Genotypic Influences on Metabolic Alterations during Inflammation and the Nutritional Outcome

Robert Francis Grimble

*Institute of Human Nutrition, School of Medicine, University of Southampton, UK*

**Introduction**

Inflammation is part of the immune response, whose function is to combat pathogens and restore health following injury and surgery. Inflammation also occurs in a less helpful role during cancer, exposure to environmental pollutants, radiation, and allergens, and during chronic inflammatory disease. This latter group of inflammatory conditions includes diseases such as rheumatoid arthritis, Crohn’s disease, asthma and psoriasis. During the inflammatory response metabolites are released from endogenous sources to supply the immune system with substrate to support its activity. Key features of the inflammatory response are: (i) immunonutrition from endogenous sources; (ii) loss of lean tissue components; (iii) creation of a hostile environment for pathogens, and (iv) ultimate inhibition of the process by endogenous systems (Fig. 1). The strength and duration of the response are modulated by pro-inflammatory and anti-inflammatory cytokine production, from the immune system. Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) fall into this first category and interleukin-10 (IL-10) into the second. In addition to pro- and anti-inflammatory cytokines, a wide range of immunomodulatory cytokines (e.g. IL-2, IL-4) are produced. IL-1, IL-6 and TNF-α are unique among cytokines in directly initiating metabolic changes in the host. In addition, the activity of macrophages in producing cytokines, nitric oxide and other mediators of inflammation is influenced by natural resistance-associated macrophage protein (NRAMP1). Neuroendocrine responses occur, and heat shock proteins (HSP) are produced with the objective of terminating the
The Metabolic Effects of Pro-Inflammatory Cytokines

Many of the signs and symptoms experienced during infection and following injury and surgery, such as fever, loss of appetite, weight loss, negative nitrogen and mineral balance and lethargy, are caused directly and indirectly by pro-inflammatory cytokines. Indirect effects of cytokines are mediated by actions upon the pituitary and adrenal glands and endocrine pancreas, resulting in increased secretions of the catabolic hormones adrenaline, noradrenaline, glucocorticoids and glucagon. Insulin insensitivity thus occurs, further contributing to a ‘catabolic state’. Muscle protein is catabolized to provide amino acids for synthesizing new cells and proteins for the immune response and for conversion to glucose (a preferred fuel for the immune system).
Table 1. Major acute phase proteins and their functions

<table>
<thead>
<tr>
<th>Acute phase protein</th>
<th>Function</th>
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<tr>
<td>$\alpha_1$-Proteinase inhibitor</td>
<td>Inhibition of proteinase released during the inflammatory response</td>
</tr>
<tr>
<td>$\alpha_1$-Antichymotrypsin</td>
<td></td>
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<tr>
<td>$\alpha_2$-Macroglobulin</td>
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<tr>
<td>C-Reactive protein</td>
<td>Removal of antigens from the host</td>
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<td>Serum amyloid A</td>
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<tr>
<td>Proteinase inhibitors</td>
<td>Suppression of the immune response</td>
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<tr>
<td>$\alpha_1$-Glycoprotein</td>
<td></td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Antioxidant properties</td>
</tr>
<tr>
<td>$\alpha_1$-Acid glycoprotein</td>
<td></td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td></td>
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<tr>
<td>Haptoglobin</td>
<td>Binding and transport of metals and biologically active compounds</td>
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<tr>
<td>Transferrin</td>
<td></td>
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<tr>
<td>$\alpha_1$-Acid glycoprotein</td>
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The extent of this process is highlighted by the major increase in urinary urea excretion ranging from 9 g/day in mild infection to 20–30 g/day following major burn or severe traumatic injury. These two extremes of urea excretion represent daily losses of 56 and 188 g of body protein, respectively. Acute phase proteins, which focus the actions of the immune system on the invader and help to protect healthy tissue from damage, are synthesized in increased amounts by the liver. Important acute phase proteins and their respective functions are highlighted in Table 1. The liver focuses its activities on acute phase protein synthesis by reducing synthesis of its main protein product, serum albumin, and also a number of other secretory proteins such as retinol-binding protein and transferrin. The serum albumin concentration is often regarded as an index of protein nutritional status. Thus the fall in albumin concentration that occurs during infection and inflammatory disease is often misunderstood as a sign of protein deficiency and complicates the assessment of protein status in patients. Clinically, in virtually every instance a low serum albumin reflects the presence, or recent occurrence, of a systemic inflammatory response. Raised concentrations of catabolic hormones and insulin insensitivity increase fat catabolism. The fatty acids produced help to satisfy the increased energy needs of the infected person (the resting metabolic rate increases by 13% for every 1°C rise in body temperature). The biochemistry of an infected/injured individual is thus fundamentally changed in a way which will maximize nutrient supplies from within the body for use by the immune system. The changes in temperature are mediated by the interaction of pro-inflammatory cytokines with specialized neurones in the hypothalamus. The actions of cytokines on the hypothalamus also brings about a loss of appetite. This may be transient in nature, or prolonged and profound, as is the case in chronic infections such as tuberculosis, or during cancer. It is interesting to
Influence of Genotypic Influences on Metabolic Alterations during Inflammation

