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Glutamine: essential for immune nutrition in the critically ill

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Critically ill patients on intensive care units are at an increased risk of sepsis, which is a major cause of mortality in these patients. Recent evidence suggests that impairment of the functioning of the immune system contributes to the development of sepsis in such patients. In particular, monocytes show reduced expression of HLA-DR antigen, associated with impaired antigen presenting capability and decreased phagocytic activity; lymphocytes show decreased proliferation in response to mitogens and T-helper cells show a shift in the Th1/Th2 ratio consistent with impaired immunity. The amino acid glutamine becomes conditionally essential in the critically ill, yet such patients frequently have a marked deficiency of glutamine; the reasons for this are still unclear. Glutamine is required by the cells of the immune system both as a primary fuel and as a carbon and nitrogen donor for nucleotide precursor synthesis. *In vivo* studies have demonstrated that glutamine is essential for optimal immune cell functioning for monocytes, lymphocytes and neutrophils. A number of trials of patients fed by the enteral or parenteral route have shown improved infectious morbidity when supplemented with glutamine. However, the exact mechanism of glutamine action in these patients remains to be determined.

Glutamine: Immune system: Critical illness: Sepsis

Introduction

Following critical illness, sepsis remains a major cause of morbidity and mortality. Previously, the pathogenesis of sepsis and the systemic inflammatory syndrome (SIRS) was explained in terms of persistent, uncontrolled inflammation. However it is becoming increasingly apparent that counter regulatory mechanisms exist and this has been called the compensatory anti-inflammatory response syndrome (CARS). The relative magnitude of these responses determines the patient's response to a critical illness (Bone, 1996). Evidence is now accumulating to suggest that critically ill patients may be predisposed to infectious complications because of impaired function of the immune system resulting from CARS predominance, and this impairment may occur very early on in the illness. It is now established that specific nutritional supplementation can influence the immune response, a concept known as immunonutrition and much of this work has centred on the provision of the amino acid glutamine. Glutamine is the most abundant amino acid in the body and has the highest plasma concentration (typically 0·6 mmol/l) of all the amino acids. It comprises about half the total free amino acid pool in skeletal muscle and under normal conditions is regarded as a non-essential amino acid as it can be produced in sufficient amounts to meet the body's needs (van Acker *et al.* 1999). Glutamine provides a source of energy through its partial oxidation in a process known as glutaminolysis (McKeehan, 1982) and also provides carbon and nitrogen for precursors of nucleotide synthesis; it is also a precursor of intracellular glutathione, hepatic glucose and urinary ammonia. During times of catabolic stress such as critical illness however, it becomes conditionally essential as a fuel for the cells of the immune system and the gastrointestinal system (Lacey & Wilmore, 1990; Wernerman & Hammarqvist, 1999). Deficiencies of glutamine supply seen in critical illness states are associated with impairment of the immune system and increased susceptibility to infection.

Glutamine deficiency in the critically ill

During critical illness, glutamine has been considered to be a 'conditionally essential' amino acid, with an increase in uptake in the kidneys, immune cells and intestinal mucosa. However, this increased demand is not met in severe illness, and such patients show significantly decreased plasma levels of glutamine. Plasma glutamine has been shown to be significantly decreased in patients with burns (Stinnett *et al.*

Abbreviations: BCG, Bacillus Calmette-Guerin; CARS, compensatory anti-inflammatory response syndrome; EN, enteral nutrition; GSH, glutathione; HSP, heat shock proteins; IFNγ, interferon gamma; IL, interleukin; LPS, lipopolysaccharide; PHA, phytohaemagglutinin; ROI, reactive oxygen intermediates; ROS, reactive oxygen species; SIRS, systemic inflammatory syndrome; Th, T-helper; TPN, total parenteral nutrition.

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1982; Parry-Billings *et al.* 1990), major trauma (Askanazi *et al.* 1980) and in a heterogeneous cohort of critically ill patients (Griffiths *et al.* 1997). Skeletal muscle is the main source of glutamine and there is a dramatic decline in muscle free glutamine levels in critical illness (Askanazi *et al.* 1980). It has been demonstrated by Mittendorfer *et al.* (1999) that the depletion of intramuscular glutamine in critically ill patients with burn injuries is not due to synthesis impairment or decreased appearance from protein breakdown but rather due to accelerated export from muscle cells. However there is evidence that the net intramuscular *de novo* synthesis of glutamine (the balance between glutamine synthesis and degradation) may be limited by a reduced availability of intramuscular glutamate as a precursor (Biolo *et al.* 2000). Increased transamination of glutamate to form alanine occurs and it is speculated that this is linked to the need to clear excess pyruvate. Addition of glutamine to total parenteral nutrition after abdominal surgery is associated with a significantly smaller fall in intracellular muscle glutamine than in control patients who received TPN (Hammarqvist *et al.* 1989).

