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How does polyunsaturated fatty acid biosynthesis regulate T-lymphocyte function?

B. A. Fielding*, P. C. Calder[†,‡], N. A. Irvine[†], E. A. Miles[†], K. A. Lillycrop[§], J. von Gerichten* and G. C. Burdge[†]

*Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK;
[†]School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK;
[‡]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, University of Southampton, Southampton, UK;
[§]Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton, UK

Abstract

Impaired regulation of immune function characterised by chronic inflammation together with a declining protective immune response is a major challenge to healthy ageing. It is therefore important to understand the mechanisms that regulate immune function and the impact of ageing upon such processes. Appropriate induction and resolution of the immune response require adequate availability of polyunsaturated fatty acids (PUFAs) for incorporation into cell membranes. However, humans are unable to synthesise PUFAs *de novo* and are dependent upon dietary intake for pre-formed PUFAs or synthesis by the liver from the essential fatty acids, linoleic acid (LA, 18:2*n*-6) and alpha-linolenic acid (aLNA, 18:3*n*-3). We have shown that activation of peripheral blood mononuclear cells increases PUFA biosynthesis from essential fatty acids via a mechanism that involves altered epigenetic regulation of a key gene in the pathway. Moreover, induction of PUFA synthesis is directly involved in the regulation of lymphocyte activation and proliferation. The aim of the Biotechnology and Biological Sciences Research Council responsive mode award described in this paper, 'How does polyunsaturated fatty acid biosynthesis regulate T-lymphocyte function?', is to determine how PUFA biosynthesis regulates T-cell function and the effect of ageing on this process. The project will identify points of regulation in the biosynthetic pathway and how these might influence the capacity for up-regulation of PUFA synthesis in older individuals. We will use stable isotope tracers of LA and aLNA to determine whether newly synthesised PUFAs are preferential substrates for synthesis of lipid mediators and whether they are involved in formation of membrane microdomains that mediate cell signalling.

Keywords: eicosanoids, epigenetics, healthy ageing, immune function, polyunsaturated fatty acids, stable isotopes

Correspondence: Dr. Barbara Fielding, Faculty of Health and Medical Sciences, University of Surrey, The Leggett Building, Guildford, Surrey GU2 7WG.
E-mail: b.fielding@surrey.ac.uk

The role of polyunsaturated fatty acids in the immune response

Leukocytes include all white blood cells [neutrophils, monocytes, T-lymphocytes (T cells), B lymphocytes

and so on]. It is possible to isolate these cell types from human blood; one fraction commonly prepared is referred to as peripheral blood mononuclear cells (PBMCs) which are a mixture of lymphocytes and monocytes, with T cells being present in the greatest proportion. Leukocyte membranes are characterised by high levels of polyunsaturated fatty acids (PUFAs), in particular arachidonic acid (AA, 20:4*n*-6), which are substrates for the synthesis of lipid mediators (such as eicosanoids) that are released in response to immunological stimuli such as pathogens and cytokines. The eicosanoids, including leukotrienes, prostaglandins and lipoxins, are subsequently recognised by specific cell receptors, usually G protein-coupled receptors. An appropriate balance of specific eicosanoid species, derived from different PUFAs, is required to activate pro- and anti-inflammatory pathways, in order to achieve 'resolution' of the immune response (Lone & Tasken 2013). PUFAs also influence the biophysical properties of the lipid bilayer (Burdge 2018). This report describes studies that we will carry out to investigate the role of PUFAs in T cells. Some of the previous work that has informed our present research has been carried out using PBMCs, as noted below (*e.g.* Sibbons *et al.* 2018).

Sources of polyunsaturated fatty acids for incorporation into leukocyte membranes: Dietary fatty acids

PUFAs can be obtained from the diet, and those of chain length >18 carbons are primarily obtained by consuming animal foods including oil-rich fish, meat and some dairy products. As a consequence of habitual intakes of these foods, leukocytes are exposed to an environment that contains pre-formed PUFAs of both the *n*-3 and *n*-6 series. By way of explanation, *n*-3 is a notation used to describe the position of the first double bond, counting from the methyl end, although the first carbon is sometimes referred to as the omega (ω) carbon. Using this shorthand notation, AA, 20:4*n*-6, has a chain length of 20 carbons, four double bonds, and the first double bond is inserted six carbons from the methyl end. This notation is helpful in understanding synthetic pathways.

