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Clinical Nutrition and Metabolism Group Symposium on 'Nutrition in the severely-injured patient'

The scientific basis of immunonutrition†

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Substrates with immune-modulating actions have been identified among both macro- and micronutrients. Currently, the modes of action of individual immune-modulating substrates, and their effects on clinical outcomes, are being examined. At present, some enteral formulas are available for the clinical setting which are enriched with selected immune-modulating nutrients. The purpose of the present paper is to review the scientific rationale of enteral immunonutrition. The major aspects considered are mucosal barrier structure and function, cellular defence function and local or systemic inflammatory response. It is notable that in critical illness the mucosal barrier and cellular defence are impaired and a reinforcement with enteral immunonutrition is desirable, while local or systemic inflammatory response should be down regulated by nutritional interventions. The results available from clinical trials are conflicting. Meta-analyses of recent trials show improvements such as reduced risk of infection, fewer days on a ventilator, and reduced length of intensive care unit and hospital stay. Thus, a grade A recommendation was proclaimed for the clinical use of enteral immune-modulating diets. Improvement in outcome was only seen when critical amounts of the immune-modulating formula were tolerated in patients classified as being malnourished. However, in other patients with severe sepsis, shock and organ failure, no benefit or even disadvantages from immunonutrition were reported. In such severe conditions we hypothesize that systemic inflammation might be undesirably intensified by arginine and unsaturated fatty acids, directly affecting cellular defence and inflammatory response. We therefore recommend that in patients suffering from systemic inflammatory response syndrome great caution should be exercised when immune-enhancing substrates are involved which may aggravate systemic inflammation.

Immunonutrition: Immune-modulating substrates: Enteral nutrition

The interrelationship between nutrition and the immune system has become the focus of ever increasing attention as an increasing number of substrates are being identified as having an immune-modulating function. Immunonutrients might be identified among macro- and micronutrients. Amino acids such as glutamine, arginine, cysteine and taurine, as well as nucleotides, are important immune-modulating substrates. Lipids that may be involved include monounsaturated and polyunsaturated fatty acids (PUFA), as well as short-chain fatty acids. Numerous substrates that interact with the immune system have been identified among vitamins and trace elements (vitamins A, C and E, Zn and Se). Based on experimental observation, many immunomodulatory effects have been claimed for a long time, but their clinical significance has only been recognized since the early 1980s. Indeed, the levels of inclusion of immune-modulating substrates clearly exceeds the amount used in a simple prevention of deficits. However, 'pharmacological' immunonutrition should simultaneously satisfy both the metabolic and immunological needs of the patient. The rationale for writing the present review is to examine the scientific basis of immunonutrition in the

Abbreviations: EPA, eicosapentaenoic acid; GSH, glutathione; IL, interleukin; LT, leukotriene; NOS, NO synthase; PUFA, polyunsaturated fatty acids; SIRS, systemic inflammatory response syndrome.

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context of enteral feeding interventions in the severely injured patient.

Areas of immune defence and their modulation by defined substrates

In order to simplify this task, the immune defence system can be subdivided into three sites of action representing potential targets for specific nutritional substrates: (1) mucosal barrier function; (2) cellular defence function; (3) local or systemic inflammation (Fig. 1).

The mucosal barrier function of the intestinal mucosa represents the first line of defence against translocating pathogens, and it is already considered important in relation to early enteral nutrition of critically ill patients (Gardiner *et al*. 1995). Indeed, sufficient availability of suitable substrates is currently considered the major tool in maintaining the structure and functionality of the mucosal barrier.

Cellular defence function includes the specific and non-specific cellular immune response. Following invasion of pathogens it represents the second line of defence, consisting of granulocytes, macrophages, lymphocytes and plasma cells. The complex interactions between these effector cells are coordinated through the release of cytokines and other mediators. Nutritional substrates can modulate the cellular and humoral defence system via modified mediator formation or by interference with signal transduction.

Essential components of the inflammatory immune response are represented by the activation of cascade systems, such as the coagulatory or the complementary system. Moreover, mediators are involved which include cytokines, eicosanoids, platelet-activating factor and NO, as well as vasoactive amines and kinines. Systemic inflammatory response is manifest at the endothelium, the smooth vascular and bronchial muscles, and at platelet aggregation. This response may impair microcirculation, pulmonary gas exchange, vascular permeability, coagulation, as well as substrate utilization, and thus may influence organ function. Thus, a selective quantitative and qualitative choice of the supply of certain defined nutritional substrates which serve as the precursors of mediators may modulate the severity of the inflammatory immune response.

