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SYMPOSIUM ON 'THE INTERACTION BETWEEN NUTRITION AND INFLAMMATION'

Nutrition, immune function, and inflammation: an overview

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The account of acute inflammation is one of the classical clinical descriptions in medicine. However, those early physicians describing *Rubor et tumor cum calor et dolore* (Celsus in 50 BC) could hardly have imagined the complexity of the events that they were observing.

In a healthy individual, the response to tissue injury and infection is rapid and efficient, with resolution occurring before involvement of the specific immune system. However, when the inflammatory stimulus is overwhelming, or if the patient is already malnourished or chronically ill, a series of inter-related events are set in motion. Among these are the interaction of immune cells, phagocytes and release of powerful cytokines that affect the utilization of nutritional substrates in the body. Moreover, antigen processed by macrophages is presented to lymphocytes, thus eliciting a specific immune response.

The present paper summarizes our present understanding of the pathophysiology of inflammation and explores the objectives for nutritional support in the presence of immunodysfunction. The rationale for adjunctive nutritional support is explained, and modified regimens, tailored both to provide metabolic substrates and to enhance immunity to disease, are given.

Pathophysiology of the inflammatory response

The immune system is phylogenetically separated into innate or non-specific immunity and the adaptive or specific immune system. While understanding that both processes are often working together, events in each system will be considered individually at a cellular and molecular level.

Non-specific immunity. Tissue injury leads to release of Hageman factor (XIIa-XII) and to the degranulation of tissue mast cells. Factor XII initiates the process, causing complement activation and bradykinin production locally. Micro-organisms and endotoxin may also trigger this pathway, directly activate complement, or cause mast cell degranulation (Fig. 1) (Schlesinger, 1975). This mechanism ensures the presence of inflammatory mediators such as the complement factors C3a and C5a, bradykinin, and histamine. Damaged cell membranes release products of arachidonic acid (AA) metabolism, such as prostaglandins (PG) and leukotrienes (LT) (Goetzl, 1981), both of which are eicosanoids having important mediating functions in acute inflammation. The net effect is to increase vascular permeability, which causes the accumulation of acute-phase

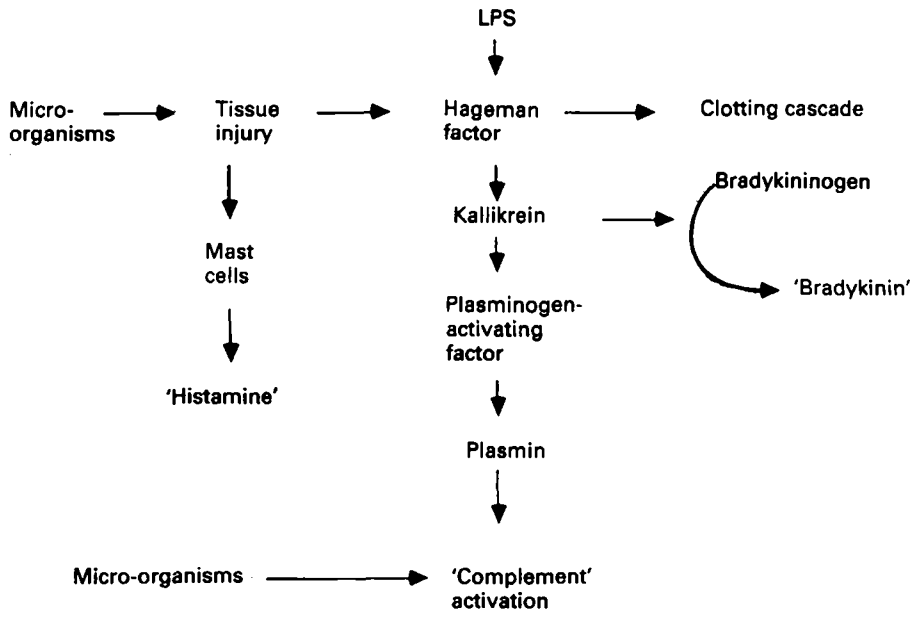

Fig. 1. Production of inflammatory mediators. Tissue injury leads to the release of Hageman factor and the degranulation of tissue mast cells. Micro-organisms and endotoxin lipopolysaccharides (LPS) may also trigger this process.

proteins and immune-complexes, and to promote the cellular phase of the acute inflammatory response through margination, diapedesis and chemotaxis of leukocytes.

The cellular phase is initially dominated by the neutrophil, whose suicide function is to phagocytose and to kill pathogenic micro-organisms. This phagocytic function begins via cell binding to the target cell through a non-specific mechanism. The cell–bacteria linkage is improved if the bacteria are coated with opsonins, such as complement or immunoglobulin (Ingham *et al.* 1981). Neutrophils and macrophages carry receptors for the C3 subunit of complement and the Fc portion of immunoglobulin (Schlesinger, 1975). Perturbation of the cell membrane through binding to the Fc or C3 receptor causes the neutrophil to go into a higher state of activation, with a metabolic oxidative burst that results in the production of hydrogen peroxide and toxic oxygen free-radicals (Lehrer, 1988). The products perform the intracellular killing of bacteria but also lead to the death of neutrophils and the release of toxic waste products.

Changes in the cell membrane associated with Fc and C3 receptors in both macrophages and neutrophils cause the release of PG and LT (Borgeat & Samuelsson, 1979*a*; Goetzl, 1981). These further serve to enhance and to prolong the inflammatory phase. Clearly, however, these mechanisms, if allowed to continue, lead to increased tissue damage and are ultimately detrimental to the host.

Antigen presentation: the specific immune response. In addition to mediating the acute-phase response, the macrophage is responsible for presenting and processing antigen, thus triggering the specific immune response. The macrophage is involved in antigen presentation and carries major histocompatibility complex class II (MHC) surface antigens. The presence of foreign antigen (Ag) in association with monocytes or macrophages leads to the production of cytokines such as interleukin-1 (IL-1) and

cachectin or tumour necrosis factor (TNF) (Gery & Wahsman, 1972). These two powerful cytokines are responsible for the metabolic effects of the acute-phase response, and IL-1 is also essential in the activation signal to T-cells from the antigen-presenting cell. The key interaction is the MHC-restricted stimulation of T-helper cells, which carry Ag receptors (T_3+) and MHC class II Ag receptors (T_4+) (Weiss *et al.* 1986).

The processing of Ag by antigen-presenting cells causes cell membrane activation of AA metabolism, leading to the release of LT and PG with consequent increase in intracellular calcium ions and the production of IL-1 (Kennedy *et al.* 1980). IL-1 produced from macrophages stimulates protein kinase C (*EC* 2.7.1.37; PKC) in T-cells, thereby enhancing the production of lymphokines such as interleukin-2 (IL-2), γ-interferon (γ-INF), macrophage-activating factor, chemotactic factor and migration-inhibiting factor (Weiss *et al.* 1986). LTB_4 produced by this mechanism raises intracellular Ca^{++} and further promotes IL-1 activity. However, excess PGE_2 production can inhibit the transcription and the translation of IL-1 by raising intracellular cAMP (Knudson *et al.* 1986).