There is substantial evidence that dietary PUFAs are incorporated into lymphocyte, and other leukocyte, membranes and can modify the T-cell response to mitogens and antigens (Calder 2009). For example, increased consumption of fish oil by humans results in enrichment of lymphocyte membranes with *n*-3 PUFAs, in particular eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) leading to suppression of some immune responses, including cell proliferation and inflammation (Calder 2009). This, at least in part, is the result of displacement of AA from membrane phospholipids by EPA leading to reduced formation of pro-inflammatory eicosanoids including 2-series prostaglandins (Calder 2009). Furthermore, dietary PUFAs are able to influence lymphocyte function via non-membrane pathways. EPA has been shown to up-regulate the expression of PPARα, PPARβ and PPARγ in murine $CD4^+$ T cells (Unoda *et al.* 2013). Dietary supplementation of human volunteers with EPA and DHA induced changes in the DNA methylation of genes in PBMCs related to sex, PUFA type and CpG locus (Hoile *et al.* 2014).

Sources of polyunsaturated fatty acids for incorporation into leukocyte membranes: Synthesis from essential fatty acids

Humans can synthesise PUFAs from the essential fatty acids, linoleic acid (LA, 18:2*n*-6) and alpha-linolenic acid (aLNA, 18:3*n*-3) by a well-established pathway that is located primarily in the endoplasmic reticulum of liver cells and which involves alternating desaturation and carbon chain elongation reactions (Sprecher & Chen 1999). The initial rate-limiting Δ6 desaturation (catalysed by Δ6 desaturase; encoded by FADS2) is followed by chain elongation by elongase 5 (encoded by ELOVL5) and Δ5 desaturation (catalysed by Δ5 desaturase; encoded by FADS1), which creates a double bond at the 5th position from the carboxyl end of the fatty acid (see Fig. 1). These reactions yield AA from LA or EPA from aLNA. Two rounds of chain elongation by elongases 5 or 2 (the latter encoded by ELOVL2) followed by Δ6 desaturation produces 24 carbon intermediates which can be translocated to peroxisomes where two carbons are removed by a single cycle of β-oxidation to yield 22:5*n*-6 from 18:2*n*-6 or 22:6*n*-3 from 18:3*n*-3 (Sprecher & Chen 1999). Hepatic synthesis of DHA is undetectable in men (Burdge *et al.* 2002), but readily detected in women (Burdge & Wootton 2002). However, it is becoming clear that extrahepatic PUFA synthesis may also be important (Burdge 2018), especially in immune cells. There is limited evidence from five studies conducted prior to 1995 that leukocytes can convert essential fatty acids to longer chain PUFAs (Chapkin *et al.* 1988; Anel *et al.* 1990a, 1990b; Chapkin & Coble 1991; Calder *et al.* 1994), although these studies were constrained by the analytical techniques available at

the time. Murine macrophages were shown to convert 18:2*n*-6 to its elongation product 20:2*n*-6, but not to longer chain PUFAs (Chapkin *et al*. 1988). Others reported elongation of 18:3*n*-6 to 20:3*n*-6, but not Δ5 desaturation of 20:3*n*-6 to 20:4*n*-6 in activated macrophages (Chapkin & Coble 1991). Activation of human lymphocytes increased synthesis of unidentified triene (three double bonds) and tetriene (four double bonds) PUFAs (Anel *et al*. 1990a; Calder *et al*. 1994). Furthermore, 20:4*n*-6 concentration was lower in splenocytes from FADS2 null mice (Calder *et al*. 1994). We have shown using stable isotope tracers that conversion of aLNA to longer chain PUFAs was undetectable in quiescent PBMCs. However, stimulation with the T cell mitogen concanavalin A (Con. A) increased [^{13}C]18:3*n*-3 uptake and conversion (Sibbons *et al*. 2018). The first two reactions were reversed in PBMCs compared to the 'conventional' hepatic pathway (Voss & Sprecher 1988), such that the first reaction was carbon chain elongation by an unidentified enzyme followed by Δ8 desaturation, rather than the expected Δ6 desaturation step, possibly by Δ6 desaturase, namely the protein product of FADS2, acting on 20:3*n*-3 (Fig. 1). This suggests that the operation and regulation of the pathway may differ between leukocytes and other tissues (Burdge 2004).

Regulation of polyunsaturated fatty acid synthesis and T-lymphocyte proliferation