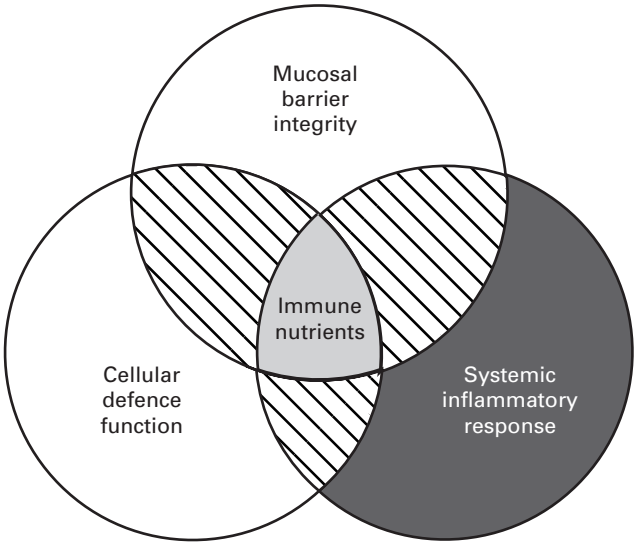

Fig. 1. Schematic representation of the three areas of immune defence affected by immunonutrients.

The actions of pathogens on the systemic immune response are illustrated in Fig. 2. Although a boost of cellular defence functions may initially take place; in the long term this boost is followed by the suppression of these functions, an effect described by the term 'immune paralysis'. Within the framework of these events experimental and clinical data lend credence to the idea of understanding defined substrates as 'pharmacologically effective agents' by which the cellular defence function can be restored or the systemic inflammatory response alleviated. Consequently, glutamine, arginine, nucleotides and PUFA are considered of primary relevance.

Glutamine

Glutamine is the most prevalent free amino acid in the human body. In skeletal muscle glutamine constitutes >60 % of the total free amino acid pool (Bergström *et al*. 1974). It is a precursor that donates N for the synthesis of purines, pyrimidines, nucleotides, amino sugars and glutathione (GSH), and is the most important substrate for renal ammoniagenesis (regulation of the acid–base balance). Glutamine serves as a N transporter between various tissues, and represents the major metabolic fuel for the cells of the gastrointestinal tract (enterocytes, colonocytes; Windmueller, 1982; Souba, 1991) as well as for many rapidly proliferating cells, including those of the immune system (Calder, 1994). Consequently, the morphological and functional integrity of the intestinal mucosa appears to be protected by sufficient availability of glutamine. There is much evidence that hypercatabolic and hypermetabolic situations are accompanied by marked depressions in muscle intracellular glutamine. This response has been shown to occur after elective operations, major injury, burns, infections and pancreatitis, irrespective of nutritional attempts at the time of repletion. A reduction in the muscle free glutamine pool (approximately 50 % of the normal level) thus appears to be a hallmark of the response to injury, infection and malnutrition (for references, see Fürst, 1994*a*). This response creates a glutamine-depleted environment, the consequences of which include enterocyte and immunocyte starvation (Bode & Souba, 1994). It has been suggested that glutamine becomes a conditionally essential amino acid during episodes of catabolic stress such as injury and sepsis.

Numerous experimental studies support this hypothesis. Glutamine-supplemented enteral or parenteral nutrition solutions are associated with increased intestinal mucosal thickness and DNA and protein content, reduced bacterial translocation after radiation (Souba, 1991), weakened adverse effects of experimentally induced enterocolitis (Rombeau, 1990), preserved intestinal mucosa during parenteral nutrition (Babst *et al*. 1993) and enhanced rat mucosal hyperplasia after small bowel resection (Klimberg *et al*. 1990). *In vitro*, glutamine has been shown to induce

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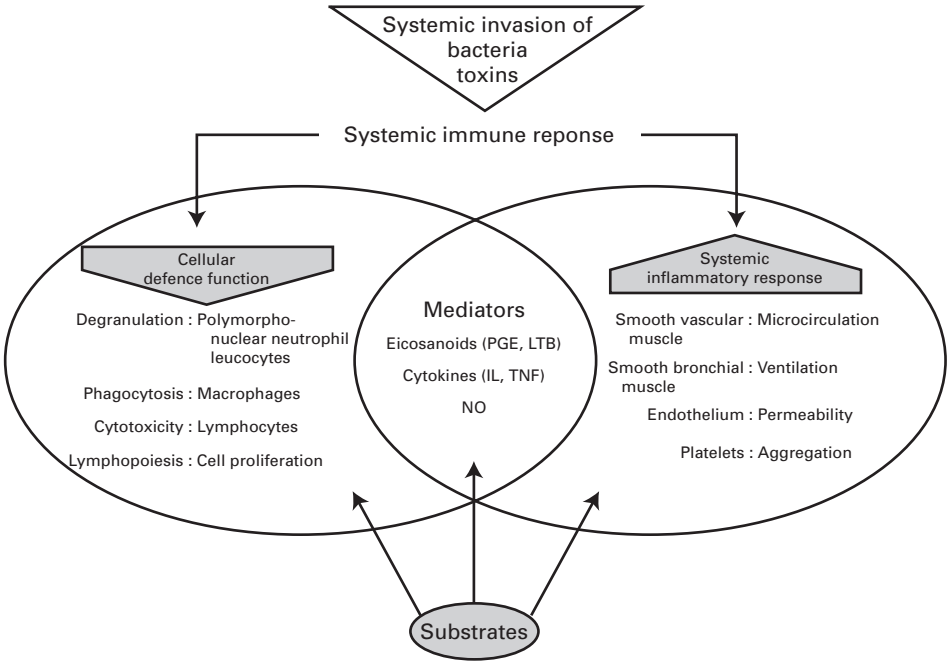

