

Symposium on ‘Evidence-based nutrition’

Nutritional modulation of immune function

R. F. Grimble

Institute of Human Nutrition, School of Medicine, University of Southampton, Southampton SO16 7PX, UK

The inflammatory response to injury and infection, although an essential part of immune function, carries the risk of severe tissue depletion and immunosuppression. These outcomes increase morbidity and delay recovery. Evidence is accumulating that single-nucleotide polymorphisms in the genes controlling pro-inflammatory cytokine production adversely influence the response. Immunonutrition provides a means of modulating the inflammatory response to injury and infection, and thereby improves clinical outcome. *n*-3 Polyunsaturated fatty acids (*n*-3 PUFA), glutamine, arginine, S amino acids and nucleotides are important components of immunonutrient mixes. While animal model studies suggest that all these components may exert a beneficial effect in patients, the number of large randomized placebo-controlled trials utilizing immunonutrition is fairly limited and the observed effects are relatively small. Meta-analyses suggest that while immunonutrition may not reduce mortality rates, a reduction in hospital length of stay, decreased requirements for ventilation and lower infection rates are achieved by this mode of nutrition. The present paper discusses some underlying reasons for the difficulty in demonstrating the clinical efficacy of immunonutrition. Paramount among these reasons is the antioxidant status and genetic background of the patient. A number of studies suggest that there is an inverse relationship between inflammation and T-cell function. Immuno-enhancive effects have been shown in a number of studies in which *n*-3 PUFA, glutamine and N-acetyl cysteine have been employed. All these nutrients may exert their effects by suppressing inflammation; *n*-3 PUFA by direct suppression of the process and glutamine and N-acetyl cysteine by acting indirectly on antioxidant status. Glutamine and nucleotides exert a direct effect on lymphocyte proliferation. Preliminary data suggests that not all genotypes are equally sensitive to the effects of immunonutrition. When further studies have been conducted to discern the precise interaction between each individual's genotype of relevance to the response to injury and infection, and immunonutrients, the level of precision in the application of immunonutrition will undoubtedly improve.

Immunonutrition: Immune function: Single-nucleotide polymorphisms: *n*-3 Polyunsaturated fatty acids: N-acetyl cysteine

It is generally accepted that a high proportion of patients in hospital are malnourished and that malnourishment impairs immune function (McWhirter & Pennington, 1994). In addition, a major burden of ill health exists in the population due to overactivity in the inflammatory arm of their immune system, as is evident in rheumatoid arthritis, inflammatory bowel disease and asthma. Furthermore, it is becoming increasingly apparent that inflammation plays an important part in atherosclerosis (Ross, 1993). The capacity for nutrients to modulate the actions of the immune system and

to affect clinical outcome has thus become an important issue in clinical practice and public health.

The application of nutrients for this purpose is referred to as ‘Immunonutrition’. A working definition of ‘Immunonutrition’ might be ‘modulation of the activities of the immune system, and the consequences on the patient of immune activation, by nutrients or specific food items fed in amounts above those normally encountered in the diet’. At present there are a relatively limited number of nutrients employed in ‘immunonutrition’ (Table 1). These nutrients

Abbreviations: IL, interleukin; LT, leukotriene; PBMC, peripheral blood mononuclear cells; PG, prostaglandin; PUFA, polyunsaturated fatty acids; TNF, tumour necrosis factor.

Corresponding author: Professor Bob Grimble, fax +44 23 8059 7302, email rfg1@soton.ac.uk

have been initially identified in studies on animal models, but are now widely used in clinical practice (Grimble, 1998a; O'Flaherty & Bouchier Hayes, 1999). While the animal studies have indicated the mechanisms by which immunonutrition may work, evidence of clinical efficacy is controversial (Fig. 1).

In this presentation I will attempt to review the evidence for the efficacy of immunonutrition, the limitations of the evidence for immunonutrition being effective in practice, the mechanisms whereby immunomodulation is occurring and the underlying biological reasons for the difficulty in demonstrating the efficacy of immunonutrition in clinical trials.

Table 1. Immunomodulatory nutrients and their functions

Immunonutrient	Function
<i>n</i> -3 Polyunsaturated fatty acids	Act as anti-inflammatory agents, reverses immunosuppression
S amino acids	Enhance antioxidant status via glutathione synthesis
Glutamine	Nutrient for immune cells, improves gut barrier function, acts as a precursor for glutathione
Arginine	Substrate for NO synthesis, stimulates growth hormone synthesis, improves helper T-cell numbers
Nucleotides	RNA and DNA precursors, improves T-cell function

The nature of the immune response and adverse effects associated with its operation