Note that the body fat regulatory hormone, leptin, is induced by TNF-α but, as of yet, no direct role for leptin has been found in the significant weight loss that occurs during infection and cancer.

Major changes occur in plasma iron, copper and zinc concentration. The changes in iron and zinc are often misinterpreted as indicating mineral deficiency, but are very likely to be due to major redistribution of these elements within the body in an attempt to ‘starve’ blood-borne microbes of nutrients. However, micronutrient deficiency can be precipitated by the response to infection as urinary loss of many micronutrients is accelerated following infection and injury. The resultant deficiencies in zinc, iron and copper, which may occur, have deleterious effects on general immune function and wound healing.

The cytokines also stimulate the synthesis of oxidant molecules (hydrogen peroxide, nitric oxide, hydroxyl radical, hypochlorous acid, and superoxide anion), which damage the cellular integrity of the invading organism.

Many of these complex metabolic and physiological changes create a hostile environment for invading organisms, which results in the balance being swung from being in favor of the invading organism to being in favor of the infected individual.

**Systems for Suppressing Pro-Inflammatory Cytokine Production**

Pro-inflammatory cytokines induce the production of a cascade of other cytokines from lymphocytes. The cascade has modulating actions on the lymphocyte function (IL-2 stimulates lymphocyte proliferation, IL-8 attracts immune cells to the site of invasion, IL-4 alters the class of immunoglobulin produced). While IL-10 has no direct metabolic effects, it indirectly influences the metabolic events which characterize the inflammatory response by suppressing pro-inflammatory cytokine production. There is evidence that a poor outcome can arise from an excessive systemic inflammatory response. This syndrome is referred to as the systemic inflammatory response syndrome (SIRS) and is manifested by increased TNF-α concentrations in sepsis, or following burn injury. However there is a compensatory anti-inflammatory response syndrome (CARS), which is reflected in the increased production of IL-10 which, if excessive, can also have a deleterious outcome. From a growing body of research data, it has been hypothesized that the relative balance between SIRS and CARS has an impact on survival. This is probably why attempts to block pro-inflammatory cytokine production with monoclonal antibodies have met with little success in reducing mortality.

Proteins originally identified in heat-stressed cells (HSP) are produced by cells exposed to pro-inflammatory cytokines. HSP have a general anti-inflammatory influence and suppress pro-inflammatory cytokine production. These autoregulatory mechanisms are very important biological phenomena.
for, while cytokines are essential for effective operation of the immune system, they exert a high metabolic cost on the body and can exert damaging and lethal effects (Fig. 2).

**Fig. 2.** Key features of the inflammatory response, which influence recovery from injury and infection. + = Stimulatory influence; − = inhibitory influence.

### Damaging and Life-Threatening Effects of Cytokines

Although cytokines are essential for the normal operating of the immune system, if produced at the right time and in the right amounts, high production of IL-1 and TNF-α has damaging effects upon the host [1]. In conditions such as cerebral malaria, meningitis and sepsis, they are produced in excessive amounts and are an important factor in increased mortality. A number of clinical studies have shown that not only is a capacity for high production of pro-inflammatory cytokine deleterious to health and survival, but that enhanced production of anti-inflammatory cytokines is also disadvantageous. It would appear that the ratio of pro- to anti-inflammatory cytokines might be a
more predictive determinant of outcome than the level of production of either type of cytokine alone. Indeed, in a recent study on sepsis, patients’ high IL-6 to IL-10 ratios were associated with greater mortality than lower ratios and, in a study on patients admitted to hospital with fever, mortality was significantly higher in patients with high plasma IL-10 to TNF-α ratios than in those with more equitable ratios [2, 3]. Pro-inflammatory cytokines play a major damaging role in many inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, asthma, psoriasis, systemic lupus erythematosus, and in cancer. They are also important in the development of atheromatous plaques in cardiovascular disease [4].