Fig. 2. Effects of invading pathogens on the systemic immune response and its modulation by substrates with immune-modulating action. PGE, prostaglandin E; LTB, leukotriene B; IL, interleukin; TNF, tumour necrosis factor.

heat shock protein 70 and its RNA transcription in intestinal epithelial cells, thereby reducing cytolysis, induced by heat and oxidation (NH_2-Cl; Wischmeyer *et al*. 1997; Chow & Zhang, 1998). Thus, glutamine supplementation reduced heat shock-induced cell death. This effect, together with the maintenance of cell growth, may play a key role in the prevention of intestinal mucosal atrophy.

In addition, glutamine supplementation has been reported to restore mucosal immunoglobulin A and enhance upper respiratory tract immunity (Li *et al*. 1997), prevent gut-derived sepsis in obstructive jaundice (Houdijk *et al*. 1997), reverse gut-derived sepsis due to prednisone administration (Gennari & Alexander, 1997) and enhance bacterial clearance in peritonitis (Furukawa *et al*. 1997).

Glutamine supplementation augments the cytotoxic activity of natural killer and lymphokine-activated killer cells (Alverdy, 1990; Babst *et al*. 1993; Horig *et al*. 1993) as well as adequate lymphocyte, killer cell and macrophage proliferation (Griffiths & Keast, 1990; Parry-Billings *et al*. 1990) and function (Griffiths & Keast, 1990; Wallace & Keast, 1992; Calder & Newsholme, 1992; Calder, 1994; Juretic *et al*. 1994). Leucocyte glutaminase activity is high, thus indicating a high rate of glutamine utilization (Calder, 1994). Recumbent immunocompetent cells already show a distinctive glutamine metabolism, that further increases by immunological provocation (Calder, 1994). All these effects emphasize that cellular defence function can be reinstated by glutamine repletion *in vitro* and *in vivo*.

In experimental studies supplemental glutamine preserves hepatic and intestinal mucosal stores of GSH and maintains plasma concentrations (Hong *et al*. 1992; Harward *et al*. 1994; Denno *et al*. 1996; Yu *et al*. 1996). In the gut GSH is involved in the detoxification of reactive oxygen species and pro-oxidative nutrients. An experimentally induced intestinal GSH deficiency has been shown to be associated with impaired mucosal integrity and function (Martensson *et al*. 1990; Kelly, 1993). It has also been shown that lumen and lymphatic concentrations of lipid hydroperoxides were related to intestinal GSH status (Aw, 1997). Experimental feeding with glutamine results in a considerable increase in its gut fractional uptake and a marked increase in intestinal GSH fractional release, indicating increased intestinal GSH production (Cao *et al*. 1998). The biochemical explanation for these findings is based on the fact that the highly charged glutamic acid molecule, one of the direct precursors of GSH, is poorly transported across the cell membrane, whereas glutamine is readily taken up by the cell. Glutamine is then deaminated and thus can serve as a glutamic acid precursor (Hong *et al*. 1992). Obviously, glutamine-mediated GSH synthesis might be one of the most important factors in the systemic inflammatory response. It is proposed that tissue GSH synthesis is a crucial factor in causing the reversal of the clinical biochemical signs of critical illness.