The body is well equipped to focus a powerful set of biological processes and agents on invading organisms. Reference to a standard immunology text gives details of the diversity of these events; however, among them, three key processes occur which influence patient outcome. These processes are initiated by secretion of the pro-inflammatory cytokines interleukin (IL) 1, IL-6 and tumour necrosis factor (TNF) α . These processes are: (1) creation of a hostile environment (for pathogens); (2) provision of nutrients for the immune system from endogenous sources; (3) strengthening of the protective and control systems against damage to healthy tissue by the immune response (Grimble, 1998a; Fig. 2). Inhibitory systems come into play, with the objective of terminating the response once its primary purpose of defeating pathogens has been achieved. The control systems include secretion of anti-inflammatory cytokines (e.g. IL-10), production of cytokine receptor antagonists (e.g. IL-1ra), secretion of glucocorticoids and down regulation of nuclear factor κ -B activation by enhancement of antioxidant defences (Grimble, 1998a). There are a number of foci at which the response may exceed its healthful confines. These foci are: (1) immunosuppression and hyperinflammation; (2) oxidant damage; (3) excessive loss of tissue components. The relationship between excessive loss of lean tissue mass and mortality is well recognized. In patients dying of sepsis there is clear evidence of an imbalance in pro- and anti-inflammatory cytokine production, a failure to maintain antioxidant defences and high levels of activation nuclear factor κ -B (Cowley *et al.* 1996; Arnalich *et al.* 2000).

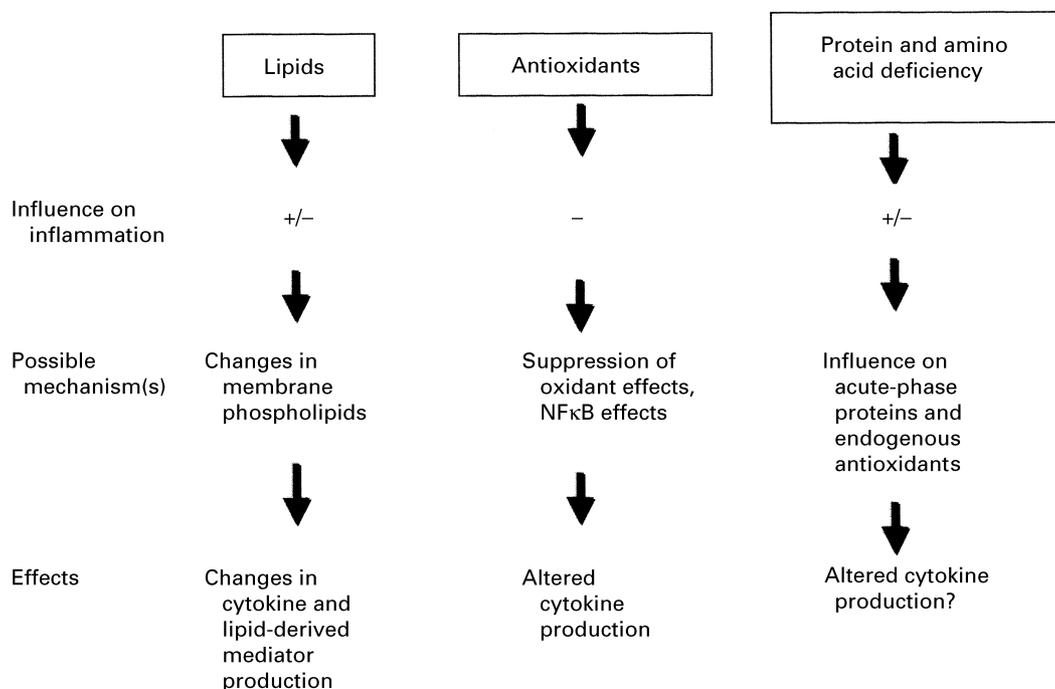


Fig. 1. Overview of the modulatory effects of nutrients on the response of animal models to inflammatory stimuli and the mechanisms underlying modulatory effects. +, Stimulatory; -, inhibitory; NF κ B, nuclear factor κ B.

