 1995; Gabert et al., 2011). 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., 2003; Coakley et al., 2009; Devillard et al., 2009; McIntosh et al., 2009; Gorissen et al., 2010). The variable production of positional and geometric isomers of CLA may affect 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 fecal sample, while the fecal 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 affect 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 other researchers (Duncan et al., 2008; Daniel et al., 2014) 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 effect on the immune system and influence of the microbiota

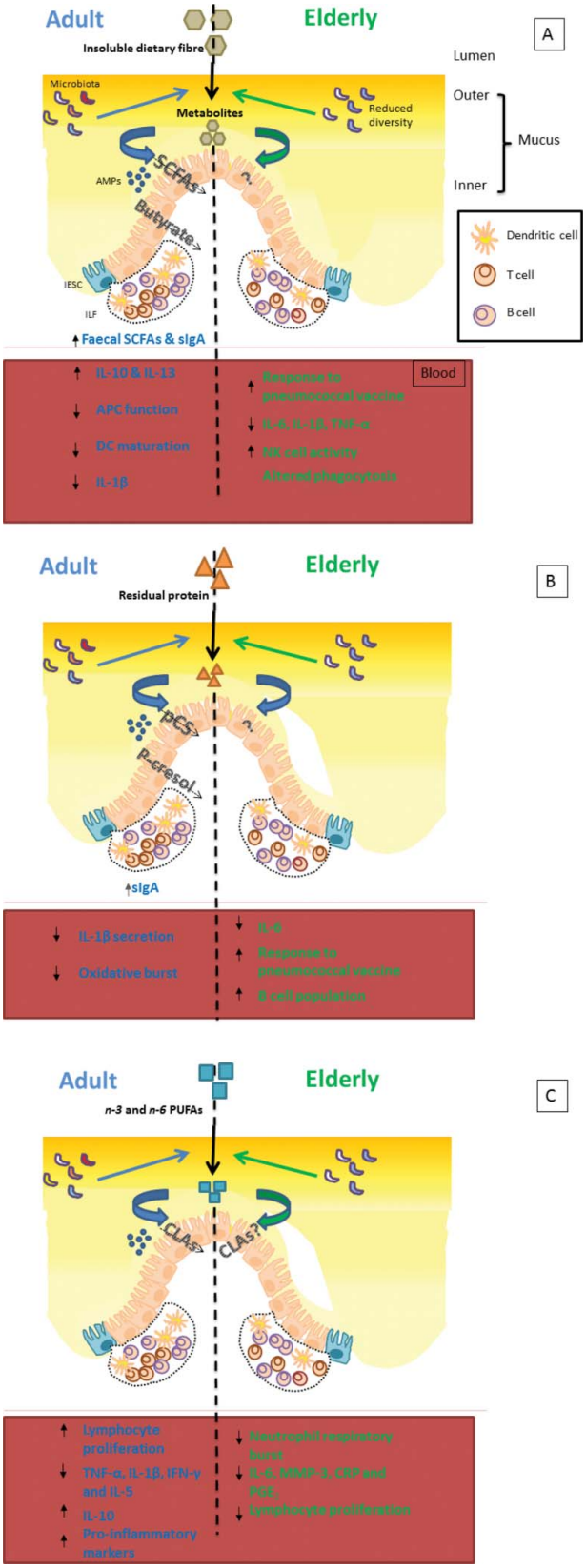
According to various lines of investigation, three possible metabolic pathways can produce bacterial metabolites that influence the immune system with evidence of their effect in healthy adults and elderly populations. The first involves the production of SCFA by bacterial fermentation of complex plant-based 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) (Fig. 1A). These products of bacterial fermentation are thought to promote the establishment of regulatory T (T_{reg}) cells that are important for 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 T_{reg} cells (Atarashi et al., 2011, 2013). Also, segmented filamentous bacteria, which are prominent in the small intestine, have been shown to bind intestinal epithelial cells (IECs) and provide them with SCFAs, including butyrate (Hamer et al., 2008), which 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 fiber-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 provisions of SCFAs and butyrate have immunosuppressive effects on antigen presenting cells and, in particular, dendritic cells (DC) (Bohmig et al., 1997; Berndt et al., 2012; Liu et al., 2012). Other in vitro model studies suggest that while SCFAs are important, the plant fibers 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 effect of probiotics and prebiotics (galacto-oligosaccharides (GOS)) during pregnancy and after birth has shown that the treated infants displayed reduction in IgE-associated eczema and atopic eczema (Kukkonen et al., 2007). These findings were interpreted as a positive effect of biotic therapy on the development of these diseases via effects on the microbiota, since the GI tract of the supplemented infants was colonized with higher levels of beneficial lactobacilli and bifidobacteria. However, as this study primarily focused on the probiotics provided to infants as opposed to co-administered GOS, the observed effects may have been due to a synergistic effect of both probiotics and prebiotic (synbiotics). Similarly, provision of xylo-oligosaccharides and inulin has been shown to increase fecal 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 fermented by resident bacteria to produce phenols, ammonia, nitrates, and branched chain fatty acids (BCFAs) as well as SCFA and gases (Fig. 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 was 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) as 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 for an aging population. Significantly increased oxidative burst activity of monocytes and lymphocytes was observed from ex vivo *p*-cresyl sulfate (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 fecal 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 reduction 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 acid (PUFA) origin, which are metabolized by the microbiota to produce CLA isomers (Fig. 1C). The potential of the colon to contribute to fatty acid absorption has not been studied in detail to date. Bifidobacterium species can metabolize 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 difference in immune parameters after CLA supplementation (Kelley et al., 2000, 2001; Albers et al., 2003; 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 reduce 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 3 g 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 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 ($\geq$10 IU/L) in their serum to be considered protective against hepatitis B infection, this did not reach statistical significance. In a crossover study to determine whether these two main isomers have differing effects, subjects were given capsules containing varying doses of each isomer with no difference 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* PUFA has also been be associated with reduction in secreted TNF receptors (sTNFR1 and sTNFR2) in plasma but this effect is only apparent with high intakes of *n-3* PUFA. Without *n-3* PUFA, *n-6* PUFAs appear to be detrimental and elevate levels