Impaired immune cell function in the critically ill

Monocytes and macrophages

Monocytes are precursor cells for tissue macrophages and both are of central importance in the initiation, development and outcome of the immune response. Expression of class II major histocompatability complex antigens (HLA-DR and related antigens) is required for monocytes to present an antigen to lymphocytes and act as accessory cells. HLA-DR is also a marker of monocyte activation and thus the ability to phagocytose opsonized organisms. The monocytes of patients with septic shock have significantly less HLA-DR expression compared with those from normal subjects (Lin *et al.* 1993). It has been accepted until recently that patients with sepsis show a biphasic immunological pattern in which the early hyperinflammatory phase is counterbalanced by an anti-inflammatory response which may lead to a later hypoinflammatory state, characterised by monocyte deactivation (Kox *et al.* 2000). An important concept however is that many critically ill patients following trauma or major surgery have reduced HLA-DR expression on monocytes very early during the course of their illness (Wakefield *et al.* 1993; Ditschkowski *et al.* 1999; Giannoudis *et al.* 1999). Recently, immunoparalysis of monocytes has also been described for other groups of intensive care patients (Peters *et al.* 1999). Hershman *et al.* 1990 have shown that the downregulation of HLA-DR correlates with clinical outcome and sepsis in such patients. The reason for monocyte deactivation in CARS is not fully understood but there is increasing evidence that interleukin 10 (IL-10) plays a central role. Giannoudis *et al.* (2000) have demonstrated that immediate IL-10 expression following trauma regulates HLA-DR expression and is associated with sepsis development. Recovery of the monocyte function of patients destined to develop sepsis was markedly delayed compared with control subjects in this study.

Lymphocytes

In experimental models and in critically ill patients, various deficiencies of lymphocyte function have been described. These include depletion in total circulating lymphocyte numbers and particularly CD4+ lymphocytes, and a lowered proliferative response to mitogenic stimuli (Levy *et al.* 1984; Lin *et al.* 1993). CD4+ cells can differentiate into two types of effector cells and the pattern of circulating cytokines in critical illness determines CD4 Th (T-helper) lymphocyte subset predominance (Mossmann & Coffman, 1989). Th1 cells produce IL-2 and interferon gamma (IFNγ) favouring cell mediated immunity whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10h or IL-13, favouring humoral immunity. The Th2 antibody mediated immune response predominates in those patients with sepsis and it has been suggested that this may be due to markedly increased circulating concentrations of IL-10 (Sherry *et al.* 1996). A study by O'Sullivan *et al.* (1995) showed that patients with trauma and burns had increased levels of IL-4 and IL-10, suggesting a predominantly Th2 response, together with decreased levels of IL-12, a Th1-promoting cytokine. Ferguson *et al.* (1999) showed that the median ratio of Th1/Th2 ratio was 0·46 in patients with sepsis, and significantly lower than 2·5 in non-septic control patients. The decrease in the Th1/Th2 ratio and consequent Th2 cytokine pattern dominance is associated with decreased resistance to infection (Neidhardt *et al.* 1997).

Neutrophils

Polymorphonuclear neutrophils have both regulatory and phagocytic functions. These cells synthesise and export cytokines and inflammatory mediators and also operate in the first line of defence against invading organisms. Excessive activation of neutrophils and consequent free radical release leads to organ dysfunction via tissue and endothelial cell damage (Fujishima & Aikawa, 1995). However there is also evidence for neutrophil dysfunction in various forms during critical illness. Expression of the surface marker CD11b on neutrophils is decreased in patients who die of sepsis compared with controls (Muller Kobold *et al.* 2000), up-regulated CD11b being regarded as a marker of neutrophil activation. Release of the proinflammatory cytokine IL-8 from neutrophils in septic patients is significantly reduced when stimulated *in vitro* with lipopolysaccharide (LPS), when compared with neutrophils from healthy volunteers (Marie *et al.* 1998). Superoxide production by neutrophils has been shown to be reduced in critically ill patients with sepsis (Tschaikowsky *et al.* 1993). Simms & D'Amico (1997) have also shown that the development of nosocomial infection on a surgical intensive care unit is associated with auto-oxidative injury to neutrophils (measured as a reduction in opsonic receptor expression induced by neutrophils themselves). Despite these observations, the exact prevalence of neutrophil dysfunction in critical illness is less well defined than for monocytes and lymphocytes.

Glutamine and the immune system

Dependence of immune cells on glutamine

A striking feature of the cells of the immune system is that despite their differing function and cell biology, all are dependent on glutamine in a similar manner (Newsholme *et al*. 1999). All of these cells show high rates of glutamine consumption in culture; most of the glutamine is not fully oxidised (for energy) but converted to glutamate, lactate and aspartate. The high rates of glutamine utilisation are in excess of the needs for energy and nucleotide precursors and it has been hypothesised that the high rate of glutaminolysis provides for precision in regulating changes in the rate of synthesis of nucleotides (Newsholme *et al*. 1985). In addition, optimal phagocytic and secretory activity of immune cells may be dependent on adequate glutamine.

