Why Is L-Glutamine Metabolism Important to Cells of the Immune System in Health, Postinjury, Surgery or Infection?[1,2]

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ABSTRACT Glutamine is normally considered to be a nonessential amino acid. However, recent studies have provided evidence that glutamine may become "conditionally essential" during inflammatory conditions such as infection and injury. It is now well documented that under appropriate conditions, glutamine is essential for cell proliferation, that it can act as a respiratory fuel and that it can enhance the function of stimulated immune cells. Studies thus far have determined the effect of extracellular glutamine concentration on lymphocyte proliferation and cytokine production, macrophage phagocytic plus secretory activities and neutrophil bacterial killing. Other cells of the immune system remain to be studied. The high rate of glutamine utilization and its importance to the function of lymphocytes, macrophages and neutrophils have raised the question "why glutamine?" because these cells have access to a variety of metabolic fuels both in vivo and in vitro. I have attempted to answer this question in this article. Additionally, knowledge of the rate of utilization and the pathway of metabolism of glutamine by cells of the immune system raises some intriguing questions concerning therapeutic manipulation of utilization of this amino acid such that the proliferative, phagocytic and secretory capacities of cells of the defense system may be beneficially altered. Evidence to support the hypothesis that glutamine is beneficially immunomodulatory in animal models of infection and trauma, as well as trauma in humans, is provided. J. Nutr. 131: 2515S–2522S, 2001.

KEY WORDS: • *glutamine* • *metabolism* • *immune system* • *infection* • *trauma*

Lymphocytes, macrophages and neutrophils play important roles in the immune and inflammatory response. Mature lymphocytes recirculate via blood and lymph through lymphoid tissues in a relatively quiescent state until stimulated to proliferate during, for example, a bacterial or viral infection. T-Lymphocytes are required to stimulate macrophage and B-lymphocyte activities mainly via production of regulatory cytokines. B-Lymphocytes produce and secrete antibodies in response to antigenic stimuli. By contrast, macrophages are terminally differentiated end-cells in which the ability to proliferate is gradually lost. They originate, as do all cells of the immune system, in the bone marrow and enter the blood as immature macrophages, termed monocytes. Monocytes enter the tissues and serous cavities of the body where they mature into macrophages and subsequently phagocytose foreign material and apoptosing host cells, they present antigen at the cell surface in association with major histocompatibility complex II (MHC II),[3] and they secrete inflammatory cytokines and free radicals when stimulated to do so. Neutrophils constitute 60% of the circulating leukocytes. They act as first-line-of-defense cells in the blood and at sites of infection. Their function is to remove and destroy foreign material by phagocytosis either alone or in cooperation with antigen-specific defenses. They digest and dismantle the phagocytosed material by production of superoxide and exposure to hydrolytic enzymes in specialized "phagolysosomes." They differ from macrophages in that they die via apoptosis after digesting foreign antigen and they cannot present antigen.

In recent years, the molecular biology of these cells and the process of chemical communication among them has attracted considerable interest, and much progress has been made in our understanding of some regulatory aspects of the immune system. This system is of fundamental importance not only in preventing or limiting infection, but also in the overall process of repair and recovery from injury. It is therefore of importance in clinical conditions of trauma, sepsis, burns and recovery from surgery.

Despite the undoubted importance of the cells of the immune system, it was surprising that until recently, relatively little was known about their metabolism, the fuels they require

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[3] Abbreviations used: BCG, bacillus Calmette-Guerin; iNOS, inducible nitric oxide synthase; LAK cells, lymphokine-activated killer cells; LPS, lipopolysaccharide; MHC, major histocompatibility complex; TCA, tricarboxylic acid.