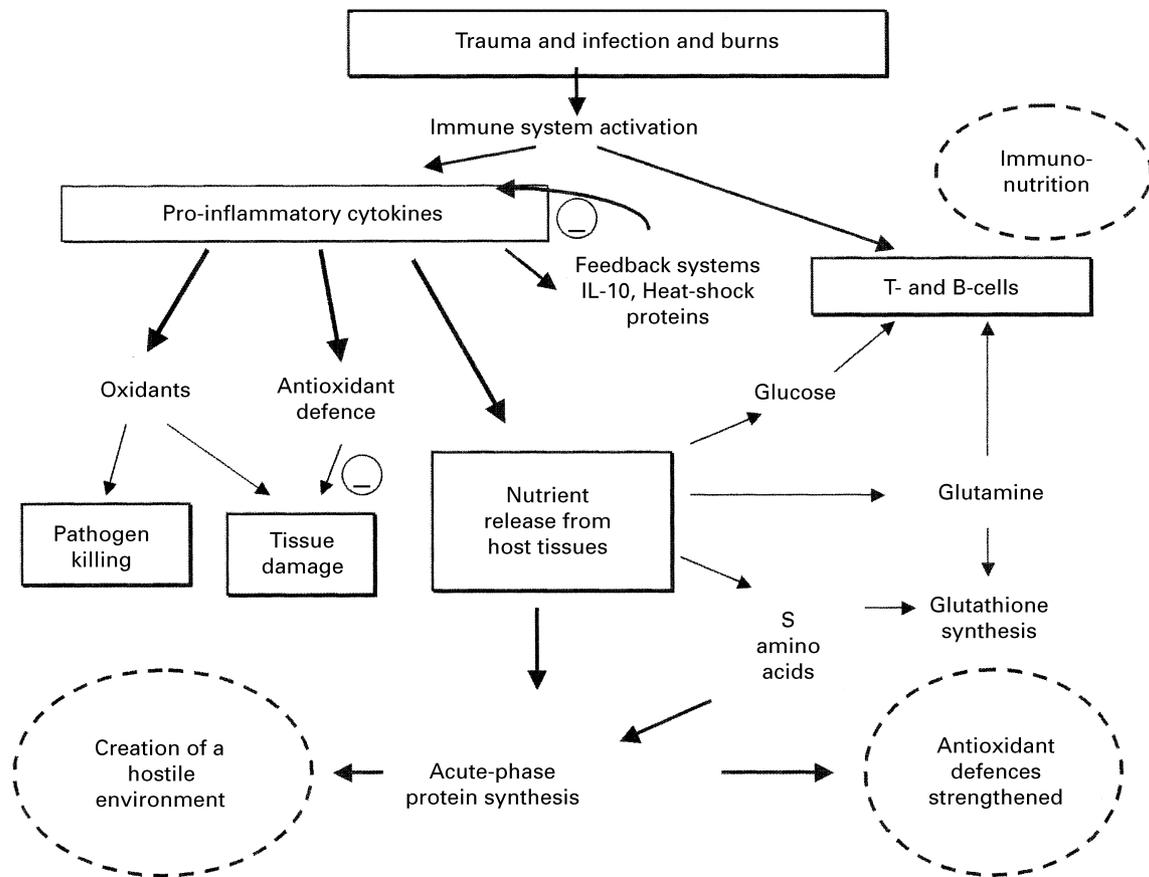


Fig. 2. Overview showing key features of the immune and metabolic response to injury and infection. (⊖), An inhibitory influence; IL, interleukin.

Thus, important targets for immunomodulation are: enhancing the cell-mediated response; altering the balance of pro- and anti-inflammatory cytokines; prevention of excessive activation of nuclear factor κ -B; facilitation of optimal activity of activator protein-1 (Jackson *et al.* 1998) and moderation of tissue nutrient depletion (Fig. 3).

Immunonutrition

During the last 20 years the pace of evolution of immunomodulatory feeds and intravenous solutions has accelerated. These products contain combinations of a number of components which have various functional attributes ascribed to them (Table 1). Various meta-analyses have been conducted on the efficacy of these products. Beale *et al.* (1999), in a meta-analysis of twelve studies containing over 1400 patients receiving enteral immunonutrition, observed that while there was no effect on mortality there were marked reductions in infection rates, time spent on a ventilator and in hospital length of stay.

Given the known functions of components of immunonutrient mixes and the potential 'trouble spots' described earlier, it could be hypothesized that the various formulations were operating at various parts of the response identified in Fig. 3. Do carefully-conducted randomised double-blind placebo-controlled clinical trials support this broad conclusion? The answer to this question is a qualified

'yes'. An increasing number of high-quality studies have been, and are being, conducted, but unfortunately very few trials make measurements on all of the linked aspects of the patient's response that determine clinical outcome (Fig. 4). Clinical indices such as infection rates, mortality rates and length of stay are often measured in the absence of functional and biochemical aspects of the response, such as T-cell function, cytokine production and antioxidant status, and vice versa. There is still a need for comprehensive studies taking into account all the linked aspects of the response and its outcome (Fig. 4).

Nonetheless, there are a number of studies which encompass a sufficient number of these aspects to be able to come to some conclusions about the impact of immunonutrition on immune function. The examples I will use are illustrative rather than comprehensive. In randomized controlled trials the administration of glutamine, either as a dipeptide during total parenteral nutrition to surgical patients or as a glutamine-enriched enteral feed to trauma patients, resulted respectively in improved N retention (less tissue depletion) and a reduction in length of stay by 6.2 d, a concomitant suppression of the rise in plasma soluble TNF receptors (reduced inflammation) and a lower incidence of bacteraemia, pneumonia and sepsis (improved immune function) (Houdijk *et al.* 1998; Morlion *et al.* 1998).