Factors Influencing Pro-Inflammatory Cytokine Production

Individual Genotype

In males and post-menopausal females, in vitro production of TNF-α by leukocytes shows remarkable constancy on an individual basis. Individuals may inherently be a ‘high’, ‘medium’ and ‘low’ producer of TNF-α [5]. IL-1, TNF-α and IL-10 production is strongly influenced by single nucleotide polymorphisms (SNPs) in the promoter region of their respective genes. SNPs in the TNF-α and lymphotoxin-α (LT-α) promoters, influence TNF-α production [6, 7]. Individuals who are homozygous for the TNF-α allele (TNF2) or for the lymphotoxin-α allele (TNFB2) show high levels of production. Homozygotes for the TNF1 or TNFB1 alleles exhibit low production with intermediate levels of production being found in heterozygotes [7]. SNPs in the IL-1 genes result in constant individual levels of production [8]. There are several SNPs in the IL-10 gene that influence production. The 1082*A allele is associated with low and the 1082*G allele with high production. Microsatellites in the NRAMP1 gene are associated with regulation of macrophage activity. As a result of activation, nitric oxide and cytokines are produced. There are 4 microsatellite alleles in the NRAMP1 gene which influence macrophage sensitivity to inflammatory agents. Alleles 1, 2 and 4 are poor promoters, while allele 3 causes high gene expression.

With the existence of these strong genetic determinants of pro- and anti-inflammatory cytokine production, it could be postulated that within each individual there is a pro- and anti-inflammatory cytokine balance that is genetically determined, i.e. each individual on exposure to an inflammatory event will experience an ‘inflammatory drive’ of differing intensity, which is pre-determined by their genotype.

Increased frequencies of various cytokine gene alleles are associated with increased morbidity and mortality in a range of diseases and conditions. An increased frequency of the TNF2 allele occurs in systemic lupus erythematosus, and insulin-dependent diabetes mellitus [7–9]. Children who were homozygous for the TNF2 ‘high expression’ allele had a sevenfold increase
in the relative risk of death from cerebral malaria than children who were homozygous for the TNF1 ‘low expression’ allele [10]. Mortality among sepsis patients was over threefold higher in individuals who possessed the TNF2 allele than those without this genetic characteristic [11]. In addition, homozygosity for the TNF2 allele has been associated with disease severity in chronic hepatitis C infection and increased rejection rates of renal and heart transplants [12]. Trauma patients who were homozygous for the TNFβ2 allele had a fivefold increased incidence of sepsis and higher peak TNF-α concentrations than patients who were heterozygotic [6]. In patients, who developed sepsis and were homozygous for TNFβ2, the mortality rate and peak TNF-α concentrations were twice those recorded in patients who were heterozygous for the two lymphotoxin-α alleles [13]. A SNP at – 511 in the IL-β gene has been associated with an increased risk of cardiovascular disease [14]. Allele 3 of the NRAMP1 gene has been linked to autoimmune disease and high resistance to infection, and allele 2 to low resistance to infection, but low incidence of autoimmune diseases [15, 16]. Thus there will be differences in how each individual responds to inflammatory agents in his environment due to the mix of SNPs for cytokine genes that he possesses. The mix of SNPs would have a bearing on individual susceptibility to developing diseases with an inflammatory basis, their capacity to kill microbes and the likelihood of experiencing an adverse outcome to the inflammatory response, which follows surgery and infection.

Oxidant Stress and Antioxidant Defense

Cytokine production is strongly influenced by other participants in the inflammatory response. During inflammation, oxidants, intended to create a hostile environment for invading pathogens, also enhance pro-inflammatory cytokine production by activation of nuclear factor kappa B (NFκB). The activated transcription factor migrates from the cytoplasm to the nucleus, where it binds to response elements of a number of genes associated with inflammation, including those for pro-inflammatory cytokines, adhesion molecules and acute phase proteins [17]. Antioxidant defenses may also be upregulated during inflammation by this mechanism, since the gene for γ-glutamylcysteine synthetase (rate-limiting enzyme in glutathione synthesis) contains an NFκB-binding site. Functionally this action not only protects healthy tissue from the damaging effects of oxidants but also suppresses activation of NFκB [18]. However, a wide range of diseases and trauma results in depletion of antioxidant defenses. Likewise elective surgery causes transient reduction in plasma and muscle glutathione concentrations, which returns to normal values within 48 h of surgery [18]. This phenomenon has important functional consequences since glutathione is a major component of total antioxidant defense. Not only does it act as an antioxidant in its own right, but, by cyclical oxidation and reduction, is able to maintain other components of defense in a chemically reduced state, in which form they are able to quench oxidant molecules.
The importance of upregulation of antioxidant defenses during inflammation is illustrated in animal model studies, where prior administration of diethyl maleate, which binds irreversibly to reduced glutathione, results in normally nonlethal doses of recombinant TNF-α exerting lethal effects [19]. Furthermore, in patients it has been observed that, after the onset of sepsis, a fall in antioxidant potential of blood occurs, which returns to normal within 5 days in survivors. In nonsurvivors, however, the potential fell to lower concentrations than in survivors and did not return to normal. A recent study indicates that enhanced activation of NFκB might play a prominent part in the deleterious interaction between oxidants, antioxidant defense and mortality. In sepsis patients, nonsurvivors showed almost double the level of NFκB activation in peripheral blood mononuclear cells (PBMCs) to that seen in survivors [2].