Figure 1. Pictorial representation of key differences in the GI environment of adults and elderly individuals in their response to the consumption of different dietary components. (A) Insoluble fiber reaching the colon is metabolized by the microbiota to produce short chain fatty acids (SCFAs), such as butyrate, which can affect local immune cells to alter cytokine secretion and T cell priming by dendritic cells (DCs). In the elderly, consumption of fiber 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-cresylsulphate (pCS) and p-cresol because 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 due to 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 CLAs) are involved, although since CLAs regulate synthesis of prostaglandin E2 (PGE_2), these 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.

of sTNFR1 and sTNFR2 (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 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 fiber to SCFAs (Smith et al., 2013) together with the elevated production of beneficial SCFAs in adult human studies after increased fiber 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 four-fold increase in their antibody response to Pneumovax II was significantly increased when consuming five portions per day (Gibson et al., 2012). The consumption of fructo-oligosaccharides by elderly subjects was shown to reduce IL-6 mRNA expression and phagocytic activity of granulocytes and monocytes (Guigoz et al., 2002). Similarly, administration of beta-galacto oligosaccharides to elderly subjects 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 and 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 fiber in the diet are 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 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 recommended daily amount (RDA) for adults is 0.8 g protein per kg weight regardless of age, although 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 with their baseline measure, while a protein intake of 2.94 g per kilogram body cell mass per day, which is the RDA, achieved a 47% increase in DTH reaction to dermally applied antigens. This suggests that achieving the RDA of 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 United Kingdom (Bates et al., 2014) imply that adults aged over 65 years had a lower intake of meat than that by younger adults (Bates et al., 2014), and with age people consume lower amounts of protein, when, in fact, their requirements need to be more.

To date, there is little research on the effect of protein consumption on 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 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 population.

The source of protein may also be relevant to immune health. Whey protein in comparison to soymilk protein has been shown to increase antibody response to pneumococcal vaccines, and in particular to the four pneumococcal serotypes that cause the most severe infections in adults (Freeman et al., 2010). However, protein intake between the intervention and control groups in this study was significantly different when the supplement was excluded, which may affect these findings. This is significant, as soy milk given to a group of postmenopausal women increased 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., 2006). Other observations also suggest a detrimental effect of fish oils on the aged immune system, even in 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 two 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 of four times per week had no effect 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. In addition, a multi-ethnic study found that 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 synthesis of PGE_2 is regulated by CLA, as CLA administration to cultured human macrophages significantly reduced PGE_2 production (Stachowska et al., 2009). Therefore, CLA, a metabolite produced by intestinal microbiota from consumption of PUFA, 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 and changes in immune parameters because of alterations in dietary intake, respectively. These should provide valuable data and may assist in understanding how dietary intake might influence outcomes of the aging process, and whether parameters of immune function can be improved in elderly subjects.

Conclusions

More studies need to be carried out in aging 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 aging. Such studies are made more difficult and complicated by confounding factors attributable to the aging 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.

In addition, 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 intake of red meat. 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 because of the elevated intake of fruit and vegetables and whole grains, thus increasing the proliferation of T_{reg} cells. In addition, fat intake and its source is important with the Med diet fat being derived from oily fish and olive oil, which promote 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 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 the most recognized findings to date comes from animal models and the induction and expansion of T_{reg} 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 sulfur amines and BCFA from bacterial fermentation on immune function. The effects of CLA isomers on the immune system are also less established but the data obtained to date are promising. More research is clearly required to establish and prove these effects in both models of aging and large-scale human intervention studies. This is especially important since the majority of the data available so 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 the consumption of dietary fiber and manage protein intake so that recommended intakes are, as a minimum, achieved, and the sources of fat intake be considered so as not to include potentially deleterious quantities.

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