Membrane activation of T-cells leads to activation of phosphodiesterase (*EC* 3.4.1.10), which promotes phosphoinositide hydrolysis with the formation of inositol phosphate, inositol triphosphate and diacylglycerol. Such cell membrane activation coincidentally elevates cellular Ca^{++} (Nishizuka, 1984; Weiss *et al.* 1986). This rise in cytosolic Ca^{++} in turn activates PKC and triggers protein synthesis of IL-2 and other lymphokines (Weiss *et al.* 1986). The production of IL-2 and the increased expression of IL-2 receptors by T-lymphocytes are the essential step that drives T- and B-cells into clonal proliferation (Fig. 2). With clonal proliferation and maturation, the development of a predominantly cell-mediated immune response often occurs with the activation of cytotoxic cells, both specific (cytotoxic T-cells and K-cells) and non-specific (highly activated macrophages and natural killer cells).

Interleukin-1 and tumour necrosis factor: the acute-phase response

The acute-phase response initiated by IL-1 or TNF, or both, includes fever, redistribution of trace elements, skeletal muscle catabolism, increased hepatic acute-phase protein synthesis, endocrine alteration, and neutrophilia (Dinarello, 1984*a*,*b*, 1988) (Fig. 3). These events redistribute the body's endogenous resources and create an environment in which systemic sepsis or illness has the best chance of being defeated by a boosted immune system. The price is metabolic catabolism; the pay-off is enhanced immunity.

Evidence indicates that IL-1 is involved in triggering the transcription and synthesis of acute-phase proteins by the liver (Ramadori *et al.* 1985). The acute-phase proteins support the infected organism by metabolic as well as functional activities. The protease inhibitors, such as α_2-antitrypsin and α_2-macroglobulin, can accumulate at the site of injury to prevent additional damage caused by the release of proteolytic enzymes from already damaged tissues and from the phagocytic cells (Wannemacher *et al.* 1975; Dinarello, 1984*b*).

The C-reactive proteins and albumin may provide essential nutrients to facilitate wound healing, or may aid in transporting trace metal co-factors (Pepys & Baltz, 1983). Fibrinogen increases the tensile strength of the wound and stimulates fibroblast proliferation and growth (Dinarello, 1984*a*,*b*). Other important roles for these acute-phase proteins are modulation of the rate of structural protein synthesis, hormonal transport, local modulation of humoral effects, neutralization of the potentially toxic products of the inflammatory response, inhibition of microbial invasion, and localization of bacteria.

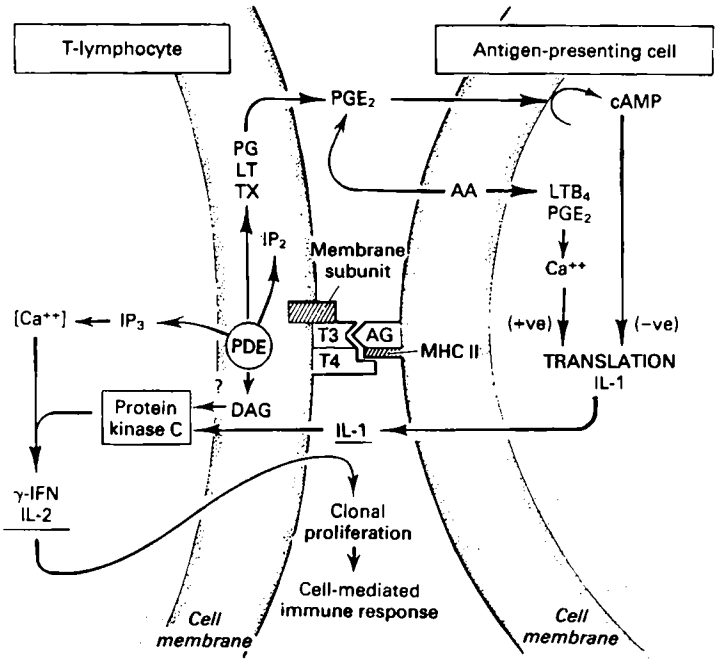

Fig. 2. Antigen presentation. Schematic representation of antigen presentation to T-lymphocytes. The processing of antigen (Ag) by antigen-presenting cells causes cell membrane activation of arachidonic acid (AA) metabolism and release of prostaglandins (PG), leukotrienes (LT) and thromboxanes (TX) with consequent increase in the intracellular calcium (Ca^{++}) and the production of interleukin-1 (IL-1). IL-1 integrates the entire immune network by activating T-lymphocytes to synthesize lymphokines, interleukin-2 (IL-2), and γ-interferon (γ-INF). Membrane activation of T-lymphocytes leads to activation of phosphodiesterase (*EC* 3.4.1.10; PDE), which promotes phosphoinositide hydrolysis with the formation of inositol phosphate (IP_2), inositol triphosphate (IP_3), diacylglycerol (DAG), and raises cellular Ca^{++}. These events also activate protein kinase C (*EC* 2.7.1.37) leading to the synthesis of lymphokines. The production of lymphokines drives activated T- and B-cells into clonal proliferation and maturation. Excessive production of prostaglandin E_2 (PGE_2) can negatively inhibit the formation of IL-1 via cAMP, thus inhibiting the lymphocyte response. MHC, major histocompatibility complex; T4 and T3, T-helper cells; +ve, stimulation; −ve, inhibition.

The significance of fever induced by IL-1 or TNF, or both, during the inflammatory process and sepsis is not clear. There is good evidence that IL-1 is more active at elevated temperatures than at normal temperature (Hoffman-Goetz & Kluger, 1979*a*,*b*; Hoffman-Goetz *et al.* 1981; Duff & Durum, 1982). In vitro studies performed by Baracos *et al.* (1983) demonstrated that the muscle proteolysis stimulated by IL-1 was enhanced at an elevated temperature. Furthermore, immunological reactions, including antibody synthesis (Brucher *et al.* 1973; Jampel *et al.* 1983) and lymphocyte response to antigens and mitogens (Ashman & Nahmias, 1977) have been reported to be strongly temperature-sensitive.

Redistribution of zinc by IL-1 or TNF, or both (Dinarello, 1984*a*, 1988) is believed to be correlated with the increased production of acute-phase proteins (Sugarman, 1983). Zn metalloenzymes are known to participate in many metabolic processes, including carbohydrate metabolism and the synthesis and degradation of lipid, protein, and nucleic acid (Li & Vallee, 1980). Reduction of plasma iron in response to IL-1 may maintain the bacteriocidal activity of neutrophils and aid the development of cellular immune

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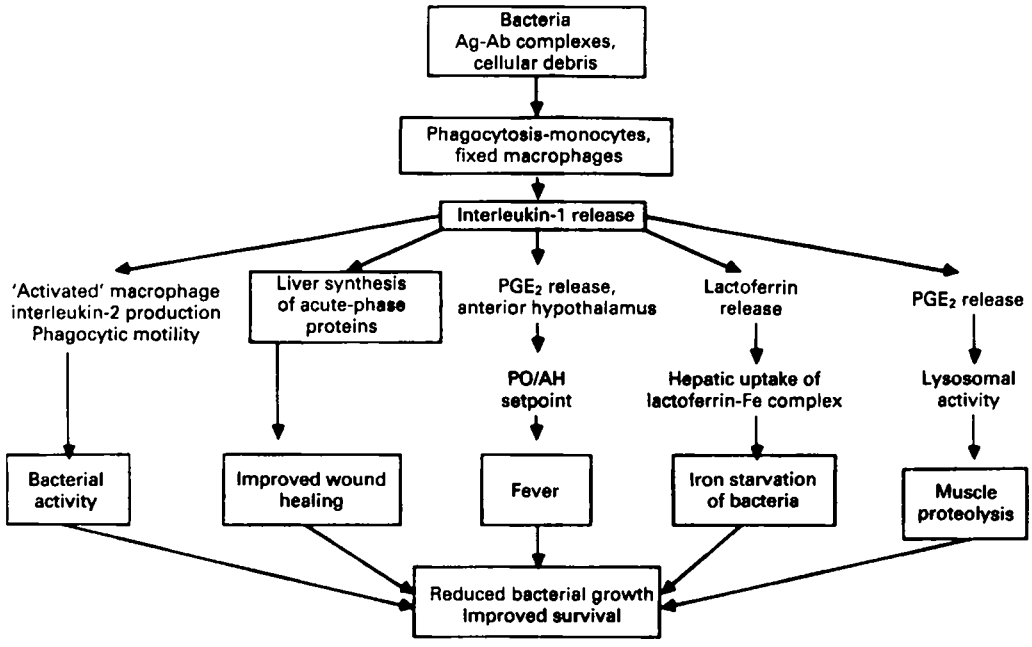