The mRNA expression of FADS1 and FADS2, and ELOVL4 and ELOVL5 was significantly higher in stimulated compared to unstimulated PBMCs indicating that capacity for PUFA synthesis is regulated in PBMCs, at least in part, at the level of transcription rather than being primarily the result of increased 18:3*n*-3 uptake. Elongase (ELOVL) 2, which catalyses conversion of 22:5*n*-3 to 24:5*n*-3, which precedes 22:6*n*-3 synthesis, was not detected (Sibbons *et al*. 2018). This is consistent with the lack of detectable enrichment of 22:6*n*-3 in stimulated cells and suggests truncation of the pathway after 22:5*n*-3. The fatty acid 22:5*n*-3 did not accumulate in activated cells (Sibbons *et al*. 2018), which suggests further conversion to an unidentified metabolite. As expected, treatment of mitogen-stimulated PBMCs with the Δ6 desaturase inhibitor SC26196 increased the level of 20:3*n*-3, but reduced the amount of 20:4*n*-3. The findings also showed for the first time that treatment with SC26196 decreased both the proportion of lymphocytes that underwent division and the mean number of cell divisions of the original cell population by approximately 10% each (Sibbons *et al*. 2018). The partial reduction in proliferation is consistent with the partial inhibition of PUFA synthesis. This demonstrated for the first time a functional role of PUFA synthesis in T cells, specifically that PUFA synthesis is involved in the regulation of T-cell activation and proliferation.

Elongase 4 (encoded by ELOVL4) catalyses elongation of 22 carbon PUFAs to very long-chain PUFAs (VLCPUFAs) of chain length ≥24 carbons (Vasireddy *et al*. 2007). One possible interpretation of the increase in ELOVL4 expression in stimulated PBMCs

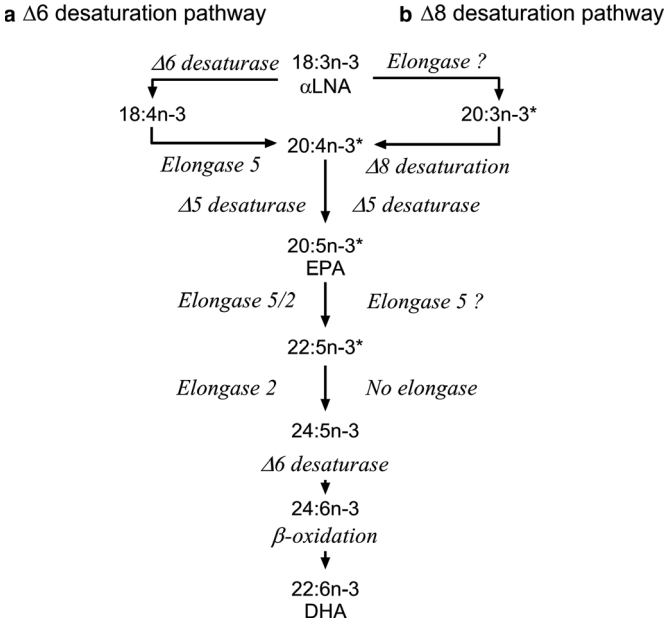

Figure 1 Alternative pathways of *n*-3 fatty acid synthesis in activated T cells [adapted from our published work in peripheral blood mononuclear cells: Sibbons *et al*. (2018)]; the asterisk indicates the main accumulated products of synthesis from 18:3*n*-3, alpha-linolenic acid, aLNA. For simplicity, the full names of not all individual fatty acids are given. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

is a need for greater capacity for conversion of 22 carbon PUFAs, such as 22:5*n*-3 (Wetzel *et al*. 1991; Suh & Clandinin 2005), to VLCPUFAs. *n*-3 VLCPUFAs are specific components of membrane microdomains that are critical for cell signalling processes involved in T-lymphocyte activation (Iwabuchi *et al*. 2010). Thus, it is possible that up-regulation of ELOVL4 facilitates *n*-3 VLCPUFA synthesis to support the formation of microdomains required for T-cell activation.

Stimulation of PBMCs also induced a significant increase in methylation of twenty CpG loci in a 611 bp region between 667 and 1278 bp upstream of the FADS2 transcription start site (Sibbons *et al*. 2018). DNA methylation is typically associated with suppression of transcription (Kovesdi *et al*. 1987; Comb & Goodman 1990; Jane *et al*. 1993; Gaston & Fried 1995), although some studies have reported the opposite (Hu *et al*. 2013; Clarke-Harris *et al*. 2014; Nordin *et al*. 2014). A possible explanation for this could be that transcriptional activation in the presence of increased DNA methylation is the consequence of blocking of the binding of repressive transcription factors (Clarke-Harris *et al*. 2014).

Current and future research objectives

The Biotechnology and Biological Sciences Research Council (BBSRC) responsive mode joint awards (BB/S00548X/1 and BB/S005358/1) entitled 'How does polyunsaturated fatty acid biosynthesis regulate T-lymphocyte function?' build on our recent work showing that PUFA biosynthesis from essential fatty acids is directly involved in the regulation of T-cell activation and proliferation. The purpose of this project is to determine the mechanism by which PUFA synthesis in human T cells regulates their activation and proliferation. The project will address a number of objectives that will be met through a series of interrelated experiments.