A number of roles have been ascribed to glutamine as an immunonutrient. These roles are: (1) as an essential nutrient

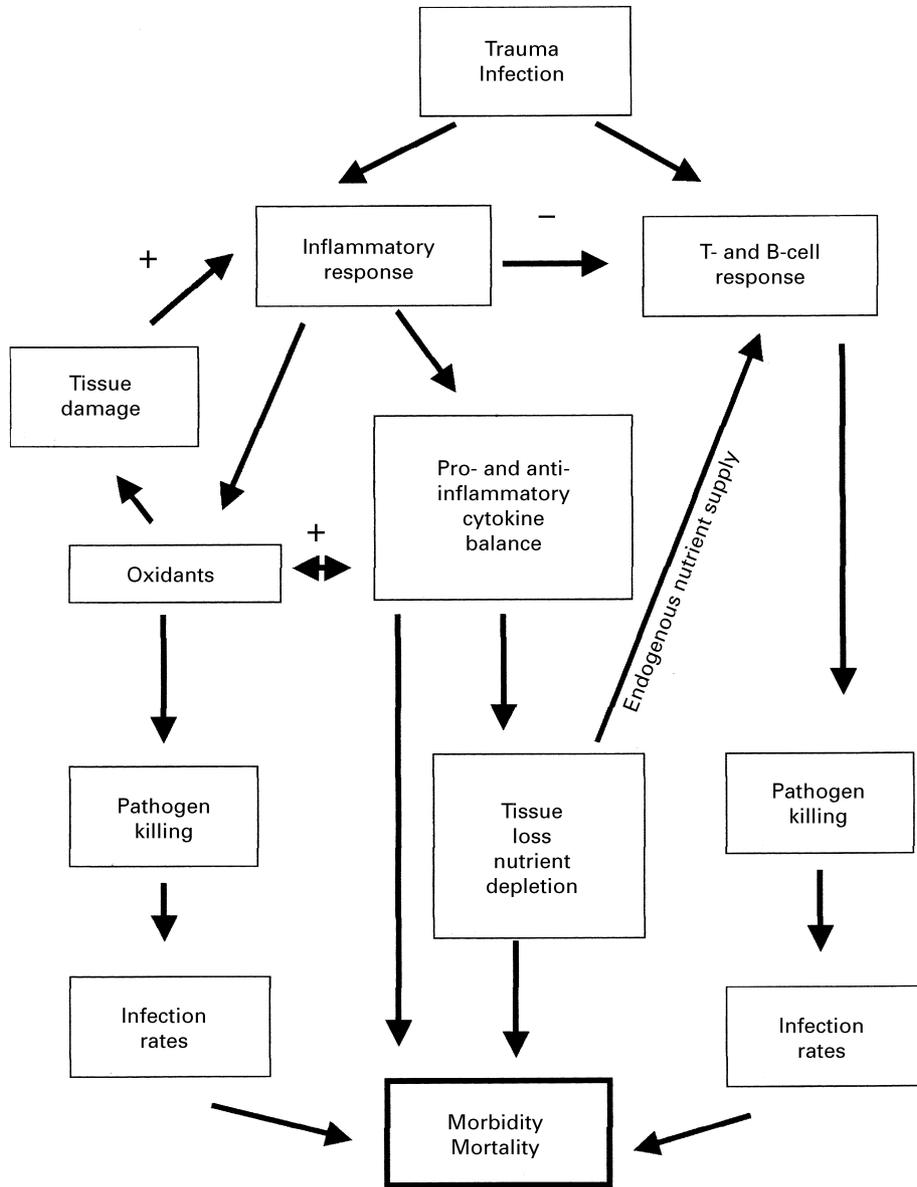


Fig. 3. Features of the response to injury and infection which influence clinical effects and outcome. +, Stimulatory effect; -, inhibitory influence.

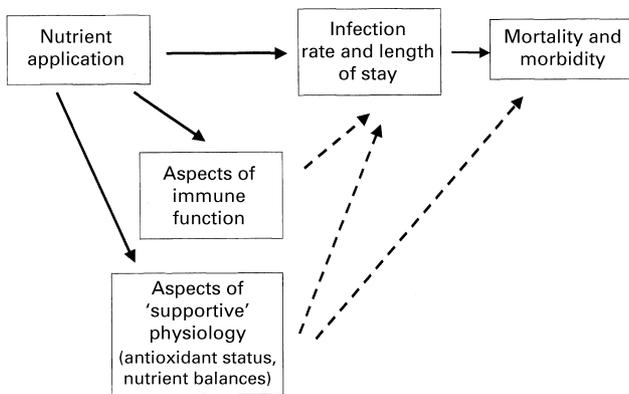


Fig. 4. Key areas which are influenced by immunonutrition. (→), Link variables which are frequently measured in clinical trials; (- ->), correlations which would strengthen the evidence obtained from trials.

for immune cells; (2) as an important modulator of gut barrier function; (3) as a substrate for glutathione synthesis. A number of reviews have been written about the first two of these roles (Newsholme *et al.* 1985; Elia, 1992). Let us consider the last of these roles. Could glutamine be exerting an anti-inflammatory influence via glutathione, and thus enhancing immune function? (see Fig. 5). Certainly, in a study in rats glutamine supplementation resulted in an increased production of glutathione by the gut (Cao *et al.* 1998), and total parenteral nutrition with glutamine raised plasma glutathione concentrations in these animals (Denno *et al.* 1996). A number of studies in which antioxidant status has been raised indicate that improvement of antioxidant status is associated with an increase in cellular aspects of immune function. Supplementation of the diet of healthy subjects and smokers with 600 mg α -tocopherol/d for