Regarding the clinical application of glutamine, impressive confirmation that enteral glutamine therapy is effective in preventing infective complications has been reported recently in sixty patients with severe multiple trauma (Houdijk *et al*. 1998). In a randomized controlled study enteral glutamine nutrition at 25–30 g/d (Houdijk, 1998) was commenced within 4 h of trauma via a naso-duodenal tube for a minimum of 5 d. There was a significant reduction (< −50 %) in the 15 d incidence of pneumonia, bacteraemia and severe sepsis. As a measure of the systemic inflammatory response, the group receiving glutamine showed lower levels of soluble tumour necrosis factor receptors. The strength of this study lies in the relatively homogeneous population of patients studied, and that it does

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not suffer the confounding factors present in multi-centre studies (Griffiths, 1999). However, the results of this fascinating study require confirmation. In a current study of a more heterogeneous group of intensive care unit patients able to tolerate enteral feeding (Jones *et al.* 1999), many of whom were already infected on admission, there was no suggestion of reduced mortality, but total post-intervention hospital costs were significantly reduced in both enteral and parenteral glutamine recipients.

In conclusion, the enteral route may be ideal when given early to the non-infected patient to improve gut-associated lymphoid tissue function and the immune defence against infection. For the already-severely-stressed or infected intensive care unit patient enteral supplements alone may be inadequate, and parallel parenteral support is likely to be required. It has been clearly demonstrated that during intensive care, the patient's parenteral supplementation of enteral nutrition does not increase the risk to the patients and may even ensure a better overall outcome (Bauer *et al.* 1998).

Nucleotides

Nucleotides are important components for the synthesis of DNA, RNA and adenine nucleotides. Adequate nucleotide synthesis requires sufficient amounts of purines and pyrimidines. In healthy subjects they are efficiently absorbed from the diet which normally contains 1–2 g/d. Purines and pyrimidines are either derived from *de novo* synthesis or from RNA turnover by means of so-called 'salvage pathways'. In the case of adequate protein intake, *de novo* synthesis is the main source of nucleotides; glutamine being the major N donor (Szondy & Newsholme, 1990). The role of nucleic acids is critical because expression of the synthesizing enzymes in the *de novo* pathway is apparently impaired during catabolic stress (Grimble, 1994). During episodes of infection following injury and trauma the demand for nucleotides is increased in order to facilitate the synthetic capacity of the immune cells (Jyonouchi, 1994; Kulkarni *et al.* 1994). The absence of nucleotides (purines and pyrimidines) in the diet results in a selective loss of T-helper lymphocytes and a suppression of interleukin (IL) 2 production (VanBuren *et al.* 1994).

Parenteral solutions and the majority of enteral diets do not contain nucleotides. In clinical nutrition an adequate supply of nucleotides may be a critical factor in promoting intestinal function and immune status, as suggested by the findings of numerous experimental studies (Kulkarni *et al.* 1994; VanBuren *et al.* 1994; LeLeiko & Walsh, 1995; Cosgrove, 1998). In the experimental setting dietary nucleotide removal was associated with impaired mucosal integrity and function, which could be partly prevented or reversed by oral or intravenous supply of these substrates (LeLeiko *et al.* 1987; Nunez *et al* 1990; Iijima *et al.* 1993). Decreased availability of nucleotides is associated with impaired T-cell function (VanBuren *et al.* 1983, 1990; Carver *et al.* 1990; Pizzini *et al.* 1990), weakened natural killer cell activity (Carver *et al.* 1990), delayed rejection of allogenic transplants (VanBuren *et al.* 1983), decreased mortality from graft *v.* host reactions (Kulkarni *et al.* 1984), suppressed lymphocyte proliferation (VanBuren *et al.* 1983; Kulkarni *et al.* 1989) as well as reduced IL-2 production (VanBuren *et al.* 1994). Moreover, reduced phagocytosis (Fanslow *et al.* 1988) and an impaired clearance of experimentally applied pathogens (Kulkarni *et al.* 1986) were induced by dietary removal of nucleotides. Most of these effects could be reversed by resumption of the dietary supply of nucleotides (Pizzini *et al.* 1990).

The question of whether the demand for nucleosides and nucleotides can exceed the endogenous synthetic capacity in human subjects remains to be answered, and implications with regard to impaired organ and system function are yet to be evaluated (Grimble, 1994). Notably, there is no relevant experimental or clinical evidence that nucleotides or nucleosides may enhance the systemic inflammatory response.