Because *n*-3 and *n*-6 PUFA biosynthesis use the same enzyme pathway (Fig. 1), it is important to understand the effect of changing the relative amounts of competing essential fatty acid substrates in the diet on T-cell function. We will use stable isotope tracers of LA and aLNA to model the effect of variation in dietary essential fatty acid intake to explore whether the modified PUFA synthesis pathway in T cells exhibits the same preference for aLNA as the pathway described in liver. These experiments will be performed using T cells isolated from the blood of young healthy men and women aged 18–30 years. This novel work will determine which phase of T-cell activation is associated with increased capacity for PUFA synthesis and will include a detailed investigation into the underlying molecular mechanisms, including epigenetic processes. We will also establish if newly synthesised PUFAs are used preferentially for synthesis of lipid mediators, including eicosanoids, and/or VLCPUFAs. The latter would be a mechanism to link PUFA synthesis to immune cell activation, as VLCPUFAs are critically involved in the assembly of membrane microdomains which facilitate T-cell signalling processes (Fig. 2). We will specifically investigate these processes using stable isotope tracers and siRNA knockdown of key enzymes in the biosynthetic pathway.

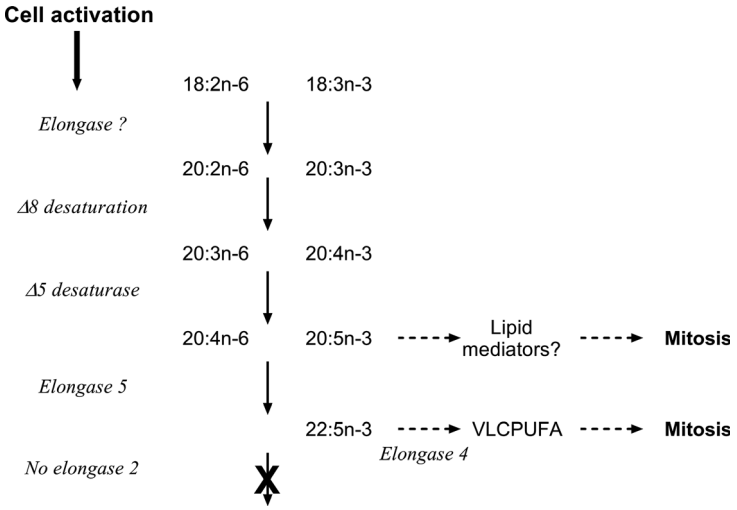

Figure 2 Suggested mechanism of up-regulation of T-cell proliferation by polyunsaturated fatty acids (PUFAs) in T cells in response to cell activation involving production of lipid mediators via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, and very long-chain PUFA (VLCPUFA) synthesis [adapted from Sibbons *et al*. (2018)].

After establishing the main processes that link PUFA synthesis to activation of T cells from young individuals, we will then investigate whether this is altered by increasing age by comparing the main findings of experiments involving cells from young individuals with those obtained from older individuals aged 65–75 years. At the end of the project, we aim to have established how PUFA (VLCPUFA) synthesis regulates T-cell activation in individuals of different ages.

Potential outcomes from the project

The number of older individuals in the UK has risen in recent years, increasing the burden on the economic and functional capacity of healthcare systems. In 2016, there were nearly 12 million people over the age of 65 years, representing 18% of the total population, which had risen from 15.8% in a period of only 25 years (Buttriss 2019). It is anticipated that this project will deliver findings that will lead to improved immunological health, thus reducing the burden of age-related immunological disease. For example, the findings of this study may suggest new explanations for the beneficial effects of modifying dietary fatty acid intake on immune function. Such knowledge may lead to the design of better nutritional strategies to improve immune health, potentially at the level of populations. Other possible outcomes from this project include identification of novel targets for the development of pharmaceuticals to treat immune dysfunction. For example, non-steroidal anti-inflammatory drugs are currently the primary treatment of chronic inflammatory disease, but these medicines can produce significant side effects, particularly in older individuals (NICE 2018). Hence, characterisation of the mechanisms that mediate the effect of PUFA synthesis on T-cell response may contribute to meeting the need for improved, less toxic immune-therapeutic agents.

Conclusions

The work leading up to the current project demonstrated for the first time that T-cell activation involves induction of PUFA synthesis via altered transcription of specific genes, possibly mediated by epigenetic processes. The current work will determine the mechanism that links PUFA synthesis to T-cell activation and explore whether this process changes with increasing age. The PUFA synthesis pathway in T cells seems to differ from that reported in other tissues. One long-term goal of this work is to develop nutritional interventions targeted at leukocytes to ameliorate dysregulation of immune function in ageing.

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Conflict of interest

The authors declare no conflicts of interest with the contents of this article.

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