**Underlying Features of the Adverse Effects Associated with Inflammation**

From the foregoing discussion it can be seen that the body is well equipped to focus a powerful set of biological processes and agents upon invading organisms. Three key processes, initiated by pro-inflammatory cytokines, occur during the inflammatory response and influence patient outcome. These processes are: (i) creation of a hostile environment (for pathogens); (ii) provision of nutrient for the immune system from endogenous sources, and (iii) strengthening of the protective and control systems against damage to healthy tissue by the immune response (Fig. 1). There are, however, a number of foci at which the response may exceed its healthful confines (Fig. 2). These are: (i) immunosuppression and hyperinflammation; (ii) oxidant damage, and (iii) excessive loss of tissue components. The outcomes of these three situations are increased morbidity and mortality. The relationship between excessive loss of lean tissue mass and mortality is well recognized. A loss of lean tissue in excess of 50% of normal body content is incompatible with good rates of survival from severe infection, injury and surgery. In patients dying of sepsis there is clear evidence of an imbalance in pro- and anti-inflammatory cytokine production, a failure to maintain antioxidant defenses and high levels of activation of NFκB [2, 20]. High levels of inflammation exert a suppressive effect on T-cell function. This may partly be due to the inhibitory effect of Th1 responses on Th2 activity, and to the immunosuppressive effects of high levels of prostaglandin E2 production during the inflammatory response. As a consequence of such immunosuppression, morbidity and mortality rates will be adversely affected. Certainly, a number of studies, using antioxidant and immunonutrient mixes which would improve antioxidant defenses, show evidence of reduced inflammation and improved T-cell function [12].
Table 2. Frequently used immunonutrients and their functions

<table>
<thead>
<tr>
<th>Immunonutrient</th>
<th>Mode of action</th>
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</thead>
<tbody>
<tr>
<td>n-3 polyunsaturated fatty acids</td>
<td>Act as anti-inflammatory agents and reverse immunosuppression</td>
</tr>
<tr>
<td>Sulfur amino acids and precursors</td>
<td>Enhance antioxidant status via glutathione status</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Nutrient for immune cells, improves gut barrier function, precursor for glutathione synthesis</td>
</tr>
<tr>
<td>Arginine</td>
<td>Stimulates nitric oxide production and growth hormone secretion, improves helper T-cell numbers</td>
</tr>
<tr>
<td>Nucleotides</td>
<td>RNA and DNA precursors, improve T-cell function</td>
</tr>
</tbody>
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Indeed meta-analysis of the effectiveness of immunonutrition indicates a mean reduction in hospital length of stay of 2.9 days and a reduction in infection rates. However, mortality was not affected [21].

Thus important targets for immunomodulation are: (i) enhancing the cell-mediated response; (ii) altering the balance of pro- and anti-inflammatory cytokines; (iii) prevention of excessive activation of NFκB, and (iv) moderation of tissue nutrient depletion (Fig. 2). At present there is a relatively limited number of nutrients included in immunonutrient mixes for moderating the inflammatory response and improving immune function (Table 2).

Variability in Responsiveness to Immunonutrients

Due to their anti-inflammatory properties, n-3 polyunsaturated fatty acids (PUFAs) are key components of immunonutrient formulations [12, 22]. However, it is not possible to discern the contribution of n-3 PUFAs to the general anti-inflammatory and immuno-enhancing effects demonstrated in trials using such formulations. Peri-operative feeding of colorectal cancer patients with an arginine-enriched enteral feed containing n-3 PUFAs resulted in a decrease in the post-operative rise in IL-6- and IL-1-soluble receptors, an increase in IL-2 receptor-α, an improvement in delayed hypersensitivity responses and a decrease in infection rates [23]. In a study on post-operative cancer patients, the same dietary formulation resulted in not only a fall in IL-6 but a rise in IL-2-soluble receptors, indicating how the immuno-enhancement may have been achieved [24]. Studies on inflammatory disease have also shown the anti-inflammatory influence of n-3 PUFAs given in the
form of fish oil. However, not all studies have shown a beneficial effect. In rheumatoid arthritis and psoriasis significant clinical improvements have been reported, however the oil is less efficacious in systemic lupus erythematosus and produced no benefit in asthma [22].