Monocyte and macrophage function

Monocytes and macrophages play an important role in the innate immune system response as a non-specific, first line defence and they are able to ingest and eliminate infectious agents. Macrophages are terminally differentiated cells derived from circulating blood monocytes but are highly active and characterised by high rates of protein secretion, however they are unable to synthesise glutamine themselves. Very high rates of glutamine utilisation occur in macrophages as shown by Newsholme *et al.* (1987). Studies by Parry-Billings *et al.* (1990) with mouse peritoneal macrophages showed that rates of phagocytosis declined at all glutamine concentrations *in vitro* below 0·6 mmol/l and that human lymphocyte proliferation was similarly reduced with decreasing glutamine concentrations. Wallace & Keast (1992) further examined the role of glutamine in adequate monocyte function using thioglycollate-elicited peritoneal macrophages from mice. A decrease in culture medium glutamine from 0·5 mmol/l to 0·125 mmol/l was accompanied by a 25 % decrease in RNA synthesis as measured by ^{3}H-uridine incorporation, demonstrating the importance of adequate glutamine for nucleotide synthesis. IL-1 secretion from macrophages was also dependent on the presence of glutamine in the media, but only 0·03 mmol/l was required to restore sufficient IL-1 secretion. Blood monocyte-derived macrophage antigen expression and function in healthy volunteers is influenced *in vitro* by glutamine (Spittler *et al.* 1995). Monocyte culture in glutamine concentration of less than 0·6 mmol/l was associated with a significant decrease in cellular ATP levels and lowering the glutamine concentration from 2 mmol/l to 0·2 mmol/l reduced monocyte HLA-DR expression by 40 % and decreased tetanus toxoid-induced antigen presentation.

Lymphocyte function

Glutamine acts as both a precursor of nucleotide synthesis and a primary fuel for lymphocytes, and high rates of utilisation occur even in resting lymphocytes (Ardawi, 1988). Chang *et al.* (1999*a*) have demonstrated that glutamine supplementation significantly enhanced phytohaemagglutinin (PHA)-stimulated lymphocyte proliferation; the production of both intracellular Reactive Oxygen Species (ROS) and glutathione (GSH) were also enhanced following glutamine supplementation. O'Riordain *et al.* (1994) showed that T-cell DNA synthesis was increased in post-operative patients who received glutamine-supplemented total parenteral nutrition (TPN), but in this study, other lymphocyte functions and markers were not examined. Studies by Chang *et al.* (1999*b*) have shown that the addition of glutamine to lymphocytes *in vitro* in glutamine free media stimulated with live attenuated bacillus Calmette-Guerin (BCG) favours a Th1 response shown by the production of IFNγ. Further evidence that glutamine is important in facilitating the IL-2 response has come from studies of mice fed with glutamine-supplemented diets (Kew *et al.* 1999). Mice receiving supplemental glutamine showed significantly greater IL-2 production from concanavalin-stimulated lymphocytes compared with mice fed control diets. There was no significant increase in IL-4 production in cells from mice supplemented with glutamine.

Neutrophil function

The importance of glutamine for optimal neutrophil functioning is less well defined compared with what is known about the requirements for optimal function of lymphocytes and macrophages. Neutrophils play a pivotal role in host defence by phagocytosing and destroying invading bacteria. Although the energy substrate for neutrophils is glucose it is suggested by Vlessis *et al.* (1995) that neutrophils are able to utilise glutamine when glucose is restricted. A study of the effect of glutamine on neutrophil function in neutrophils from paediatric burn patients by Ogle *et al.* (1994) showed evidence of improved bactericidal function following glutamine supplementation *in vitro.* At glutamine concentrations of 1 mmol/l and above, bactericidal function was significantly enhanced when the killing of *Staphylococcus aureus* (*S. aureus*) was examined. However, no net uptake of glutamine by the cells could be detected. This paper also claimed that glutamine did not affect phagocytic activity or C3b receptors, but no conclusion can be drawn because of the very small sample size of patients used for this aspect of the study. Pithon Curi *et al.* (1997) have shown that rat neutrophils grown in isolated culture utilise glutamine at a higher rate than glucose. Furukawa *et al.* (1997) investigated the role of glutamine in the killing of *Escherichia coli* (*E. coli*) *by* neutrophils from postoperative patients. Neutrophils were cultured with opsonized *E. coli* in media supplemented with different concentrations of glutamine. The number of viable *E. coli* decreased by 26 % when media was supplemented with 1 mmol/l glutamine compared with media supplemented with 0·5 mmol/l glutamine. However, different concentrations of *in vitro* glutamine supplementation caused no significant changes in the number of viable *E. coli* when cultured with neutrophils from healthy volunteers. The plasma concentrations of glutamine were significantly lower in patients than in controls.