TABLE 1

Rates of utilization of glucose or glutamine and of production of lactate, glutamate, aspartate and $^{14}CO_2$ by isolated incubated mouse macrophages, rat lymphocytes or rat neutrophils[1]

Addition to incubation medium	Glucose	Glutamine	Lactate	Glutamate	Aspartate	$^{14}CO_2$ production
			nmol/(h · mg protein)			
Mouse macrophages						
Glucose	−355[2]	—	632	—	—	11
Glutamine	—	−186	33	137	25	9
Rat lymphocytes						
Glucose	−42	—	91	—	—	1.5
Glutamine	—	−223	9	132	59	6.1
Rat neutrophils						
Glucose	−460	—	550	—	—	2.4
Glutamine	—	−770	320	250	68	6.5

[1] Data from Ardawi and Newsholme (1983), Newsholme, P. et al. (1987) and Pithon-Curi et al. (1997).
[2] Negative sign indicates utilization.

to carry out their functions, the rates of utilization and fates of these fuels and any implications for the overall metabolic homeostasis of the animal. Indeed, it was not until the pioneering work of Eric Newsholme's laboratory in the early/mid 1980s that it was established that immune cells such as lymphocytes and macrophages could utilize glutamine at high rates in addition to glucose (Ardawi and Newsholme 1983, Curi et al. 1986, Newsholme, P. et al. 1986 and 1987). It was generally thought at that time that glutamine was only a quantitatively important fuel for cells of the intestine, liver and some tumors. The importance of glutamine metabolism for immune cell function has recently become apparent and is discussed in detail in this review.

Glutamine metabolism in isolated cells

The importance of glutamine to cell survival and proliferation in vitro was first reported by Ehrensvard et al. (1949) but was more fully described by Eagle et al. (1956). Glutamine had to be present at 10- to 100-fold in excess of any other amino acid in culture and could not be replaced by glutamic acid or glucose. This work led to the development of the first tissue culture medium that contained essential growth factors, glucose, 19 essential and nonessential amino acids at approximately physiologic concentrations and a high concentration of glutamine (2 mmol).

It is now clear that glutamine is utilized at high rates by isolated cells of the immune system such as lymphocytes, macrophages and neutrophils [**Table 1**; also see Newsholme et al. (1999), Calder and Yaqoob (1999) and Wilmore and Shabert (1998) for reviews]. Although the activity of the first enzyme responsible for the metabolism of glutamine, glutaminase, is high in these cells, the rate of oxidation is low. Much of the glutamine is converted to glutamate, aspartate [via tricarboxylic acid (TCA) cycle activity], lactate and under appropriate conditions CO_2 (Table 1).

The importance of glutamine to the function of immune cells in vitro.

T-Lymphocytes. The major functions of T-lymphocytes in vivo are to proliferate in response to antigenic stimuli, to produce cytokines essential to the propagation of the immune response and to up-regulate specific cytokine receptors on the T-cell surface, which will further enhance rates of proliferation. The concentration of extracellular glutamine appears to regulate T-lymphocyte proliferation **(Fig. 1)**, the rate of interleukin (IL)-2 production and IL-2 receptor expression **(Table 2)**.

B-Lymphocytes. The differentiation of B-lymphocytes into antibody synthesizing and secreting cells is glutamine dependent and increases significantly over a range of physiologic glutamine concentrations (Crawford and Cohen 1995). This effect could not be mimicked by a variety of other amino acids.

Lymphokine-activated killer cells (LAK cells). Glutamine has been reported to support the potential of LAK cells to kill target cells (Juretic et al. 1994). These authors concluded that glutamine deficiency limited the number of activated cells generated in response to a stimulus.

Macrophages. In contrast to lymphocytes, which have the potential for rapid division, macrophages are terminally differentiated cells that have lost their ability to divide. However, they are metabolically active cells that have high rates of

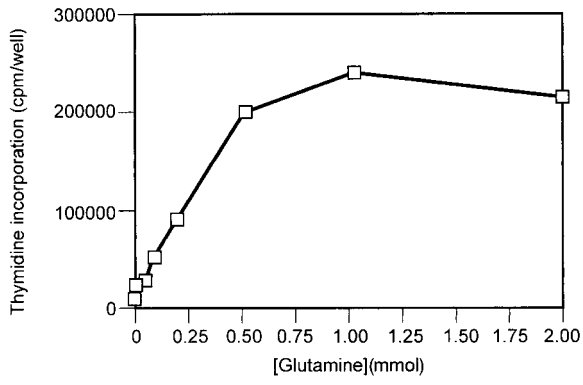

FIGURE 1 Lymphocyte proliferation is dependent on extracellular glutamine concentration. Lymphocytes obtained from rat spleens were incubated in vitro in RPMI medium containing antibiotics. They were exposed to the T-cell mitogen concanavalin A at the start of the incubation in medium containing various extracellular glutamine concentrations. Proliferation is expressed as increase in radioactively labeled thymidine incorporation into DNA during the last 18 h of a 66-h incubation. [Adapted from Yaqoob and Calder (1997).]