Fig. 3. Diagrammatic representation of the acute-phase responses to tissue injury, bacteria, antigen-antibody (Ag-Ab) complex, and monocyte or macrophage activation.

competence (Bullen, 1981). Plasma levels of copper, in the form of caeruloplasmin, are elevated by IL-1 (Dinarello, 1984*a*,*b*, 1988). Caeruloplasmin is an allosteric enzyme capable of oxidizing catecholamine and serotonin (Kampschmidt, 1981). Furthermore, it acts as a scavenger of the superoxide ions (Jacka *et al.* 1983) generated by stimulated phagocytosis, hence limiting tissue damage.

Many of the hormonal changes induced by IL-1 or TNF during the inflammatory process have potential roles in promoting substrate mobilization, namely by glycogenolysis and lipolysis (Dinarello, 1984*a*,*b*, 1988). Such a hormonal milieu is predominantly characterized by the elevation of the counter-regulatory hormones insulin, glucagon, corticosterone and catecholamines (Dinarello, 1984*a*, 1988). Amino acids released from peripheral tissues in response to the catabolic hormones provide the major energy substrate utilized by the liver for gluconeogenesis, oxidation, and synthesis of acute-phase proteins (Wannemacher *et al.* 1975; Wannemacher, 1977).

Inflammation is generally beneficial to the host until its clinical manifestations cause vascular endothelial damage, haemorrhage and thrombosis. In certain chronic inflammatory diseases such as arthritis, cancer, and inflammatory bowel diseases, prolonged or recurrent neutrophil, platelet, and complement activation in the presence of endotoxaemia and tissue injury may further exaggerate macrophage response to antigen and trigger the release of cytokines. The latter in turn activate polymorphonuclear leukocytes to adhere to endothelial cell surfaces (Pohlman *et al.* 1986; Cybulsky *et al.* 1988) (Fig. 4). Phagocytic stimulation of the neutrophils subsequently induces a local hyper-acute release of potent inflammatory mediators such as eicosanoids, toxic oxygen metabolites, histamine, bradykinin and proteolytic enzymes, causing cell wall oedema, disruption, tissue necrosis and organ failure (Fantone & Ward, 1982; Janoff & Carp, 1982; Movat & Wasi, 1985).

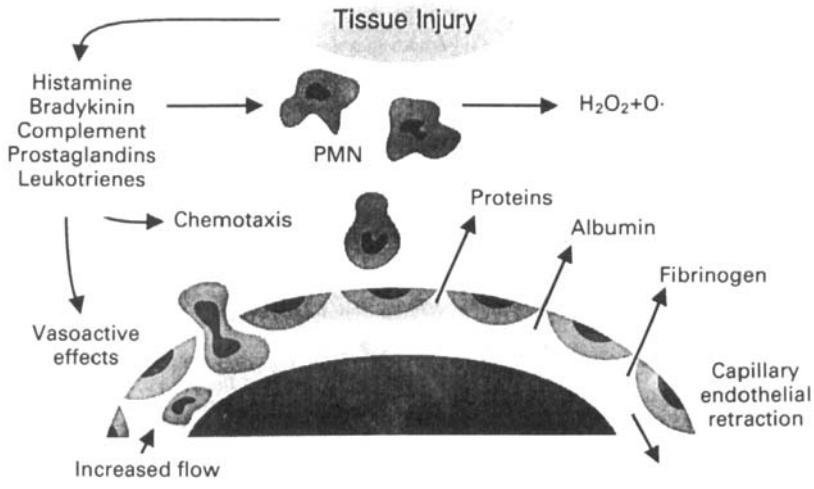

Fig. 4. Initiation of inflammation. Tissue injury causes the release of inflammatory mediators such as histamine, bradykinin, complement, prostaglandins, and leukotrienes. These mediators increase vascular permeability allowing the exit of proteins, albumin, and fibrinogen from the circulation. Polymorphonuclear neutrophils (PMN) marginate, diapedese, and migrate along the chemotaxic gradients to the damaged area to kill pathogenic micro-organisms by releasing lysosomal enzymes and hydrogen peroxide (H_2O_2) and oxygen free-radicals (O·).

Inflammatory mediators

E. coli and endotoxin are the most frequent stimuli of inflammation associated with microhaemorrhage, microthrombosis, and vascular injury (Cybulsky & Movat, 1982; Cybulsky *et al.* 1988). They can be translocated and disseminated into the circulation via the gastrointestinal tract, resulting in profound systematic effects via generation of cytokines. Evidence indicates that neutrophil-mediated vessel injury initiated by IL-1 and TNF is associated with increased vascular damage and haemorrhage (Pohlman *et al.* 1986; Cybulsky *et al.* 1988). Both IL-1 and TNF can promote the adhesion of leukocytes to endothelial cells and trigger inflammatory events. The high level of IL-1 detected in the joint effusions of patients with rheumatoid arthritis and other inflammatory diseases (Bodel & Hollingsworth, 1969; Fontana *et al.* 1982) has been associated with cartilage breakdown by the stimulation of collagenase released from the synovial cells and chondrocytes (Pettipher *et al.* 1986). IL-1 has been recognized as an activator of T-lymphocytes and a stimulant of various non-lymphoid cells, including the endothelial cell, where procoagulant activity is induced.

TNF increases neutrophil adherence to endothelial surfaces by increasing the number of ligand binding sites on neutrophils and on endothelial cells (Pohlman *et al.* 1986; Cybulsky *et al.* 1988). Accelerated production of grafted lymphocytes in graft *v.* host disease leads to a local release of TNF in the cutaneous and intestinal mucosa which, in turn, induces alterations in the epithelial cells and enhances the inflammatory reaction (Piquet *et al.* 1987).

The most potent inflammatory mediators are the eicosanoids produced from AA. Their central role in inflammation has been recognized since Vane (1971) first associated the mechanism of action of non-steroid anti-inflammatory drugs with the modulation of PG biosynthesis via the inhibition of the cyclo-oxygenase enzyme prostaglandin

synthetase (Needleman, 1978). Both thromboxane A_2 (TXA_2) and PGF_2-α cause vasoconstriction by promoting the aggregation of platelets and the release of platelet mediators (Moncada & Vane, 1979). PG, thromboxanes (TX) and LT are present in the synovial fluid of patients with rheumatoid arthritis (Klickstein *et al.* 1980), acute respiratory distress syndrome (Matthay *et al.* 1984), allergic rhinitis (Creticos *et al.* 1984), and solid tumours (Jaffe, 1974). PGE_2 stimulates osteoclastic bone resorption (Klickstein *et al.* 1980) and also acts synergistically with other mediators, including serotonin and bradykinin, to increase vascular permeability (Wedmore & Williams, 1981).