A further study by Furukawa *et al.* (2000) examined the role of glutamine in the production of reactive oxygen intermediates (ROI) and phagocytosis *in vitro* by neutrophils from postoperative patients. These patients were found

to have significantly decreased serum glutamine levels. Using a washed whole blood culture technique, they found that neutrophil ROI production and phagocytosis of fluorescent beads was significantly greater in the presence of 2 mmol/l glutamine than with lower or no glutamine supplementation. This study could be criticised on the grounds that no glucose at all was available to neutrophils once in culture, unlike the *in vivo* situation. A recent finding of interest is the effect of decreased plasma glutamine on the expression of heat shock proteins (HSP) in neutrophils. The heat shock response in inflammatory cells is supposed to provide self-protection of immune cells against ROI. Weingartmann *et al.* (1999) showed that compared to neutrophils from healthy volunteers, neutrophils in patients with polytrauma showed no HSP70 expression, in association with decreased plasma levels of glutamine. In lymphocytes of both patients and controls however, HSP70 expression was conserved.

Effects of glutamine supplementation in the critically ill

A number of randomised, double blind trials have demonstrated the efficacy of glutamine in reducing the infectious morbidity of patients with critical illness. Most of these studies have concentrated on glutamine supplementation to TPN, but some recent studies have also looked at glutamine-supplemented enteral nutrition (EN). Ziegler *et al.* (1992) demonstrated that the addition of glutamine to TPN initiated early following bone marrow transplantation reduced infection rates and microbial colonisation, associated with a decrease in the length of hospital stay. Griffiths *et al.* (1997) reported a significantly increased 6-month survival rate in patients who received glutamine-supplemented TPN compared with conventional TPN. Over three-quarters of the patients in this study were suffering from major sepsis on admission. Subsequent analysis showed that the excess mortality in the control patients was described by those fed for more than 5 days, who had progressively more and repeated acquired intensive infections yet encountered the same risk with a similar duration of parenteral nutrition and stay on ICU. Consistent with a possible cell mediated impaired T-lymphocyte function, control patients not given glutamine had more fungal infections and higher mortality (Griffiths *et al.* 2000). Following major abdominal surgery, Morlion *et al.* (1998) randomised patients to TPN with or without glutamine supplementation. They showed that following surgery, by day 3 the total lymphocyte count remained at normal levels in the patients receiving glutamine supplementation but declined in the control group. In addition, neutrophil function as assessed by the generation of cysteinyl-leukotrienes (potent lipid mediators containing all or part of the GSH molecule) was only maintained in those patients receiving glutamine.

Trials of enteral supplementation of glutamine have also been performed. Neu *et al.* (1997) studied enteral glutamine supplementation in very low birth weight infants. The difference in sepsis between the two groups was significant when birth weight was taken into account using a logistic regression analysis, with sepsis rates of 10 % for glutamine-supplemented infants and 30 % for control infants. Houdijk *et al.* (1998) conducted a randomised trial of glutamine-enriched EN on patients with multiple-trauma, using infectious morbidity during the first 15 days as a primary endpoint. Those patients randomised to glutamine showed a significant decrease in the incidence of pneumonia, bacteraemia and sepsis, and significantly higher levels of plasma glutamine than in control patients. Measurement of the soluble TNF (Tumour Necrosis Factor) receptors p55 and p75 as markers of a systemic inflammatory response demonstrated that patients with glutamine supplementation had a lower systemic inflammatory response than in control patients. However it is notable that despite the placing of nasoduodenal tubes for enteral feeding, maximum plasma glutamine levels were not achieved until day 5 even in the glutamine supplementation group. The same group also analysed endocrine and metabolic mediators from this study and found that the reduction in infectious morbidity could not be explained by modulation of the humoral stress response (Houdijk *et al.* 1999).

Conclusion

Recent evidence has accumulated to suggest that some critically ill patients may be at increased risk of infection because of impaired immune function early in the course of their illness. Studies have demonstrated that there is good evidence for monocyte deactivation and increasing evidence for lymphocyte and neutrophil dysfunction. Many of these functions have been shown to be dependent on the presence of glutamine, which is often deficient in supply in the critically ill. It would therefore seem logical that consideration should be given to giving glutamine very early on in the course of a critical illness to firstly remedy any immune system dysfunction and secondarily to prevent any further deactivation of immune cells. Despite this impressive evidence for glutamine supplementation in the critically ill, none of these trials have investigated whether it is a single or combination of mechanisms by which glutamine has exerted its effect, and further studies are needed to clarify these mechanisms.

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