TABLE 2

The effect of glutamine on T-lymphocyte function

Source of lymphocytes	Authors	Effect of increasing extracellular glutamine concentration
Rats	Ardawi and Newsholme (1983) Szondy and Newsholme (1989)	Increase in rate of proliferation in response to a mitogenic stimulus
Mice	Griffiths and Keast (1990) Yaqoob and Calder (1997)	
Humans	Parry-Billings et al. (1990) Chuang et al. (1990)	
Rats	Calder and Newsholme (1992)	Increase in interleukin (IL)-2 production in response to mitogenic stimulus
Mice	Yaqoob and Calder (1997)	
Humans	Rhode et al. (1996) Yaqoob and Calder (1998)	
Rats	Yaqoob and Calder (1997)	Increase in IL-2 receptor expression

phagocytosis, pinocytosis, protein secretion, free radical secretion (superoxide, nitric oxide), eicosanoid production and membrane recycling and synthesis. All of these processes are linked to the overall function of the macrophage, which is to destroy foreign material via exposure to free radicals and hydrolytic enzymes, antigen presentation to T-lymphocytes (in association with MHC II molecules) and activation of lymphocyte subpopulations via cytokine secretion. It has been demonstrated that when the extracellular glutamine concentration was reduced from 2 to 0.2 mmol, MHC class II expression was reduced by 40% and a decreased level of tetanus toxoid–induced antigen presentation was observed (Spittler et al. 1995). In addition phagocytosis of complement opsinized *Escherichia coli* was decreased in the low glutamine environment (Spittler et al. 1995). Glutamine availability has also been reported to influence the phagocytic uptake of unopsinized yeast cell walls (Parry-Billings et al. 1990a) and of opsinized sheep red blood cells (Wallace and Keast 1992) by mouse macrophages.

Macrophages can be found in various states of activation in vivo. However, macrophages obtained from experimental animals or matured from human moncytes may be specifically stimulated to produce a fairly homogenous population. For example, intraperitoneal injection of bacillus Calmette-Guerin (BCG)-vaccine into mice will lead 4–7 d later to a peritoneal population of *activated* macrophages. These cells are characterized by their enhanced free radical and cytokine secretory activity, ruffling of their membranes, phagocytic capacity and increased metabolism compared with the resident peritoneal cavity macrophage population, which has received no equivalent stimuli. The rate of glutamine utilization of these different macrophage populations had not been determined until relatively recently (Murphy and Newsholme 1998). It is now widely accepted that macrophage activation in vivo (via exposure to BCG) or in vitro [via exposure to lipopolysaccharide (LPS)] leads to a significant increase in glutamine utilization **(Table 3)**.One possible explanation for enhanced rates of glutamine utilization is to satisfy the large demand for arginine by activated macrophages. Macrophages are unable to utilize extracellular arginine after activation because the enzyme arginase is rapidly secreted from the cells under these conditions (Murphy and Newsholme 1998), which subsequently depletes the extracellular arginine concentration. Arginine is the immediate precursor of nitric oxide synthesis catalyzed by the enzyme inducible nitric oxide synthase (iNOS) in the macrophage. The secretion of arginase,

TABLE 3

Glutamine utilization rates in resident and activated murine macrophages[1,2,3]

	Resident macrophage		Activated macrophage	
	Time of preincubation (h)			
Conditions	24	48	24	48
	nmol glutamine consumed/(h · mg protein)			
5 mmol glucose + Arginine				
Control	20.7 ± 0.3	37.9 ± 4.2	73.8 ± 9.4	95.5 ± 12.7
+LPS	26.7 ± 3.0†	71.0 ± 9.3†	100.4 ± 15.0	118.1 ± 23.5
5 mmol glucose − Arginine				
Control	56.1 ± 6.1	104.6 ± 10.2	126.8 ± 18.6	121.1 ± 4.0
+LPS	74.1 ± 6.4*	127.5 ± 15.9	168.7 ± 26.4	136.6 ± 5.1*

1 Cells were isolated and purified via adherence to tissue culture plastic as previously described. After purification, cells were preincubated for 24 or 48 h at 37°C, in 95% air and 5% CO_2, in selected tissue culture media supplemented with a combination of 2 mmol glutamine with 5 mmol glucose and with or without 0.4 mmol L-arginine. After preincubation, the media were replaced and the cells were incubated for 1 h under the conditions described in the table in fresh media; glutamine utilization was determined and was linear over this period.