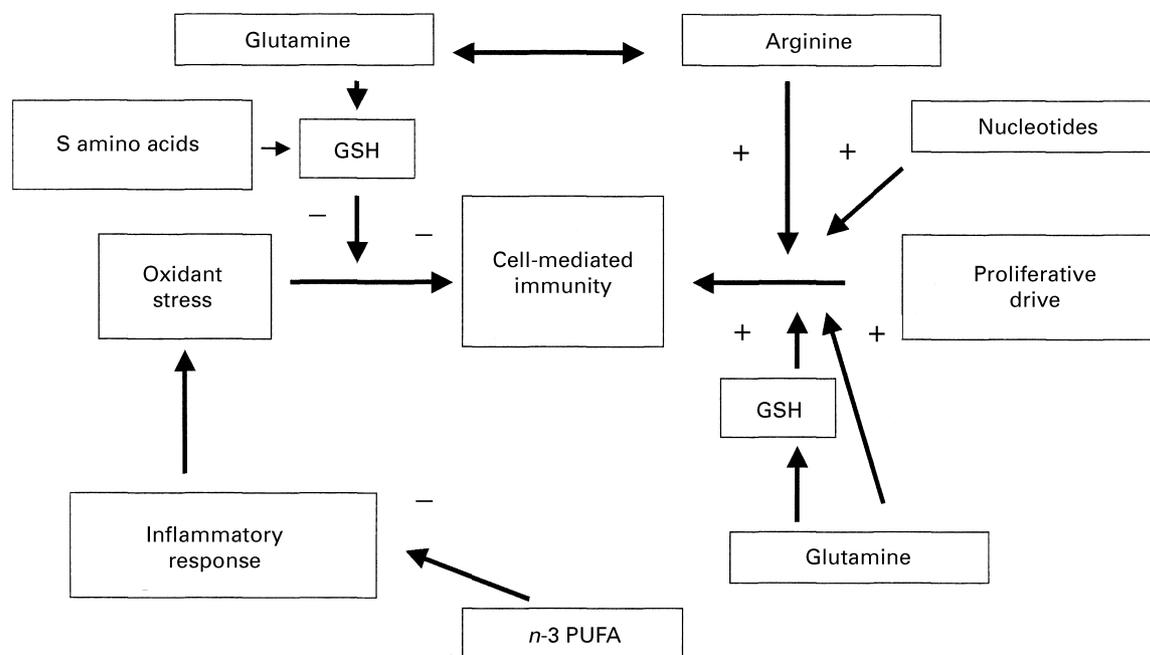


Fig. 5. Overview of the points at which immunonutrients counteract the deleterious influence of oxidant stress on cell-mediated immunity and enhance T lymphocyte activity. +, Stimulatory or supportive effect; –, an inhibitory influence; GSH, glutathione; *n*-3 PUFA, *n*-3 polyunsaturated fatty acids.

4 weeks suppressed the ability of peripheral blood mononuclear cells (PBMC) to produce TNF- α (Mol *et al.* 1997). The same dose given to healthy elderly subjects for 235 d increased delayed-type hypersensitivity and raised antibody titres to hepatitis B (Meydani *et al.* 1997). An enteral feed enriched with vitamin E, vitamin C and taurine given to intensive-care patients decreased total lymphocyte and neutrophil content in bronchio-alveolar lavage fluid (decreased inflammation) and resulted in a reduction in organ failure rate, a reduced requirement for artificial ventilation and a reduction of 5 d in the requirement for intensive care (Gadek *et al.* 1999). These results highlight the associated phenomenon of reduced inflammation and improved immune function. *In vitro* studies support this inverse relationship. PBMC taken from healthy young subjects and incubated with glutathione show decreased prostaglandin (PG) E₂ and leukotriene (LT) B₄ production (reduced inflammation) and an increase in mitogenic index and IL-2 production (enhanced immune function) (Wu *et al.* 1994).

Thus, inclusion of antioxidants or substances which increase glutathione synthesis in immunonutrient mixes would seem to be beneficial. While all antioxidants are important, due to the linked nature of antioxidant defence (Fig. 6), glutathione plays a pivotal role as it acts directly as an antioxidant and maintains other components of defence in a reduced state. Furthermore, glutathione may have a more specific effect on the function of lymphocytes via the thioredoxin system (Dröge *et al.* 1994). Unfortunately, surgery, a wide range of diseases which have an inflammatory component and ageing and protein–energy malnutrition decrease reduced glutathione concentrations in blood and other tissues (Luo *et al.* 1996; Boya *et al.* 1999; Loguercio *et al.* 1999; Nuttall *et al.* 1999; Reid *et al.* 2000; Micke *et al.* 2001). Within 24 h of elective abdominal

surgery muscle glutathione content falls by over 30%. Values return to normal 72 h post-operatively. A smaller perturbation in blood glutathione occurs over a shorter time course.