**Mechanisms Underlying the Variable Response to Fish Oil**

The question arises as to why an anti-inflammatory effect is not found in all studies in which n-3 PUFAs have been given and, in those studies where n-3 PUFAs show this effect, why an anti-inflammatory influence is not demonstrable in all subjects. The answer to these questions may impact upon why formulations enriched with n-3 PUFAs are not efficacious in all patients.

The study of Endres et al. [25] focused attention on fish oil, as a potential anti-inflammatory nutrient, particularly in its capacity to reduce pro-inflammatory cytokine production. In the study 9 subjects were given 18 g fish oil/day for 6 weeks. A statistically significant fall in *ex vivo* IL-1 and TNF-α production from stimulated PBMCs was noted. However the data showed large standard deviations indicating that, within the 9 subjects, there were both ‘responders’ and ‘nonresponders’ to the anti-inflammatory effects of fish oil. Since that time other studies, also on relatively small numbers of subjects, have shown either an inhibitory effect of fish oil supplements on *ex vivo* pro-inflammatory cytokine production [26] or no effect [27]. We have investigated the effects of feeding 6 g fish oil/day for 12 weeks on *ex vivo* TNF-α production by PBMCs in 111 young men [28]. Surprisingly, fish oil resulted in a lowering of TNF-α production in 51% of the subjects and in an increase in production in 49%. In *in vitro* studies on PBMCs, cultured with prostaglandins (PGs) and leukotrienes (LTs), it was shown that PGE2 suppresses and LTB4 enhances TNF-α production [25, 29]. As a result of supplementation, with n-3 PUFAs, arachidonic acid (AA) in the cell membrane will be replaced by eicosapentaenoic acid (EPA). AA is the precursor for a number of eicosanoids including PGE2 and LTB4. EPA, however, is the precursor for PGE3 and LTB5. These latter eicosanoids have lower bioactivity than the E2 and B4 varieties. Thus, theoretically, substitution of EPA for AA in the membrane of the PBMCs might result in a lessening of the inhibitory, or stimulatory, influence of the respective eicosanoids. TNF-α production would thus either rise or fall. An additional cause of variability in response might lie with genetic influences, referred to earlier in this article. We investigated whether all individuals, with each of the possible combinations of TNF-α and LT-α alleles, were equally sensitive to the effects of fish oil supplementation, and found that this was not the case [12]. Thus sensitivity to the anti-inflammatory effects of fish oil is influenced by individual genotypic characteristics. Whether this situation is also true for other immunonutrients is at present unclear.
Fig. 3. Major determinants of the intensity of the inflammatory response to injury and infection. + = Stimulatory influence; − = inhibitory influence.

**Improving the Efficacy of Immunonutrition**

While meta-analyses indicate that immunonutrition can be efficacious in some groups of patients, when applied without specific knowledge of the precise requirements or metabolic status of the patients, improvements in efficacy will only occur if patients are carefully monitored in terms of their antioxidant status and level of depletion of tissue nutrient stores. When further studies have been conducted to discern the precise interaction between each individuals, genotype of relevance to the response to injury and infection and immunonutrients, the level of precision in the application of immunonutrition will undoubtedly improve (Fig. 3).

**Conclusion**

From the diverse range of studies mentioned above, it would thus appear that pro-inflammatory cytokines, although essential for immune defense, exert lethal effects when produced in large amounts, that certain genotypes are associated with high or disproportionate pro-inflammatory cytokine production, increased morbidity and mortality, that inflammation depletes lean tissue and antioxidant defenses, and that, in the absence of good antioxidant defense, oxidants enhanced pro-inflammatory cytokine production resulting in increased morbidity and risk of mortality. Furthermore the depletion of lean tissue, which occurs as a normal part of the inflammatory response,
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carries with it the risk of precipitating a state of malnutrition. The precise way in which inflammation, cytokine genotype and antioxidant defense interact in individuals to determine the outcome of the inflammatory response is unknown. In order for treatment of inappropriate levels of inflammation to be targeted effectively, the nature of these interactions needs to be identified.

Acknowledgements

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References


Discussion

Dr. Segal: Do you have any idea of the importance of the mast cell in initiating inflammation?