LTC_4, LTD_4, and LTE_4 are classified as 'slow reacting substances of anaphylaxis' (Borgeat & Samuelsson, 1979*b*). They are potent bronchoconstrictors that stimulate tracheal mucus secretion (Samuelsson, 1983; Lewis & Austen, 1984), and are detected in effusions from patients with allergy-associated conditions such as bronchial asthma (O'Driscoll *et al.* 1984), acute respiratory distress syndrome (Matthay *et al.* 1984) and allergic rhinitis (Creticos *et al.* 1984), as well as in those with chronic diseases such as cystic fibrosis (Crowell *et al.* 1981). LTB_4 is a potent chemotactic agent and may modulate the inflammatory response through induction of leukocyte accumulation, mediation of vascular permeability, and blood flow changes (Palmer *et al.* 1980; Smith *et al.* 1980).

Other inflammatory mediators, including histamine, bradykinin, and platelet-activating factor (PAF), are also produced in response to antigen–antibody reactions. Histamine released from mast cells is often responsible for hyperactivity of the airway (Rafferty & Holgate, 1987). The release of PAF can give rise to bronchial constriction, systemic hypotension, neutropenia and thrombocytopenia (Hanahan, 1986; Morley, 1986). PAF stimulates platelets to release amines and to activate the complement C5 component, which stimulates granulocyte adhesion and oxygen free-radical production, thus causing tissue damage.

It is clear from this synopsis that ample opportunities exist for the interaction of inflammation with metabolism and nutrition in which dietary manipulations may alter immune mechanisms. These events will now be reviewed.

Interrelationships of nutrition, immune function and inflammation

Protein–energy deprivation and immunodysfunction. In the presence of infectious disease and inadequate nutrition, a vicious cycle of malnutrition through alteration in metabolism will worsen immune function. A wealth of evidence indicates that malnutrition impairs immune competence (Chandra, 1979, 1983). The effect of malnutrition is not simply to reduce the availability of endogenous stores of nutrients utilized by the host in defence processes; it may also lead to increased susceptibility to secondary infection through reduced synthesis of IL-1 and impaired cell-mediated immunity.

Early work by Hoffman-Goetz & Kluger (1979*a*,*b*) and Hoffman-Goetz *et al.* (1981) showed that rabbits fed on a protein-depleted diet developed attenuated fever following a Gram-negative infection. The authors suggested that such attenuation was attributed to a reduction in the amount of IL-1 being synthesized and released from phagocytic cells in response to the pathogen. Many of the metabolic responses to IL-1 and endotoxin, including granulocytosis, fever, and acute-phase protein response, were also shown to be attenuated in the protein-depleted guinea-pig model (Drabik *et al.* 1987). A reduction in synthesis or release of IL-1 from leukocytes has been observed in non-stressed, protein-malnourished patients (Keenan *et al.* 1982). The production of IL-1 returned to normal in protein-deprived patients after adequate nutrition was re-instituted (Hoffman-Goetz & Kluger, 1979*a*,*b*).

Both experimental and clinical protein deficiency has been characterized by immune dysfunction, including reduced B-cell antibody formation (Carlomagno *et al.* 1980), T-cell responsiveness to polyclonal mitogens and specific antigens (Chandra, 1979; Gross & Newberne, 1980), and delayed-type hypersensitivity skin-test response to recall antigens (Nohr *et al.* 1986). Inhibition of macrophage function (Rose *et al.* 1982) may affect overall immunity, since macrophages play a crucial role in cellular immune responses involved in the presentation of antigen to specific T-lymphocytes and in the production of IL-1. In vivo, it has been shown that graft *v.* host responses induced by T-cells from chronically protein-deprived mice are more vigorous than those in control mice (Bell & Hazell, 1975; Malave *et al.* 1978; Rose *et al.* 1982). The secretory IgA–antibody response, such as that directed against respiratory and enteric pathogens, is also affected in protein–energy malnutrition (Koster & Pierce, 1985).

Dietary ω-6 polyunsaturated fatty acids (PUFA) in inflammation. The mechanism of action of ω-6 PUFA in many inflammatory diseases is still unclear. Alteration of membrane structure and function by dietary ω-6 PUFA manipulation may change membrane fluidity, receptor binding sites, hormonal signal transduction, and immune responsiveness to surface antigen presentation (Burns *et al.* 1979; Shinitzky & Sourojon, 1979; Traill & Wick, 1984). Alteration in membrane structure and phospholipid turnover has been associated with the activation of PKC, a phospholipid-dependent enzyme, in a process that has led to tumour initiation and production through the association of PKC with many oncogenes (Castanga *et al.* 1982; Kaibuci *et al.* 1986).

Furthermore ω-6 PUFA are believed to exert their effects indirectly by acting as precursors for the synthesis of eicosanoids of the 2- and 4-series. These eicosanoids are potent modifiers of immune function as well as being mediators of inflammation (Mertin & Hunt, 1976; Goodwin & Webb, 1980; Samuelsson, 1983; O'Driscoll *et al.* 1984). Thus, excess consumption of dietary fats rich in the ω-6 PUFA may influence immune function in disease. It has been demonstrated that increased tumorigenesis is associated with high levels of linoleate (ω-6 PUFA) in the diet (Chan *et al.* 1983; Cohen *et al.* 1984). Furthermore, diets rich in PUFA are known to depress the response of spleen cells to mitogen (Mertin & Hunt, 1976), to prolong allograft rejection, and to induce auto-antibody development (Ring *et al.* 1974).

Although the overall picture of immunodysfunction caused by lipid modification is complex, it is now clear that certain PG (in particular PGE_2) produced from ω-6 PUFA mediate a negative feedback mechanism, controlling the extent and duration of the cell-mediated immune response (Goodwin & Webb, 1980; Goodwin & Ceuppens, 1983; Herman & Robinson, 1984; Jordan *et al.* 1987). PGE_2 has been shown to inhibit various aspects of the immune response, including lymphocyte proliferation, lymphokine secretion, macrophage collagenase (*EC* 3.4.24.3) synthesis, natural killer-cell activity, and the tumoricidal activity of activated macrophages (Ring *et al.* 1974; Bankhurst, 1982; Marshall & Johnston, 1985). Deficiencies in the production of interferon and other lymphokines by rheumatoid T-cells has been attributed to an enhanced sensitivity of these cells to the suppression of lymphokine secretion by PGE_2 (Hasler *et al.* 1983).

In the presence of antigens, such as bacterial lipopolysaccharides, and an activated complement system, PGE_2 derived from macrophages thus induces immunological suppression either by inducing a subset of T-suppressor lymphocytes or by directly inhibiting T-cell function (Goodwin & Webb, 1980; Goodwin & Ceuppens, 1983; Jordan *et al.* 1987) via IL-1 suppression (Herman & Robinson, 1984; Kunkel *et al.* 1986). IL-1 is a vital immunomodulator in the integration of host immunity because it produces the necessary signal for B-cell, T-cell and non-specific immune activation; its depression will result in reduced IL-2 and in the suppression of other lymphokines (Goodwin & Webb,

1980; Weiss *et al.* 1986) (Fig. 2). Reduced IL-2 activity will in turn depress the helper: suppressor lymphocyte ratio and prolong the impairment of natural killer-cell cytotoxicity, thus causing damage to vital endothelial cells, including hepatocytes, alveolarcytes, and medullary tubules, and may eventually lead to organ failure.