Arginine

Arginine is a dibasic amino acid which the body obtains from dietary sources and by endogenous synthesis via the urea cycle. During trauma and sepsis endogenous availability of arginine is reduced (Barbul *et al.* 1983; Kirk & Barbul, 1990; Nirgiotis *et al.* 1991). Arginine is metabolized within the enterocyte via the arginase pathway to ornithine and urea. Arginine, via the formation of glutamate, may yield increased amounts of proline and hydroxyproline, which are required for the synthesis of connective tissue. Moreover, arginine is the precursor of polyamine, histidine and nucleic acid synthesis. It is a promoter of thymic growth and an endocrinological secretagogue stimulating release of growth hormone, prolactin, insulin and anti-insulinaemic hormones (Barbul, 1986). Most importantly, however, via the arginine deaminase pathway (Blachier *et al.* 1991), arginine has been shown to be the unique substrate for the production of the biological effector molecule NO. NO is formed by oxidation of one of the two identical terminal guanidino groups of L-arginine by the enzyme NO synthase (NOS). Of the three NOS isoenzymes characterized, two are constitutive, Ca^{2+}-dependent (endothelial and neuronal) and generate lesser levels of NO than their inducible counterpart (Nathan & Xie, 1994). Inducible NOS is prominent in inflammatory conditions and it is also most often implicated as the producer of NO during the immune response. According to recent reports NO plays an essential role in the regulation of inflammation and immunity (Albina, 1996).

Inhibition of NO synthesis increased intestinal mucosal permeability in experimental models of ischaemia–reperfusion intestinal injury (Kubes, 1993) and acute necrotizing enterocolitis (Miller *et al.* 1993). In addition, administration of L-arginine reversed the effect of NOS inhibition (Kubes, 1993). These results suggest that basal NO production is important in minimizing the mucosal barrier dysfunction in these models.

Arginine may also be of significance in the critically ill patient because of its potential role in immunomodulation (Kirk & Barbul, 1990; Evoy *et al.* 1998). It is hypothesized that arginine enhances the depressed immune response of individuals suffering from injury, surgical trauma, malnutrition or sepsis. In experimental animals as well as in human studies supplementation with arginine resulted in an

improved cellular response, a decrease in trauma-induced reduction in T-cell function and a higher phagocytosis rate (Kirk & Barbul, 1990).

It is notable that 5 years ago parenteral arginine was considered a novel and valuable tool to improve immunity and to beneficially influence metabolism and pathophysiology in cancer and trauma. Remarkably, in the current literature the intravenous arginine approach is almost absent, while emphasis is laid on enteral arginine nutrition. Presumably the prominent reports of the drawbacks and disadvantages of large amounts of parenteral arginine have been slowly recognized and considered (for references see Fürst & Stehle, 1995). In healthy human subjects and surgical and intensive care unit patients enteral arginine supplementation was accompanied by increased lymphocyte and monocyte proliferation as well as enhanced T-helper cell formation (Daly *et al*. 1988; Barbul, 1990; Cerra *et al*. 1990). Clinical studies have demonstrated moderate net N retention and enhanced protein synthesis compared with isonitrogenous diets in critically ill and injured patients. Following surgery for certain malignancies in elderly post-operative patients, supplemental arginine (25 g/d) enhanced T lymphocyte responses to phytohaemagglutinin and concanavalin A, and increased the CD_4 phenotype number (Daly *et al*. 1988). Interestingly, insulin-like growth factor-1 levels were about 50 % higher, reflecting the growth hormone secretion induced by arginine supplementation. A high load of oral arginine (30 g/d) improved wound healing (Barbul *et al*. 1990) and enhanced blastogenic response to several mitogens (Sodeyama *et al*. 1993). Some of these studies were also associated with *in vitro* evidence of enhanced immunoactivity (Kirk & Barbul, 1990; Brittenden *et al*. 1994*a*,*b*; Beaumier *et al*. 1995). Thus, it is probable that the observed beneficial effects of these substrates were due to improved function of the immune system rather than improved gut barrier function.

Results available from clinical trials failed to demonstrate improvements in patient outcome (for references, see Lin *et al*. 1998). There is also some concern that arginine may enhance the systemic inflammatory response due to an enhanced NO release in patients with severe systemic inflammatory response syndrome (SIRS) or sepsis. This response would lead to a negative iono- and chronotropism of the myocardium (Lowenstein *et al*. 1994), impaired coagulation (Radomski *et al*. 1990; deGraaf *et al*. 1992) and vascular dilatation leading to refractory hypotension (Lee *et al*. 1984; Lorente *et al*. 1993). Apparently, NO may exert cytotoxic effects as a non-specific effector inhibiting growth or killing off cells in an untargeted fashion (Lepoivre *et al*. 1991; Wink *et al*. 1991; Lowenstein *et al*. 1994). On the other hand, according to current knowledge, NOS and NO-mediated immunofactors as well as intracellular arginase are restricted to distinct compartments, thus supplemental arginine may not affect extracellular NO concentration (Moncada *et al*. 1991).

n-*3 Polyunsaturated fatty acids*

We are gradually understanding that lipids are more than sources of energy and building blocks for cell membranes, but may, in some circumstances, be considered as pharmacological agents provided through nutrition. This situation appears to be particularly true for the *n*-3 PUFA.