Dr. Grimble: We know that mast cells are rich in tumor necrosis factor α (TNFα) and they are positioned in the body in such a way that they are likely to be stimulated early on during injury or insult, so I think they probably play a very important role. I don’t know of any studies that have specifically looked at mast cells per se and the role they have in the inflammatory response but it is likely to be an important one.

Dr. Segal: With the overwhelming AIDS epidemic that we are seeing in South Africa, would there be a place for antioxidants or any other nutrients in terms of intervention?

Dr. Grimble: I am not a clinician and so anything I say about the clinical issue might not be sensible. However, it seems to me that HIV infection is the disease with which to test the antioxidant cytokine hypothesis. I know various studies that have looked at this using cocktails of antioxidants, and I think some of them have shown benefit, but people working on HIV know how difficult it is to design carefully controlled studies in this disease. My reading of the literature is that antioxidants are useful in reducing or preventing conversion of HIV positivity to full blown AIDS and that there is a need for much more careful research in the area. It puzzles me greatly that there have
not been more studies looking at antioxidants in HIV infection. Part of the reason for that may be the reliance on drug cocktails and the feeling that pharmacology provides more help in clinical problems than nutrition, but I believe that nutrition really holds the answer.

**Dr. Segal:** My third question is about pancreatitis. If one uses acute pancreatitis as a model of infection or inflammation, why is there reluctance to give antioxidants even though Braganza’s [1] work in Manchester has shown them to be very effective?

**Dr. Grimble:** I don’t know anything about pancreatitis. Can anyone else answer that question?

**Dr. Shenkin:** We must not confuse acute pancreatitis and chronic pancreatitis. John Braganza’s work in Manchester was specifically on chronic pancreatitis, where it is increasingly clear that antioxidant cocktails are beneficial in preventing the recurrence of episodes of pancreatitis and in reducing the need for further surgery. The acute pancreatitis situation is much more complex. My understanding of that situation is that by the time one is considering intervention, the whole proinflammatory cascade has been so activated that almost nothing you do will influence it. As far as I am aware there are no well-controlled intervention studies in acute pancreatitis that have shown any benefit of antioxidants.

**Dr. Segal:** Another question allied to that would be the timing of the intervention. Your data would suggest that the production of oxidants is a normal physiological response to trauma and obviously plays an important role in the control of that trauma or damage. At what stage should we be intervening? Is there not a stage where intervention might be harmful?

**Dr. Grimble:** I agree. The production of oxidants occurs early, and there is always the risk that there would be collateral damage to healthy tissue, and that the damaged tissues, for example peroxidized lipids or peroxidized proteins, could in themselves provide a further stimulus for the inflammatory process. Thus reducing the extent of the oxidant burst at the start could be very important. It could be that if the response was too large we would set the patient on a totally different track from the one they would have been on if antioxidant defense had been stronger.

**Dr. Labadarios:** In relation to acute pancreatitis, the only thing I would like to add is that this condition lacks any kind of homogeneity. The evidence we have, especially about selenium, doesn’t amount to much in terms of dose, frequency, or duration, and it is very difficult to come up with guidelines on its use. In relation to possible benefit, there is no evidence that mortality is affected at all.

**Dr. Meguid:** You said that antioxidants are depleted at the moment of injury. I cannot visualize how such a depletion occurs. Do these antioxidants suddenly become bound to something, or are they drained somewhere? If they were physically present, how do they become unavailable in the reaction?

**Dr. Grimble:** I didn’t want to give the impression that antioxidants were instantly depleted within half an hour of the event. I think they are progressively depleted over the following 24 h. Controlled studies on glutathione levels in muscle and plasma during surgery have shown that 24 h postoperatively there is a substantial fall, particularly in muscle glutathione, though in plasma as well, with a gradual return to normal over the next 24–48 h.

**Dr. Meguid:** Is this due to a decrease in production rate or an increase in breakdown?

**Dr. Grimble:** I think it was shown to be an increase in breakdown, so the effect is on the consumption side of the equation. When we talk about oxidant defenses we are talking about 30 or 40 different compounds, but glutathione plays a pivotal role because of its ability to move between the oxidized and reduced state and to restore
oxidized vitamin E and C to the reduced form. So whatever happens to glutathione probably sets the scene for what happens to the other antioxidants.

**Dr. Soeters:** The question about concentrations is very relevant, because there are many situations in which concentrations do not mean anything at all. Just because the concentration is low it does not mean that the flux through the pathway decreases. What actually happens with glutathione is that the flux increases – both degradation and synthesis, or oxidation and reduction, are increased at the expense of a slight lowering in the concentration. The pool of glutathione is not a major factor in the flux rate, because in a day or so you will probably deplete the whole pool, so just a lowering in the concentration does not mean that there is a decrease in synthesis or degradation, rather the reverse.