2 *P* values, where indicated, refer to the statistical difference between glutamine utilization in the presence of 15 μg/mL lipopolysaccharide (LPS) compared with its absence. * $P < 0.05$; † $P < 0.01$. Values are expressed as means ± SEM, of three or more incubations.

3 Data from Murphy and Newsholme (1998).

coupled with enhanced rates of intracellular arginine synthesis from glutamine (Murphy and Newsholme 1998, Newsholme et al. 1999), provides the conditions in which nitric oxide synthesis can occur in cells that normally have a large capacity to produce urea. The same metabolic pathway required for glutamine conversion to arginine in the mouse macrophage also exists in human monocytes (Murphy and Newsholme 1998). Nitric oxide levels determined via the stable oxidative end product, nitrite, reach significant levels after cell culture in the absence of arginine for 48 h **(Table 4)**, coincidentally the same time required to reach maximal rates of glutamine utilization (Table 3).

Wallace and Keast (1992) demonstrated that murine macrophages stimulated with LPS secreted increasing amounts of IL-1β as the availability of extracellular glutamine increased. More recently Yassad et al. (1997) and Murphy and Newsholme (1999) demonstrated that enhancement of IL-6 and tumor necrosis factor (TNF)-α secretion, respectively, by LPS-stimulated macrophages, was dependent upon extracellular glutamine availability. TNF-α, IL-1β and IL-6 are quantitatively the most important cytokines produced by LPS-stimulated macrophages. Murphy and Newsholme (1999) also demonstrated that in addition to murine macrophage TNF-α production, the production of the quantitatively important human monocyte-derived cytokine, IL-8, was also dependent upon the availability of extracellular glutamine **(Fig. 2)**.

Macrophage ATP generation and O_2 consumption rates. Macrophages are known to have a large oxidative capacity and their O_2 consumption rates [515 nmol/(h · mg protein)] are similar to those of sheep heart [696 nmol/(h · mg protein)] and rat liver [520 nmol/(h · mg protein)] in vitro as calculated by Newsholme (1987) using original data from Krebs, Johnson and Karnovsky. Additionally, Newsholme (1987) calculated

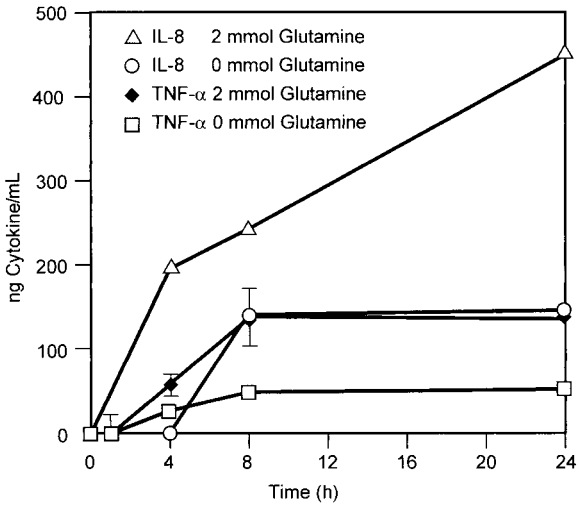

FIGURE 2 Tumor necrosis factor (TNF)-α and interleukin (IL)-8 production from murine macrophages and human monocytes, respectively, after incubation in the presence or absence of L-glutamine and lipopolysaccharide (LPS). Bacillus Calmette-Guerin (BCG)-activated murine macrophages were isolated and purified as described in Murphy and Newsholme (1999). After purification via adherence, the cells were incubated for up to 24 h in MEM tissue culture medium in the presence or absence of 2 mmol glutamine and presence or absence of 15 μg/mL bacterial LPS. TNF-α production was determined using a DuoSet immunosorbant ELISA test kit as described by Murphy and Newsholme (1999). Cytokine concentration is expressed as ng/mL, which was adjusted to reflect the amount produced from 1 mg cell protein (after cell protein assay).