Fatty acids are characterized by the number of C atoms, the number of double bonds and the position of the first double bond, calculated from the methyl end of the molecule. Thus, 18 : 2*n*-6 represents linoleic acid which serves as the precursor for the formation of the most important fatty acids of the *n*-6 series such as arachidonic acid. 18 : 3*n*-3 represents α-linolenic acid, the parent compound of *n*-3 PUFA, with the first double bond being at C-3 from the methyl end. Whereas *n*-6 fatty acid deficiency has been recognized and considered, *n*-3 fatty acid deficiency is just now being appreciated. Delayed growth, neurobiological symptoms, skin lesions, reduced visual acuity, abnormal electroretinogram and reduced learning ability represent signs of *n*-3 fatty acid deficiency. Long-chain *n*-3 PUFA such as eicosapentaenoic (20 : 5*n*-3; EPA) and docosahexaenoic acid (22 : 6*n*-3) are built up in algae and plankton and the fish living on them, rendering deep-sea fish and fish oils produced from them the main dietary source of *n*-3 PUFA for human subjects.

With the enteral or parenteral intake of increased quantities of *n*-3 PUFA, the *n*-3 : *n*-6 PUFA value in the phospholipid spectrum of the cell membrane in various tissues changes in favour of *n*-3 PUFA (Palombo *et al*. 1993; Morlion *et al*. 1996). Several laboratories have demonstrated that dietary pretreatment with *n*-3 PUFA favourably influences the pathophysiological response to endotoxins (Mascioli *et al*. 1988; Seidner *et al*. 1989) and exerts an important modulatory effect on eicosanoid and cytokine biology. The most likely way in which lipids might modulate pro-inflammatory cytokine biology is by changing the fatty acid composition in the cell membrane. As a consequence of the changes two interrelated phenomena may occur: (1) alteration in membrane fluidity; (2) alterations in products which arise from hydrolysis of membrane phospholipids (Grimble, 1998).

Changes in fluidity may alter the binding of cytokines and cytokine-inducing agonists to receptors (Stubbs & Smith, 1984; Murphy, 1990). For example, fluidity changes may alter G-protein activity, thereby changing adenylate kinase, phospholipase A_2 and phospholipase C activity (for references, see Fürst & Kuhn, 2000).

Alterations in membrane phospholipids will also directly influence the synthesis of lipid-derived mediators such as the eicosanoids, phosphatidic acid, platelet-activating factor and the secondary messengers, diacylglycerol and ceramide (Grimble, 1992, 1998; Ross *et al*. 1999). By the action of the enzyme phospholipase A_2, PUFA can be released from the membrane phospholipids and either act as a secondary messenger or alternatively serve as a precursor for the cyclo-oxygenase pathway (Kinsella *et al*. 1990). The latter pathway metabolizes arachidonic acid to the 2-series of prostaglandins, especially prostaglandins E_2 and $F_{2\alpha}$ and thromboxane A_2. EPA is also an excellent substrate for the enzyme 5-lipoxygenase. The major advantages of EPA- and docosahexaenoic acid-derived metabolites can be summarized as follows: (1) EPA-derived thromboxane A_3 is less active in platelet aggregation than thromboxane A_2; (2) leukotriene (LT) B_4 enhances chemotaxis, while other LT, e.g. LTC_4, LTD_4, and LTE_4, augment vascular permeability

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and contractility. EPA is converted to LTB_5, which has only a small proportion of the activity of LTB_4 and platelet-activating factors, resulting in decreased chemotactic migration and endothelial cell adherence. This activity would mean that *n*-3 fatty acids exert major effects on the synthesis of LT by promoting an anti-inflammatory action; (3) feeding with fish oils is associated with profound changes in immunoregulatory processes, including the production and release of various cytokines, interleukines and interferons. It is currently assumed that, partly as a result of these changes, the natural history and progression of diseases with an inflammatory or immunological component may be altered; (4) consumption of EPA and docosahexaenoic acid reduces serum cholesterol, LDL and triacyglycerol concentrations (Fürst & Kuhn, 2000).

Indeed, inflammatory symptoms of rheumatoid arthritis, psoriasis, Crohn's disease and ulcerative colitis are all ameliorated by fish-oil preparations, whether or not directly related to cytokine production. Consumption of EPA reduces the production of pro-inflammatory IL-1-α and -β and IL-6, as well as tumour necrosis factor-α and -β in response to an inflammatory stimulus (Endres *et al*. 1989, 1991; Caughey *et al*. 1996). The anti-inflammatory effects of fish oil may also include decreased production of inflammatory substances like LTB_4 and platelet-activating factors released by the action of cytokines, as well as a large reduction in cytokine-induced synthesis of prostaglandin E_2 and thromboxane B_2 in the colonic mucosa (Pomposelli *et al*. 1988; Endres *et al*. 1989; Fritsche & Cassity, 1992; Engstrom *et al*. 1996). These findings are in line with a decrease in arachidonic acid : EPA in blood mononuclear cell membranes as well as a decrease in neutrophil chemotaxis to LTB_4 (Lowry & Thompson, 1994). The combined observations may be partly explained by the finding that LTB_4 enhances blood monocyte IL-1 production after lipopolysaccharide exposure (Rola-Pleszczynski & Lemaire, 1985).