One more word about acute pancreatitis. The major thing about acute pancreatitis is whether or not it is necrotizing, and, if it is necrotizing, whether or not it is infected. Once it is infected, there is no way you can intervene any more with metabolic means – surgery is then required. Once you have major gangrene in this area only surgery can save the patient. In chronic pancreatitis, there is continuing low-grade inflammation which will ultimately kill the pancreas; in those circumstances you have the opportunity to do something with metabolic interference.

**Dr. Becker:** Can we talk about the amino acids and the glutamine/arginine controversy? We know that glutamine could have important positive effects, but equally arginine could be detrimental in cancer patients, and could even promote tumor growth. How do we get the balance right between the ‘good’ amino acids and the ‘bad’ ones in our nutritional program?

**Dr. Grimble:** It’s a weak answer, perhaps, but we just have to be aware of the dangers of what we are doing. When I first read about the early intervention studies with arginine, I wondered why those patients weren’t all dying of septic shock, because you are providing a substrate for nitric oxide. I was reassured at the time by an expert in the field who told me that it was not a substrate-limited pathway. There is indeed a metabolic interrelationship between glutamine and arginine. If I remember my biochemistry correctly, by giving glutamine you are also giving arginine, because glutamine can be converted to arginine in the body. I am not sure where we are in this particular area at present and I can’t offer any advice. If we knew more about the nature of the disease process, we might be able to start making judgments about whether there is depletion of glutamine or arginine, and what the significance might be.

**Dr. Carpentier:** Like you, I have seen the evolution of this subject over time, with many new players coming on field. I would like to suggest another. Why not add acute phase lipoproteins? These change their composition and function markedly during inflammation, probably providing cholesterol, phospholipids, and fat-soluble vitamins to damaged tissues and immune cells. There are now many reports in this field and I would like your opinion on them.

**Dr. Grimble:** I agree that my presentation was a bit like an onion, with different layers being added, and I would certainly add a lipoprotein layer in the future. I was puzzled by the early studies with TNFα, which showed that hypertriglyceridemia was part of the response. Also the interesting finding that there are high concentrations of TNFα in adipose tissue clearly points to the fact that lipid metabolism is a major factor in inflammation. The role of peroxidized lipids as proinflammatory agents is exceedingly important, and we see this in terms of atherosclerosis and of the uptake of peroxidized lipids by foam cells during the inflammatory response.

**Dr. Carpentier:** You mentioned n-acetyl cysteine (NAC). I remember an early study showing that when this was given to human subjects there was very high urinary excretion. I wonder about the bioavailability of this compound in man.
Dr. Grimble: It is true that the bioavailability of NAC in man is very poor. Studies on the administration of NAC have shown excretion of unchanged NAC in large amounts. I think in the human situation NAC simply acts as a sulfhydryl donor to help to preserve glutathione, so the sulfhydryl group of NAC is sacrificed in place of the sulfhydryl group of glutathione. I believe that pro-cysteine, or OTZ, is more bioavailable. I am aware of studies that have used pro-cysteine quite effectively and have shown a reduction in soluble TNF receptors when it is given. So it has greater potential as an anti-inflammatory than NAC, which is also very unpleasant to take.

Dr. Winter: Going back to the manipulation of the cascade and the importance of TNF, to my recollection the work with anti-TNF antibodies in septic patients was very disappointing, so should we in fact be manipulating the system at all?

Dr. Grimble: I don’t think those results should stop us from manipulating the response, but rather they suggest that we should be manipulating it a bit earlier. The TNF antibody studies were all done when it was too late – the stable door was open and the horse had fled. We can also see dangers in using TNF antibodies. For example, very useful effects of anti-TNF antibodies have been shown in Crohn’s disease, but in one study there was an increase in malignancy [2]. TNFα is an anti-cancer cytokine as well as a proinflammatory cytokine, so if we block its actions there may well be a cost.

Dr. Heymsfield: I have been exposed to another field, which is the regulation of food intake. In this, the cutting edge molecular biology techniques such as microarrays, knockouts, and replacement of genes in individual organisms can help elucidate the mechanisms and pathways involved in a very clear way. Do you see these techniques being used in your field?