Nuritional support in inflammation

Protein substrate and immune function. The overall response to inflammation can be viewed as a finely tuned integrated series of reactions that plunder fuel and amino acids from peripheral tissues in order to maintain visceral protein synthesis, wound healing, and host defences (Wannemacher *et al.* 1975; Wilmore, 1976; Blackburn *et al.* 1977; Wannemacher, 1977). Nutritional support during this acute phase should be directed towards supplementing the redistribution of amino acids.

A number of studies have demonstrated an increased demand for branched-chain amino acids (BCAA) as carbon sources for oxidative energy during stress. In addition, the liver has a reduced ability to clear aromatic amino acids while continually extracting BCAA for protein synthesis (Dinarello, 1984*a*,*b*, 1988). In vitro studies have shown that BCAA play a regulatory role both in skeletal muscle protein metabolism and in increasing protein synthesis (Fulks *et al.* 1975; Blackburn *et al.* 1979; Tischler *et al.* 1982). When given alone or combined with glucose to postoperative patients, BCAA have been shown to improve nitrogen balance and to increase whole-body protein synthesis and albumin renewal (Sukumar *et al.* 1985; Desai *et al.* 1987).

Regulation of protein synthesis is governed by variations in the amount of messenger RNA, the amount of amino acid in the cell, or the amount of energy (ATP) supply. Research in nutritional therapy should also focus on factors regulating cell protein synthesis, since many of the co-operative interactions and activities of the immune system, e.g. antibody synthesis, lymphocyte production, complement-mediated bacteriolysis, cell-mediated cytolysis, and cytokines production, are dependent on protein synthesis. The addition of certain dietary nucleotides such as RNA has been shown to improve cellular immunity and antibody production (Kulkarni *et al.* 1986), thus decreasing susceptibility to bacterial challenge.

Arginine supplementation in inflammatory disease may restore the low T_3 and growth hormone levels present in sepsis and lead to better cell preservation. Recent clinical evaluation suggests that arginine could serve as an immunomodulator because of its ability to enhance IL-2 production and lymphocyte proliferation (Reynolds *et al.* 1987). Subsequent studies have also shown that supplemental dietary arginine enhances blastogenesis to T-lymphocytes in response to mitogens (Barbul *et al.* 1981), reduces protein catabolism and enhances the immune response after burn and trauma (Sitren & Fisher, 1977; Barbul *et al.* 1984; Saito *et al.* 1987). The immunostimulatory role of arginine may be attributed to its multiple secretagogue activities in several endocrine glands, where it stimulates pituitary growth hormone and insulin (Sitren & Fisher, 1977; Barbul *et al.* 1984).

Enteral administration of a glutamine-supplemented diet can also improve and preserve mucosal cellularity, promote recovery, and contribute to improved N balance (Kapedia *et al.* 1985; Souba *et al.* 1985; Fox *et al.* 1987; Jacobs *et al.* 1987). Recent evidence has indicated that malnutrition in Crohn's disease can be attributed to the decreased intake of food due to anorexia, decreased intestinal absorption secondary to inflammation of the bowel wall, leakage of visceral protein through inflamed mucosa, and increased requirement of protein and energy as a result of the inflammatory state or its complications (Hwang *et al.* 1986). In these conditions, glutamine, a preferred small

Table 1. *Lipid classification*

Saturates		Monounsaturates	Polyunsaturates		
Medium-chain (C_6-C_{12})	Long-chain (C_{14}-C_{24})	Oleic (ω-9)	Linoleic (ω-6)	γ-Linolenic (ω-6)	Linolenic (ω-3)
Kernel oils	Cocoa butter	Olive	Maize	Blackcurrant	Linseed
Babassu	Dairy fats	Canola	Cotton	Borage	Fish oils
Coconut	Lard	Safflower (hybrid)	Soya	Primrose	Menhaden
Cohune	Tallow	Sunflower (hybrid)	Safflower (regular)		Salmon
Palm kernel	Palm		Sunflower (regular)		Mackerel
Tucum	Stearine				Tuna
MCT oils					Anchovy

Hydrogenation

Stearines	Shortenings	Margarine	Salad oils

Long-chain triglycerides

bowel oxidative fuel, may help to promote intestinal recovery and to lessen muscle wasting.

The return of cell-mediated immunity, macrophage function and perhaps IL-1 production may be brought about only by intensive nutritional supplements in the protein-malnourished patient suffering from cancer, AIDS, or inflammatory bowel diseases.

Manipulation of dietary fatty acid modulates inflammation. Today, nutritionists and physicians interested in seeking an alternative lipid substrate to modify the state of inflammation require a better understanding of the biological and physiological differences between lipids and oils. Differences in the fatty acid composition of fats and oils clearly separate them into saturates, monounsaturates, and polyunsaturates. They are further subdivided, based on their physiological and biological functions, into short-chain (C_2-C_4), medium-chain (C_6-C_{12}; MCT), and long-chain (C_{14}-C_{24}; LCT) triglycerides. The polyunsaturates may be further divided into ω-9, ω-6, ω-3 PUFA (Table 1).

In the absence of highly specific pharmacological enzyme inhibitors, the potency of these eicosanoids can be modified by supplementing diets with fats low in linoleate (ω-6 PUFA) content, such as coconut oil, or high in ω-3 PUFA, such as fish oil. Choosing lipids low in linoleic acid, such as MCT, can lower the AA content of membrane phospholipids. It has been shown that rats fed on a diet in which 40% of the energy was from coconut oil exhibited a lower urinary excretion of 6-keto-PGF_1-α and lower production of TXB_2 in the blood than rats receiving a diet containing the same proportion of safflower oil (high in ω-6 PUFA) (Croft *et al.* 1984). In an animal model, chronic feeding of coconut oil or MCT, or both, has also been shown to lower spleen phospholipid significantly and to decrease the metabolic response to *E. coli* endotoxin (Wan & Grimble, 1987). Coconut oil also lacks the ability to promote the development of mammary tumours (Chan *et al.* 1983; Cohen *et al.* 1984) in animal models when compared with safflower oil or soya-bean oil, both of which contain mainly ω-6 PUFA.

The reduction of phospholipid levels of AA (20:4ω6) can also be achieved by dietary enrichment with α-linolenic acid (18:3ω3), either from plants (such as linseed oil) or from marine fish oil, which contains high eicosapentaenoic acid (EPA) (20:5ω3). The ω-3 PUFA can deplete AA either by reversible or irreversible competitive inhibition with the

β-6-desaturase enzyme, thereby preventing the first step in the conversion of linoleic acid to AA (Magrum & Johnston, 1983, 1984).

Dietary experiments have shown that the addition of increasing amounts of ω-3 PUFA decreases PG and LT in serum tissue and macrophages (Magrum & Johnston, 1984; Lokesh *et al.* 1986). Decreased production of the 2-series PG derived from the macrophages of mice that have been fed on fish oil has been associated with a reduced susceptibility to type II collagen-induced rheumatoid arthritis (Prickett *et al.* 1984; Leslie *et al.* 1985) and has protected the WZB/NEW mouse from glomerular nephritis (Prickett *et al.* 1981).