Various compounds can be used to increase glutathione synthesis (Fig. 7). N-acetyl cysteine has been widely used. Patients with sepsis given an infusion of N-acetyl cysteine (a 150 mg/kg bolus followed by infusion of 50 mg/kg over 4 h periods) showed a decrease in plasma IL-8 and soluble TNF receptor p55, had a reduced requirement for ventilator support and spent 19 d less in intensive care than patients not receiving N-acetyl cysteine (Spapen *et al.* 1998). In a study on HIV-positive patients Brietkreutz *et al.* (2000) showed that a dose of 600 mg N-acetyl cysteine/d for 7 months resulted in a decrease in plasma IL-6, an increase in natural killer cell activity and increased responsiveness of T lymphocytes to tetanus toxin stimulation.

Variability in responsiveness to immunonutrients

n-3 Polyunsaturated fatty acids (PUFA) are key components of immunonutrient formulations, due to their anti-inflammatory properties (Endres *et al.* 1989; Gerster, 1995; Calder, 1997; Grimble 1998b). However, it is not possible to discern the contribution of *n*-3 PUFA to the general anti-inflammatory and immuno-enhancive effects demonstrated in trials using such formulations. Peri-operative feeding of colo-rectal cancer patients with an arginine-enriched enteral feed containing *n*-3 PUFA resulted in a decrease in the post-operative rise in IL-6 and IL-1 soluble receptors, an increase in IL-2 receptor- α , an improvement in delayed hypersensitivity responses and a decrease in infection rates (Gionotti *et al.* 1999). In a study on post-operative cancer patients, the same dietary formulation resulted in not only a

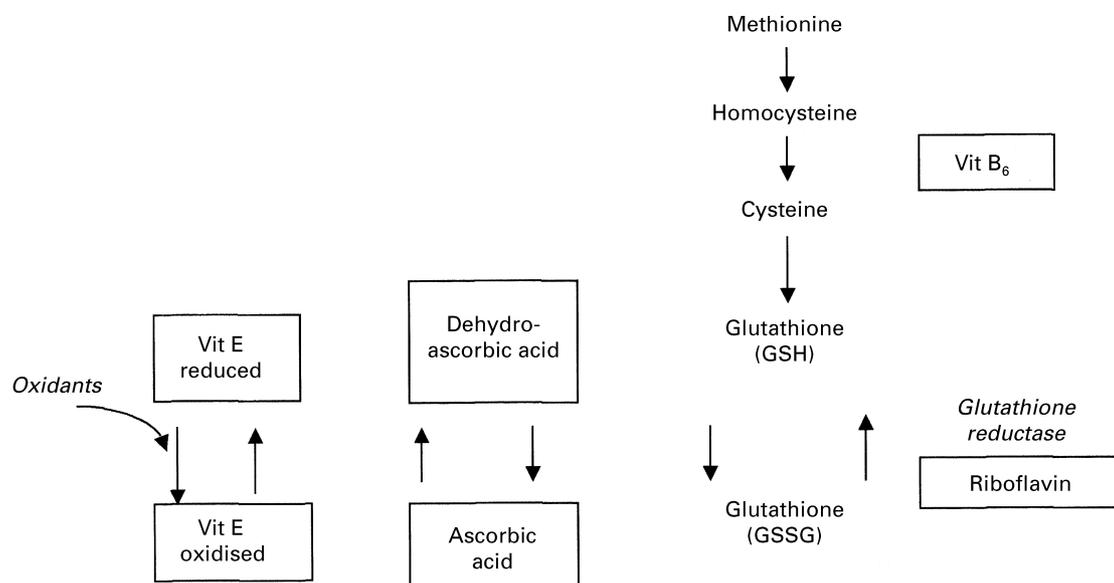


Fig. 6. The interrelationships between components of antioxidant defence and associated metabolites. Vitamin B₆ (vit B₆) and riboflavin act as cofactors for the defence system. Vit E, vitamin E; GSH, reduced glutathione; GSSG, oxidised glutathione.