Dr. Grimble: We have started to move in that direction already. For example, knockout work in animals has shown that the whole inflammatory cascade has a great deal of redundancy in it: if you knock out one of the cytokines another will take over its task. The trio TNFα, interleukin (IL)-1, and IL-6 all have effects in common and so it is very difficult to work out exactly which is the major player. The early work of Charles Dinarello [3] showed very nicely that TNFα will induce IL-1 and IL-1 will induce TNFα. It’s a very close family but it’s difficult to determine the precise role of each individual member. I’m sure that knockouts will provide insight into this. As far as microarrays are concerned, the problem is that if we apply those to human populations, which have wide genetic variability, they are so expensive; we cannot do studies which require hundreds of individuals to work out relationships. I think a more useful approach would be to look at single nucleotide polymorphisms.

Dr. Beaufrère: You mentioned that cysteine is a rate-limiting amino acid for glutathione synthesis, but you also mentioned glycine, glutamine, and threonine as important amino acids. Do you think that we will end up with a single magic amino acid or will we end up with a combination of amino acids that changes depending on the target?

Dr. Grimble: I don’t think you can pick out any one of the three key amino acids – glycine, glutamic acid/glutamine, or cysteine – it depends entirely on the nutritional status of the patient upon whom the intervention is being done. There were some interesting studies by Wilmore [4], in which he poisoned rats with paracetamol and found he was able to restore glutathione levels with glutamine. He did not try the sulfur amino acids, but his rats were well fed so their sulfur amino acid supply was totally adequate, and those would not have been rate-limiting in that scenario. If he had put the animals on a low protein diet, glutamine would probably not have been effective, and the rate-limiting amino acids would have been the sulfur amino acids. We need to be able to describe patients much more accurately in terms of the status of individual amino acids before we can apply the right amino acid intervention effectively.
**Dr. Chioléro:** A patient with very severe inflammation, such as acute respiratory distress syndrome or acute pancreatitis, cannot be considered the same as one with prolonged chronic inflammation. What might be useful in the former could be bad in the latter.

**Dr. Grimble:** I don’t think we have a situation where what is true in the first situation is bad in the second; I think we have a situation where what is true in the first is ineffective in the second. In general, nutritional interventions are either effective or ineffective, but they don’t usually cause harm. Thus maybe our strategy should be to have a general approach that will be effective in a certain proportion of our patients, but also to have targeted treatment for specific situations. We need to know the patients’ genotype, their antioxidant status, and their lean tissue mass (as an indicator of nutrient reserves during the inflammatory response), so you can apply glutamine or arginine or n-3 polyunsaturated fatty acids more effectively.

**Dr. Soeters:** I think that one of the most important issues of this meeting is whether or not the metabolic response to disease is useful. Your answer to the question of why anti-TNF antibodies didn’t work was that they should have been used at an earlier stage, but that seems to contradict the message of your talk, in that most of what you showed us suggests that what happens in the physiologic response is useful. I think I would have answered that question by saying ‘it is not appropriate to use anti-TNF antibodies in acute disease’. In chronic disease it is a completely different issue, because there the TNF response is the disease itself. Could you comment on that.

**Dr. Grimble:** I would modify what you said slightly. I would say that, if we know that the genotype of the patient is of a nature that will produce massive amounts of proinflammatory cytokines, then maybe we should consider giving antibodies. But we should be aware that, if the patient is likely to need treatment over a long period of time, there is a risk of malignancy. We have to be aware of that risk.

**Dr. Soeters:** But in the acute situation we are talking about dying or not dying.

**Dr. Grimble:** In the acute situation we should target the antibodies to particular patients.

**Dr. Leverve:** In one of your slides you showed that albumin concentrations decrease, and this is clearly stated in textbooks on the subject, but what is the evidence that albumin synthesis is reduced?

**Dr. Grimble:** It is not very good. The problem with albumin concentrations is that much of the albumin is outside the vascular system. There are massive shifts of albumin into the gut and into the interstitial space early on in inflammatory response, so we can’t interpret a reduction in concentration as being caused by a decrease in synthesis.

**De Leverve:** There are even data – I think from Beaufrère’s group – showing that in some forms of acute illness there is an increase in albumin synthesis [5].

**Dr. Beaufrère:** Yes, albumin synthesis is increased in head trauma patients. Albumin is a negative acute phase protein, and it is quite clear that, while its concentration is reduced, its synthesis is increased, as it tries to compensate for the low concentration. This probably has to do with the distribution of albumin between the vascular and extravascular compartments, as Dr. Grimble implied.

**Dr. Grimble:** The whole point about the albumin concentration is that people consider it an important indicator of outcome. I think it is generally accepted that a low serum albumin, below say 20 mg/dl, is bad news, but whether this is simply a bystander effect is not known.
Genotypic Influences on Metabolic Alterations during Inflammation

References