Both 3-series PG and 5-series LT produced from ω-3 PUFA-enriched diets are believed to be less inflammatory than those of the 2- and 4-series (Goldman *et al.* 1983; Strasser *et al.* 1985). During in vitro studies of human neutrophils, EPA has been shown to inhibit LTB_4 elaboration and to yield a structurally analogous product, LTB_5, with markedly attenuated chemotactic and aggregating activities (Goldman *et al.* 1983; Lee *et al.* 1985).

Dietary fish oil, rich in ω-3 PUFA, is remarkably hypotriglyceridaemic and reduces levels of very low density lipoprotein (VLDL) (Harris *et al.* 1984). Supplementation with this lipid in a daily diet may reduce the severity of myocardial infarction (Culp *et al.* 1980) and other inflammatory diseases. Studies on the incidence of ischaemic heart disease (IHD) in fishing populations and Eskimos (Bang *et al.* 1971; Kromman & Green, 1980) found a negative correlation between dietary and tissue EPA levels and IHD. It was suggested that the low incidence of IHD was due to EPA altering the balance between TXA_2 and PGI_2 with a net anti-aggregatory effect (Hornstra *et al.* 1981).

The anti-inflammatory and reduced immunosuppressive properties of ω-3 PUFA have recently been considered in animal studies as an ideal lipid for both enteral and parenteral nutrition. When given enterally as a post-burn lipid source, fish oil significantly lowered metabolic expenditure and improved cell-mediated immune responses, including lower adrenal weight and lower serum C3 levels as compared with those found in burned guinea-pigs fed on safflower oil (Alexander *et al.* 1986; Trocki *et al.* 1987). In an animal model of endotoxic shock, long-term enteral feeding of fish oil also prevented the development of metabolic acidosis (Pomposelli *et al.* 1988), a finding that suggests the clinical relevance to patients at risk for developing septic shock.

Clinical problems with intra-lipid in total parenteral nutrition (TPN). The administration of nutritional support to the patient with sepsis is complicated by the presence of a hormone and monokine profile that is catabolic. When glucose is used as the energy source, cholestasis and hepatomegaly secondary to hepatic lipogenesis can occur (Hall *et al.* 1982) together with attenuated secretion of VLDL-triglycerides, probably in response to impaired lipoprotein synthesis and injured hepatocytes (Young *et al.* 1983).

Based on these observations, it has been advocated that some of the glucose energy should be replaced by fat. The mixed glucose–lipid system has been shown successfully to reduce the content of triglycerides in the liver and blood while providing adequate energy (Jeejeebhoy *et al.* 1976). Nevertheless, many complications have been observed in hospitalized patients with the use of exogenous vegetable fat emulsions in TPN, including diarrhoea, hepatomegaly, impaired clotting, reduced antibody formation, impaired phagocytosis, and depressed function of the reticulo-endothelial system (RES). These clinical problems may be related to the excessive production of PG and LT from long-chain triglycerides (LCT) containing a high level of ω-6 PUFA (54–70%) in the emulsion. Under these circumstances, eicosanoids can enhance vasoconstriction, platelet aggregation, neutrophil migration, immunosuppression, cytokine depression and free-radical formation, all of which leads to secondary inflammation and sepsis.

Other clinical problems related to the administration of LCT in TPN support are related to the slow clearance of LCT from the bloodstream. When infused intravenously, the lipid, in the form of oil microdroplets, is apparently taken up by phagocytic cells, which then become clogged and are no longer capable of phagocytosis (Saba & Luzio, 1969; Curnutte & Babior, 1974; Lauser & Saba, 1982). Evidence has shown that infusion of LCT at above 50% of non-protein energy causes gross hepatomegaly and splenomegaly due to impaired macrophage function (Hamawy *et al.* 1985; Sobrado *et al.* 1985). The trapped fat globules in alveolar capillaries, as well as in the alveolar cells, may cause biochemical changes in the lung that contribute to adult respiratory distress syndrome (Freedman *et al.* 1978). Furthermore, LCT have a detergent-like property and can disrupt Gram-positive bacteria that lack the protective lipopolysaccharide coating, thus encouraging conditions favourable to the overgrowth of Gram-negative bacteria (Kodicek & Worden, 1945; Sheu & Freese, 1973).

Collectively, these studies indicate that lipid emulsions high in long-chain ω-6 PUFA as used in current TPN support can potentially 'overwork' the immune system by causing bacterial sequestration to vital organs, such as the liver, spleen and lung. Their use may also lead to over-production of eicosanoids, proteolytic enzymes, and free-radicals, further impairing the immune system and prolonging inflammation.

Problems with the clinical use of LCT in lipid emulsion have been ameliorated by the use of MCT. Having C chain lengths of C_6 and C_{12}, these fatty acids can enter the portal vein directly and, independent of the carnitine pathway, enter into the mitochondria, quickly undergoing β-oxidation to ketone bodies, thus supplying a unique and readily available fuel (Guisard & Debry, 1972; Sailor & Muller, 1981). The rapid clearance of MCT from the blood due to their smaller molecular size and greater solubility (Bach & Babayan, 1982) favours normal hepatic and splenic RES function and results in less pulmonary sequestration of bacteria (Curnutte & Babior, 1974; Freedman *et al.* 1978; Lauser & Saba, 1982; Hamawy *et al.* 1985; Sobrado *et al.* 1985).

MCT have been used clinically for therapeutic applications in a variety of conditions, including pancreatic insufficiency, bile duct obstruction, and other liver diseases. Nevertheless, there are potentially serious adverse effects from parenteral MCT, such as essential fatty acid deficiency and ketosis in susceptible patients (Bach *et al.* 1974; Gordon *et al.* 1975). The efficacy of MCT in the presence of inflammatory disease awaits further investigation.

In developing a therapeutic option, Babayan (1987), in our laboratory, has recently introduced a new product termed 'structured lipid', which consists of both medium- and long-chain fatty acids (Babayan, 1987). When administered either parenterally or enterally to burned animal models, this rearranged triglyceride has demonstrated

Table 2. *Recommendations for vitamin and mineral intakes*

Water-soluble vitamins (mg/d)		Fat-soluble vitamins (mg/d)		Trace elements (mg/d)	
Folic acid	400	Vitamin A	1·0	Zinc	2·5–4·0
Thiamin	3·0	Vitamin D	0·005	Copper	0·5–1·5
Vitamin C	100–300	Vitamin E	10	Selenium	0·1
Biotin	60	Vitamin K	10	Chromium	0·01–0·015
Niacin	40	L-carnitine	500–2000	Manganese	0·15–0·8
Vitamin B_6	4·0				
Riboflavin	3·6				
Pantothenic acid	15				
Vitamin B_{12}	5·0				

protein-sparing qualities superior to those of conventional triglycerides, as reflected in better N balance and albumin synthesis (Mok *et al.* 1984; DeMichele *et al.* 1987; Mascioli *et al.* 1987).

The combined features of LCT in the form of ω-3 PUFA (fish oil), and MCT in the form of coconut oil in a 'structured lipid' may result in decreased infection and improved survival rates by producing fewer inflammatory eicosanoids, and, at the same time, serve as a more efficient fuel.

Micronutrients. Micronutrient deficiency is often associated with defects in a number of organ systems, including the gastrointestinal, cardiovascular, haematological, and musculoskeletal systems. Administration of micronutrients such as trace elements and vitamins should be included in the nutritive support of patients who are more susceptible to deficiency as a result of increased utilization, diarrhoea, bowel and biliary losses, and excess urinary losses. A trace element and multivitamin preparation is shown in Table 2 (Brewer *et al.* 1985).