Fish oil supplementation suppresses autoimmune diseases and T-cell lymphocyte production of IL-2, and subsequent proliferation (Endres *et al*. 1993; Yaqoob & Calder, 1995). This mechanism involves down regulation of co-stimulatory molecules like leucocyte function-associated antigen, intracellular adhesion molecule-1 and CD_2 in T lymphocytes as well as co-stimulatory receptors and secretion of effector molecules from accessory cells (Calder, 1995; Harbige, 1998). It should be remembered that in critically ill patients administration of *n*-3 PUFA is associated with a reduction in the 2-series of prostaglandins, thereby boosting the cellular defence function due to the ineffectiveness of feedback inhibition induced by prostaglandin E_2 (Lee *et al*. 1984; Ninnemann & Stockland, 1984; Terano *et al*. 1984; Lokesh & Kinsella, 1987). This hypothesis is supported by experimental data showing that administration of *n*-3 PUFA during hypermetabolism is associated with increased cytokine production (Watanabe *et al*. 1991; Ertel *et al*. 1993), improved antigen presentation (Ertel *et al*. 1993), enhanced splenocyte proliferation (Ertel *et al*. 1993), improved opsonization indices (Alexander *et al*. 1986) and reduced mortality (Barton *et al*. 1991).

There are numerous studies showing suppression of T-cell-mediated immune function. This effect might be undesirable, especially in immune-suppressed individuals (Wu & Meydani, 1998). It could be demonstrated that the immunosuppression might be in part attributable to increased lipid peroxidation and decreased antioxidant (especially vitamin E) levels (Wu & Meydani, 1998). Recent studies have shown that the suppressive effect of *n*-3 fatty acid administration on T-cell function can be prevented by vitamin E supplementation (Meydani *et al*. 1991; Wu *et al*. 1996).

In conclusion, the potential clinical benefits of supplemental fish oil might be summarized as follows: reduced inflammatory response; anti-thrombotic effects; decreased reactivity to various stimuli (e.g. ventricular arrhythmias); maturation of the fetal central nervous system and retina DNA; maintenance of tissue microperfusion; increased tolerance to organ transplantation and improved function of the graft, as well as prevention of impaired cellular immunity when caused by increased prostaglandin E_2 production (Fürst & Kuhn, 2000). The latter concept, particularly, could gain considerable significance in hypermetabolism.

Clinical implication of immunonutrition: which patients benefit from immunonutrition?

Numerous clinical applications of immunonutrition have been reported (Moore, 1994; Bower *et al*. 1995; Senkal *et al*. 1995; Kudsk *et al*. 1996; Atkinson *et al*. 1998; Braga *et al*. 1998). At present, various enteral formulas are available containing substrates assumed to be beneficial, e.g. glutamine, arginine, nucleotides and *n*-3 fatty acids, as well as Se, vitamins E, C and A and β-carotene, at various concentrations (see Table 1). The clinical benefit of these immune-modulating diets on individual measures of cellular defence has been shown in post-operative or post-traumatic patients (Cerra, 1991; Daly *et al*. 1992; Moore, 1994; Kemen *et al*. 1995), yet the implication of expensive enteral preparations should be further justified. Indeed, it would be essential to demonstrate a reinforcement of cellular defence functions in association with an improvement in clinical outcome and morbidity. At present, there are numerous prospective randomized clinical studies with immune-modulating diets showing clear evidence for a reduced incidence of infectious complications, a reduced duration of ventilation, a shortened stay in the intensive care unit as well as in hospital, and reduced hospitalization costs (for references, see Zaloga, 1998). In an evaluation of a meta-analysis, two research groups, Beale *et al*. (1999) and Heys *et al*. (1999) demonstrated significant improvements in nearly all the outcome variables mentioned earlier.