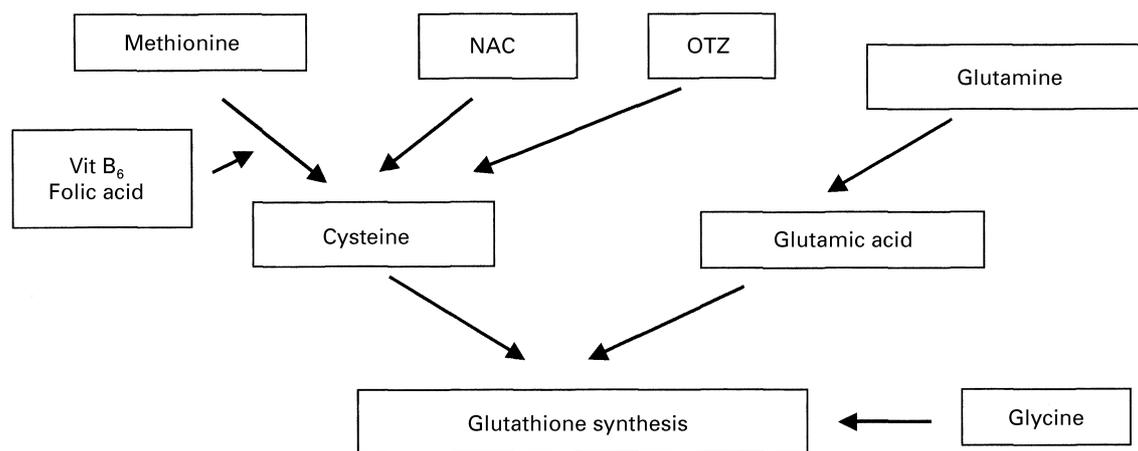


Fig. 7. Substrates which can be utilized to support and increase glutathione synthesis. OTZ, L-2-oxothiazolidine-4-carboxylate; NAC, N-acetyl cysteine; vit B₆, vitamin B₆.

fall in IL-6 but a rise in IL-2 soluble receptors, indicating how the immuno-enhancement may have been achieved (Braga *et al.* 1999). Studies on inflammatory disease have also shown the anti-inflammatory influence of *n*-3 PUFA given in the form of fish oil. However, not all studies have shown a beneficial effect. In rheumatoid arthritis and psoriasis significant clinical improvements have been reported; however, the oil is less efficacious in systemic lupus erythematosus and produced no benefit in asthma (Calder, 1997).

Mechanisms underlying the variable response to fish oil

The question arises as to why an anti-inflammatory effect is not found in all studies in which *n*-3 PUFA have been given, and in those studies where *n*-3 PUFA show this effect, why an anti-inflammatory influence is not demonstrable in all

subjects. The answer to these questions may impact on why formulations enriched with *n*-3 PUFA are not efficacious in all patients.

The study of Endres *et al.* (1989) focused attention on fish oil as a potential anti-inflammatory nutrient, particularly in its capacity to reduce pro-inflammatory cytokine production. In the study nine subjects were given 18 g fish oil/d for 6 weeks. A statistically significant ($P < 0.05$) fall in *ex vivo* IL-1 and TNF- α production from stimulated PBMC was noted. However, the data showed large standard deviations, indicating that within the nine subjects there were both 'responders' and 'non-responders' to the anti-inflammatory effects of fish oil. Subsequently, other studies, also on relatively small numbers of subjects, have shown either an inhibitory effect of fish oil supplements on *ex vivo* pro-inflammatory cytokine production (Kelley *et al.* 1999), or no effect (Yaqoob *et al.* 2000). We have investigated the

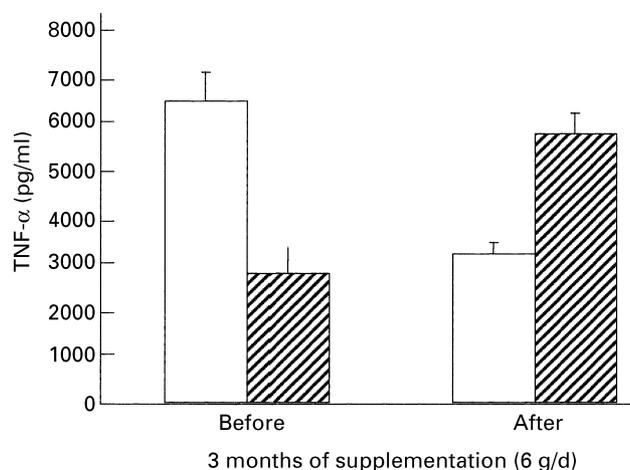


Fig. 8. The influence of a 12-week period of dietary supplementation of 111 young men with 6g MaxEPA fish oil capsules (Seven Seas Ltd, Hull, Humberside, UK)/d, on *ex vivo* production of tumour necrosis factor α (TNF- α), by peripheral blood mononuclear cells stimulated with lipopolysaccharide. (□), Subjects showing decreased TNF- α production after fish oil supplements; (▨), subjects showing increased TNF- α production after fish oil supplements.

effects of feeding 6g fish oil/d, for 12 weeks on *ex vivo* TNF- α production by PBMC in 111 young men. The results are shown in Fig. 8 (Grimble *et al.* 2001). Surprisingly, fish oil resulted in a lowering of TNF- α production in 51% of the subjects and an increase in production in 49% of the subjects. In *in vitro* studies on PBMC cultured with PG and LT it was shown that PGE₂ suppresses and LTB₄ enhances TNF- α production (Endres *et al.* 1989; Choi *et al.* 1996). As a result of supplementation with *n*-3 PUFA, arachidonic acid in the cell membrane will be replaced by eicosapentaenoic acid. Arachidonic acid is the precursor for a number of eicosanoids, including PGE₂ and LTB₄. Eicosapentaenoic acid, however, is the precursor for PGE₃ and LTB₅. These latter eicosanoids have lower bioactivity than PGE₂ and LTB₄. Thus, theoretically, substitution of eicosapentaenoic acid for arachidonic acid in the membrane of the PBMC might result in a lessening of the inhibitory or stimulatory influence of the respective eicosanoids. TNF- α production would thus either rise or fall. An additional cause of variability in response might lie with genetic influences in the patients. In studies in which cytokine production from PBMC has been measured on a number of occasions it was found that there is a high degree of constancy in production at an individual level. This phenomenon is apparent in males and post-menopausal females (Jacob *et al.* 1990). There are single-nucleotide polymorphisms in the promoter regions of cytokine genes which influence the level of expression of the respective cytokine (Hutchinson *et al.* 1999). Thus, individuals are 'hard wired' for having high, medium or low levels of production of the respective cytokine. Interestingly, single-nucleotide polymorphisms in the TNF- α and lymphotoxin- α promoters influence TNF- α production (Pociot *et al.* 1993; Majetschak *et al.* 1999). Individuals who are homozygous for the TNF- α allele (TNF2) or for the lymphotoxin- α allele (TNFB2) show high levels of TNF- α production. Homozygotes for the TNF1 or TNFB1 alleles