The abnormalities associated with Zn deficiency include impaired antibody-mediated responses to both T-cell-dependent and T-cell-independent antigens, impaired natural killer-cell activity and platelet aggregation, defective chemotaxis of leukocytes, and delayed wound healing (Weston *et al.* 1977; Gordon & O'Dell, 1980; Fraker *et al.* 1986). Zn deficiency in relapsing Crohn's disease alters the distribution of surface markers of T-helper cells and induces the production of cytotoxic T-cells; its supplementation restores the number of T-helper lymphocytes (Couvreur *et al.* 1986).

Zn is the metal component or activator of many enzymes, such as carbonic anhydrase (*EC* 4.2.1.1) and alkaline phosphatase (*EC* 3.1.3.1). Its availability governs the tissue concentrations and activities of certain Zn metalloenzymes that are involved in the synthesis of acute-phase proteins (Valle, 1959; Pekarek *et al.* 1972; Sobocinski *et al.* 1979). Thus, supplementation with Zn of the diet of malnourished patients with inflammatory disease may be essential to improve the rate of synthesis of nucleic acids and protein, thereby accelerating tissue growth and the repair processes. Furthermore, the role of Zn in the stabilization of plasma membranes (Chvapil *et al.* 1972; Chvapil, 1986) suggests its potential use as a free-radical scavenger.

Fe is an element required for the production of haemoglobin, myoglobin, and certain essential enzymes; its deficiency leads to anaemia. However, care must be taken when administering Fe to the malnourished patient with inflammation who also has low levels of plasma protein, since increased levels of free Fe in conjunction with low concentrations of serum transferrin can increase susceptibility to bacterial pathogens (Bullen, 1981).

Microvascular endothelial cell injury caused by oxidants occurs after re-perfusion of ischaemic tissues and may be attenuated by administration of free-radical scavengers. Addition of Cu, Zn, selenium, and vitamins E and C to the diet may prevent the formation of excessive oxygen free-radicals during phagocytosis and other inflammatory processes (Chow & Tappel, 1974; Bettger & O'Dell, 1981; Herman, 1981; Aust *et al.* 1985; Halliwell, 1987). These scavengers can protect hepatic mitochondria and microsomes as well as membrane lipids from peroxidative degradation (Combs *et al.* 1975). The potential role of other principal essential antioxidants, including β-carotene and vitamins A and C, in the body's defence against oxidative agents (Gey *et al.* 1987) requires further investigation. In rodents and piglets, supplementation with vitamins C and E has already been shown to reduce atherosclerotic-like lesions (Gey, 1986).

With a better understanding of the contribution of other important micronutrients, such as iodine in thyroid hormone production, magnesium in intracellular enzyme activity, and Fe in O_2 and electron transport (Brewer *et al.* 1985), nutritionists and

Table 3. *Phenotyping of T- and B-lymphocytes and their subsets*

Population	Phenotype
Total T-cells	CD2+, CD3+
Helper T-cells	CD3+, CD4+
Helper inducer T-cells	CD4+, 4B4+(CD45R)
Suppressor inducer T-cells	CD4+, 2H4+
Suppressor–cytotoxic T-cells	CD3+, CD8+
Natural killer-cells	NKH1+
Total B-cells	CD20+, CD19+, also CD5+ subset
Activated helper T-cells	CD4+, IL-2 receptor (CD25)+, CD+, HLA-Dr+
Activated suppressor–cytotoxic T-cells	CD3+, CD8+, HLA-Dr+, CD3+, CD8+, IL-2 receptor (CD25)+
Activated natural killer-cells	NKH1+, CD8+, CD11+
γ T-cell receptor T-cells	CD3+, CD4−, CD8−
Cytotoxic T-cells	CD3+, CD8+, S6F1+
IL-2 receptor on monocytes	CD14+, CD25+
LFA receptor on neutrophils	CD11+
Immature neutrophils	CD10+, CD11+
Helper inducer T-cells	CD4+, 4B4+, Leu8+
Suppressor inducer T-cells	CD4+, 2H4+, Leu8+

IL-2, interleukin-2.

physicians will be able to prescribe optimal nutritional therapy for each patient. The previously described evidence suggests that dietary manipulation of both total parenteral and enteral nutritional support may be an important therapeutic tool in regulating the inflammatory processes.

Immunotherapy

Recent availability of flow cytometric immunofluorescence methods with the use of monoclonal antibodies has revealed the pattern of immune suppression in patients with multiple organ failure (Nishijima *et al.* 1986). Abnormalities in the immune system have included decreased T-cell response to mitogens, decreased antibody concentrations, alteration of T-cell subsets, decreased levels of IL-1 and IL-2, high suppressor:helper T-cell ratios, and the presence of an immunosuppression peptide (Abraham & Change, 1986; Radosevich *et al.* 1988). Though the exact mechanism of action of the defects in immunity remains unclear, maintenance or restoration of the integrity of the immune system by the administration of immunoadjuvants in clinical medicine may help to boost the body's natural defence mechanisms.

However, before immunoadjuvants are used, a complete understanding of the T-cell and B-cell phenotypes (Table 3) and their interaction with each other is essential. Since the immune system is a highly complex and interactive network of cells and molecules, treatment of one component may perturb the entire system, resulting in significant toxicity.

Recently, immunologists have classified T-lymphocytes into different 'cluster designations' and have further subdivided them into 'effector' and 'regulatory' T-cells. The effector cells include the cytotoxic T-cells and the delayed-type hypersensitivity T-cell. They 'see' antigen in the context of the class I and class II components of MHC. The regulator T-cells are the T-helper (T_H) and the T-suppressor (T_S) cells. The former bear the CD4 markers and 'help' the B-cells to produce antibody; the latter have the CD8

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markers and 'negatively' regulate B-cells and other immune functions. Both 'T-helper' and 'T-cytotoxic' cells differentially modulate macrophage function in response to an antigen, thus determining the host's ability to combat infection.

Immunotherapy should aim at a balance between T_S and T_H cells so as to modulate the 'hyperimmune' environment of activated macrophages and killer-cells that might be harmful to tissues and vital organs. In this regard, monoclonal antibodies are useful agents for both diagnostic and therapeutic approaches. Anti-thymocyte globulin therapy has already been used effectively in depressing T-suppressor cell function in patients with severe aplastic anaemia (Radosevich *et al.* 1988). In animal models, anti-cachectin/TNF, monoclonal antibodies, and anti-serum have been successful in blocking many of the deleterious effects of septic shock during lethal bacteraemia (Beutler *et al.* 1985).

MHC antigen may also indicate another potential site of therapeutic intervention in allogenic rejection and endotoxaemia related to cell membrane injury. It has been postulated that MHC antigen on the surface of a metabolically active S+ phenotype cell is highly immunogenic, whereas the same antigen presented on the surface of the cell of the S− phenotype is non-immunogenic for the T-lymphocytes (Lafferty *et al.* 1985). The removal of the S+ cells may reduce immunogenicity in a hyperimmune environment. Modestly prolonged pancreas allograft survival has already been achieved in non-immunosuppressed recipients with anti-class II monoclonal antibodies (Lloyd *et al.* 1987) or γ-INF (Markmann *et al.* 1987) by eliminating the class II-positive dendritic cells, which are potent stimulators of allograft rejection (Lloyd *et al.* 1987).