Indeed, adequate measures for subjective clinical evaluation are a prerequisite for the classification of the manifold immunological settings. There are patients who are characterized as immune-suppressed, infection-threatened but not yet suffering from fulminant systemic infection. These patients include, post-operative tumour patients after chemotherapy and radiotherapy, surgical patients with wound infections or massive transfusion, as well as those with multiple trauma or burns; these patients include perfect candidates for immunonutrition. Patients already suffering from a severe form of SIRS or sepsis may

Table 1. Composition (g/l) of available immune-modulating diets

Diet	Reconvan*	Impact†	Immun-Aid‡	Modular Tube feeds§	Experimental Diet Abbotll
Glutamine	10	3·1	9	–	19·1
Nucleotides	–	1·3	–	–	–
Arginine	6·7	12·8	14	6·1	6·6
n-3 Fatty acids	3	3·3	1·1	5	15
Se	50	50	100	150	+
Vitamin A (mg/l)	0·7	1	0·8	47	+
β-Carotene (mg/l)	1	–	–	–	–
Vitamin E (mg/l)	10	30	336	215	+
Vitamin C (mg/l)	60	67	60	120	+

+, Levels not stated.
* Fresenius, Bad Homburg, Germany.
† Novartis, Minneapolis, MN, USA.
‡ McGaw, Irvine, CA, USA.
§Shriner's Burn Institute, Cincinatti, OH, USA.
ll Ross Laboratories, Columbus, OH, USA.

require attention when selecting suitable immunonutrients. Substrates possessing anti-inflammatory properties might be of particular value, whereas the use of pro-inflammatory substrates, such as arginine, must be avoided.

Shortcomings and pitfalls with immunonutrition

Several of the recent clinical studies have been poorly designed. Much of the criticism relates to the characterization of patients. Frequently the control diets were not isonitrogenous and/or isoenergetic. Many studies failed to establish the critical lower limit of tolerance relating to the volume of enteral nutritional preparations. This factor is of utmost importance, since immunomodulation is only beneficial in patients receiving the critical minimum amount of the enteral preparation. Furthermore, the study groups were often not stratified with regard to the severity of illness or to the expected outcome, resulting in difficulties in analysing subgroups. Similarly, different studies used different preparations with an inconsistent proportion of the individual immune-modulating substrates. The major question might be whether benefits of immunonutrition in patients suffering from shock, sepsis, and organ failure are equal to those in moderately-traumatized surgical patients. In this context it should be noted that existing meta-analyses did not show any improvements in the former group of patients, but rather demonstrated a tendency towards poorer outcome (Beale *et al*. 1999; Heys *et al*. 1999). In particular, the multi-centre study by Bower *et al*. (1995) showed an alarming tendency towards increased mortality in the most-severely-ill patients with immunonutrition. In addition, higher mortality, longer hospitalization, longer ventilation periods and increased treatment costs were seen in a subgroup of burn patients receiving the immune-enhancing formula (Saffle *et al*. 1997). Similarly, increased intensive care unit and hospital stay, increased ventilator time and increased incidence of pulmonary organ failure were reported with immune-enhancing diets (Mendez *et al*. 1996, 1997). These results might be a serious warning to the unrestricted use of immune-enhancing formulas in the most-seriously-ill patients. It should be emphasized that substrates intended to stimulate the cellular defence function would not simultaneously induce enhancement of systemic inflammation if used in patients with severe indication of critical illness.

Concluding remarks

Currently-available enteral nutrition preparations with an immune-modulating effect are first-generation products, their design being based on a 'multi-pragmatic' approach. The modulating effect of selected substrates on immune response is considered as pharmacological nutrition and is experimentally and clinically established. Pharmacological nutrition is a novel concept which has introduced a new dimension into the fascinating field of modern clinical nutrition (Fürst, 1998). It is proposed that as a result of this new approach it will be possible to improve immune function (Bower, 1990; Chandra, 1991), reduce the frequency of inflammation (Burton, 1994; Fürst, 1994*b*), improve gut barrier function (Souba *et al*. 1990; Burton, 1994; Fürst, 1994*b*) and regulate cellular hydration state (Häussinger *et al*. 1993). The rapid increase in new information relating to this exciting approach is certainly only a prelude to its use in routine clinical settings.

Special caution should be exercised when dealing with patients with most severe appearances of SIRS, sepsis and organ failure. We put forward the hypothesis that certain immune-enhancing substrates like arginine and *n*-3 fatty acids may be responsible for an undesirable outcome, as they may aggravate ongoing systemic inflammation (Gonce *et al*. 1990; Heyland *et al*. 1994). In contrast, in experimental and clinical settings no adverse effects have been reported for glutamine and nucleotides. Thus, immune-modulating interventions which include arginine and *n*-3 PUFA should be undertaken with care if administered in complex immune-pathological situations such as severe SIRS or organ failure. Moreover, the absence of clinically-available immunological monitoring, the lack of profound patho-physiological understanding, as well as the lack of objectives for influencing immune responses in patients with fulminant systemic inflammation should prompt further efforts in basic and applied clinical research.

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