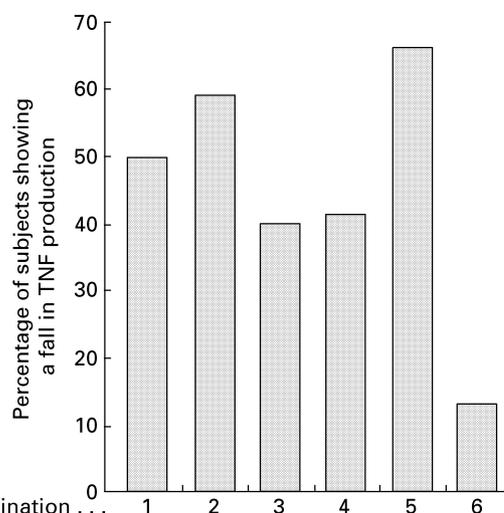


Fig. 9. Influence of tumour necrosis factor α (TNF- α) and lymphotoxin (LT)- α promoter allele combinations, of young men supplemented with 6g MaxEPA fish oil capsules (Seven Seas Ltd, Hull, Humberside, UK)/d for 12 weeks, on the ability of supplementation to suppress TNF- α production by peripheral blood mononuclear cells stimulated with lipopolysaccharide.

exhibit low production, with intermediate levels of production being found in heterozygotes. Increased mortality in malarial infection and sepsis has been noted in individuals who are homozygous for TNF2 or TNFB2 respectively (McGuire *et al.* 1994; Stüber *et al.* 1996). In addition, homozygosity for the TNF2 allele has been associated with disease severity in chronic hepatitis C infection and increased rejection rates of renal and heart transplants (Asano *et al.* 1997; Turner *et al.* 1997; Hohler *et al.* 1998).

We investigated whether all individuals with each of the possible combinations of TNF- α and lymphotoxin- α alleles were equally sensitive to the effects of fish oil supplementation (Grimble *et al.* 2001). An overview of the data is shown in Fig. 8. As reported earlier, fish oil showed an anti-inflammatory influence in 51% of our study population. However, as can be seen in Fig. 9, individuals with allele combination 1 conformed to this finding, while those with allele combinations 3 and 6 showed a greater and lesser responsiveness respectively to the anti-inflammatory influence of fish oil. Thus, sensitivity to the anti-inflammatory effects of fish oil is influenced by individual genotypic characteristics.

Improving the efficacy of immunonutrition

While meta-analyses indicate that immunonutrition can be efficacious in some groups of patients when applied without specific knowledge of the precise requirements or metabolic status of the patients, improvements in efficacy will occur if patients are carefully monitored in terms of their antioxidant status and level of depletion of tissue nutrient stores. When further studies have been conducted to discern the precise interaction between each individual's genotype, of relevance to the response to injury and infection, and immunonutrients, the level of precision in the application of immunonutrition will undoubtedly improve (Fig. 10).

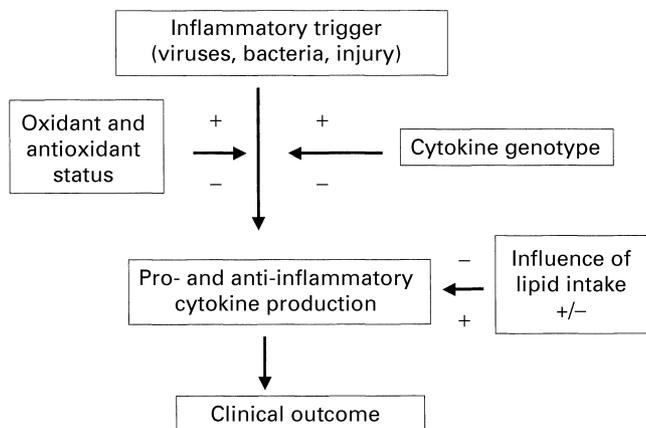


Fig. 10. Summary of nutritional and genetic influences on cytokine production and clinical outcome. +, A stimulatory effect; -, an inhibitory influence.

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