Among experimental approaches, cyclosporin has been regarded as a unique immunomodulator because of its ability to inhibit antigen presentation by human monocytes to class I MHC without affecting the expression of human leukocyte antigen-DR (activated cytotoxic T-cell) (Snyder *et al.* 1987). The immunosuppressive effects of cyclosporin occur via inhibition of IL-2 and IL-4 production, both of which are involved in the pathogenesis of graft *v.* host disease (Forman *et al.* 1987; Yee *et al.* 1988) and autoimmune diseases (Geha, 1988). Recent evidence has demonstrated that replacing conventional olive oil with fish oil as a vehicle for cyclosporin improves immunosuppressive effects and reduces nephrotoxicity (Kirkman *et al.* 1987). The immunosuppressive activity of cyclosporin is mediated through binding to Ca calmodulin, but its action is ineffective against activation mediated by PKC (Weiss *et al.* 1986).

The efficacy of immunomodulators, including γ-INF and α-INF, IL-1 and IL-2, TNF, or combinations used in clinical medicine (Fanci *et al.* 1987), is still in doubt, with reservations mainly related to the complexity of the immunoregulatory network. Nevertheless, the synergistic effects of IL-1, interferon, and IL-2 have proved successful in cytotoxicity (Dempsey & Dinarello, 1982). Evidence also indicates that the diminished tumour killing by natural killer-cells from patients with cancer can be overcome by treating with IL-1 (Dinarello *et al.* 1986) and TNF (Nathan *et al.* 1983). Adjuvant therapy with vaccines containing microbial products or lipopolysaccharide may be essential during IL-1 treatment in cancer, since IL-1 activation of T-cell or B-cell proliferation is dependent on antigen presentation (Dinarello *et al.* 1986; Fanci *et al.* 1987).

Both γ-INF and α-INF are also regarded as good antiproliferative agents (Nathan *et al.* 1983). High doses of interferon are immunosuppressive and inhibit both B- and T-cell proliferation, but low doses can stimulate the immune system, primarily by increasing the cytocidal activity of natural killer-cells, macrophages, and T-lymphocytes. TNF has anti-viral, anti-parasitic, and anti-tumour effects with a specific effect on infected cells (Creasey *et al.* 1987). These effects are similar to and synergistic with those of γ-INF. These findings have pointed to the possibility of combination therapy against a wide range of viral diseases, including AIDS.

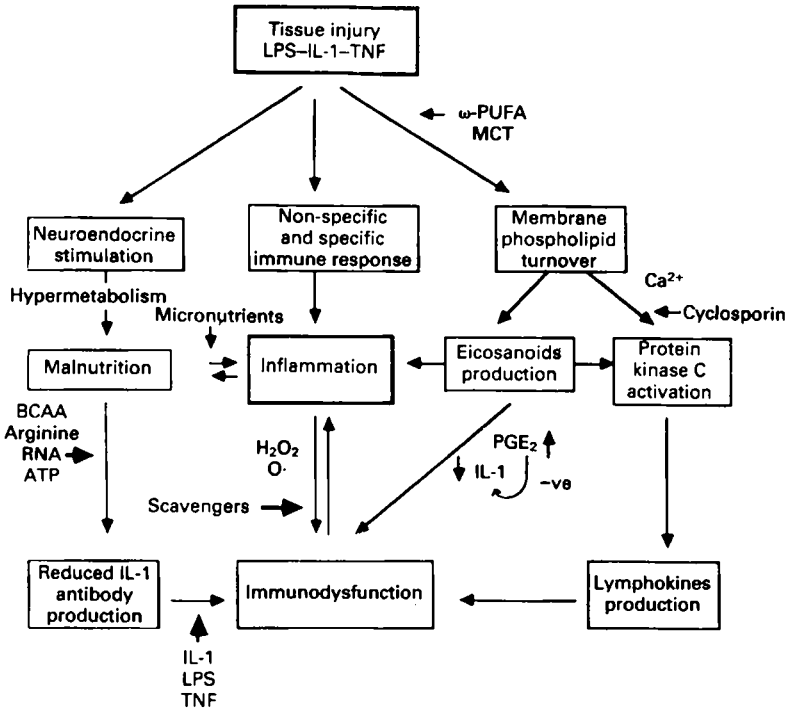

Fig. 5. Hypothetical summary of nutritional and immunological supports in inflammatory diseases. ω-PUFA, ω-polyunsaturated fatty acids; LPS, lipopolysaccharides; IL-1/TNF, interleukin-1/tumour necrosis factor; MCT, medium-chain triglycerides; O·, oxygen free-radical; PGE_2, prostaglandin E_2; −ve, inhibition; BCAA, branched-chain amino acids.

The choice of an immunopotentiator such as isoprinosine, which increases both IL-1 and IL-2 production in patients with AIDS (Tang *et al.* 1985), may be safer than direct administration of IL-2, when its side-effects are taken into account. With further knowledge about the immunological patterns of individuals following injury, infection, and sepsis, immunomodulation by use of selective diets, monoclonal antibodies and recombinant monokines and lymphokines may help to restore the host's normal defence system (Fig. 5).

Summary

The collective evidence suggests that nutritional insult to both cell-mediated and humoral immunity in the presence of protein–energy malnutrition contributes to abnormalities of inflammation. The primary goal of nutritional support in inflammatory disease is to provide adequate energy and protein to meet endogenous requirements for tissue repair, IL-1 production, and restored cellular function, thus preventing secondary infection.

Substrate provision should aim at improving the acute phase of injury while avoiding immune dysfunction. This goal may be achieved be altering the eicosanoid pathway toward a more regulated inflammatory state. In the context of allograft response, macrophages are central to the initiation of allosensitization by virtue of their ability to present antigen to T-cells. Activated T-cells may further modulate macrophage function by the secretion of lymphokines. Manipulation of macrophage eicosanoid production by dietary ω-3 PUFA may reduce cellular immune response.

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Table 4. *Feeding regimens for critically ill patients*

Macronutrients	Present	Investigational	Future
Carbohydrate (mg/kg per min)	2–5	2–5	2–5
Glucose (as % total carbohydrate)	100	50	50
Xylitol (polyol) (as % total carbohydrate)	0	50	50
Protein (g/kg per d)	1·5–2·0	1·5–2·5	1·5–2·5
EAA:NEAA	50:50	60:40	60:40
BCAA (as % total)	18–22	35–50	35–50
Glutamine* (as % protein)	0·0	0·05	>0·05
Arginine* (as % protein)	0·02	10	>10
Fat energy (as % total energy)	30–50	30–50	30–50
Long-chain triglyceride (LCT)†	100	25	0
Medium-chain triglyceride (MCT)†	75 or 50	0	0
Fish oil (FO)†	0	25	<40
Structured lipid (LCT–MCT)†	0	0	100 or 60
Structured lipid (FO–MCT)†	0	0	100
γ-Linolenic acid†	0	0	?

EAA:NEAA, ratio essential amino acids:non-essential amino acids; BCAA, branched-chain amino acids.
*Intravenous.
†As % of total energy from fat.

Nutritional support should also focus on providing essential micronutrients, with their potentially immunomodulating role, as adjunctive therapy in order to protect the host from toxic effects of free-radicals and chemicals released during inflammatory events. (Feeding regimens currently under investigation and development are presented in Table 4.)

By integrating dietary immunotherapy with the use of recombinant hormones, monoclonal antibodies, and various available monokines, an optimal outcome for each patient may be achieved. However, effective application of immunotherapy to nutritional supplementation will require accurate monitoring of immune function in individual patients in order to avoid inappropriate treatment.

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