TABLE 4

Nitrite production in resident and BCG-activated murine macrophages

	Resident macrophage		Activated macrophage	
Conditions	24 h	48 h	24 h	48 h
	nmol nitrate/(h · mg protein)			
0 mmol L-Arginine				
Control	0.0 ± 0.0	4.8 ± 0.8	0.0 ± 0.0	8.4 ± 1.2
+LPS	0.0 ± 0.0	6.6 ± 0.7†	0.0 ± 0.0	11.4 ± 1.1*
0.06 mmol L-Arginine				
Control	8.1 ± 0.8	8.8 ± 1.4	16.3 ± 1.2	16.5 ± 1.2
+LPS	11.0 ± 1.1*	10.4 ± 0.9	21.9 ± 2.9*	18.7 ± 2.8
0.4 mmol L-Arginine				
Control	14.2 ± 3.5	13.8 ± 1.2	36.5 ± 3.1	46.3 ± 2.3
+LPS	22.3 ± 1.7†	15.1 ± 0.7*	42.5 ± 0.7†	47.4 ± 0.9

[1] Peritoneal murine macrophages were isolated and purified as previously described. After purification via adherence to tissue culture plastic, cells were cultured for 4, 24 or 48 h at 37°C, in 95% air and 5% CO_2 in tissue culture media supplemented with 2 mmol glutamine and 5 mmol glucose in the absence or presence of varying concentrations of L-arginine. After preincubation, the media were replaced and the cells were incubated for 1 h under the conditions described below after which nitrite production was determined. Nitrite production was linear over this period.

[2] *P*-values, where indicated, refer to the statistical difference between glutamine utilization in the presence of 15 μg/mL lipopolysaccharide LPS compared with its absence. * $P < 0.05$; † $P < 0.01$. Values are expressed as means ± SEM of three or more incubations.

[3] Data from Murphy and Newsholme (1998).

ATP generation rates for isolated and incubated macrophages in vitro and cultured macrophages, taking into account oxygen utilized by the NADPH oxidase of these cells. The ATP generation rate in the presence of both glucose and glutamine was 930 nmol/(h · mg protein), based on known pathways of metabolism. Glucose contributed 62% and glutamine 38% to the energy requirement of the cell. Because the ATP concentration of the macrophage is ~7 nmol/mg protein (Newsholme, P. et al. 1987), then the total ATP concentration of the cell must have been turned over at least 2 times/min. The macrophage, when studied over longer periods (82 h of culture), had a similar dependency on these fuels in which glucose contributed 68% and glutamine 32% to the energy needs of the cell. The major difference in metabolism between freshly isolated cells and cultured cells is that a greater proportion of glutamine carbon is fully oxidized in culture; thus the overall rate of glutamine utilization is lower [55 nmol/(h · mg protein)].

Neutrophils and monocytes. A recent study by Furukawa et al. (2000) demonstrated that neutrophils and monocytes obtained from patients over a 7-d period after gastrointestinal operations respond to glutamine addition to cell culture medium by increasing their phagocytic activity and rate of production of superoxide (a key free radical required for bacterial killing) in a dose-dependent manner. The degree of improved neutrophil and monocyte phagocytosis and superoxide production with glutamine addition was positively correlated with severity of inflammatory stress and more severe plasma glutamine depletion in vivo (see later). The work by Furukawa et al. (2000) complements a previous study (Saito et al. 1999) in which it was shown that bacterial killing by neutrophils from postoperative patients is enhanced by glutamine in vitro. Neutrophils were cultured in either 0.5 or 1.0 mmol glu-

tamine. The number of viable *E. coli* decreased by 26% as the extracellular glutamine concentration was increased.

A recent study by Garcia et al. (1998) may throw some light on the mechanism by which glutamine may provide increased antimicrobial activity in neutrophils. They demonstrated that 2 mmol extracellular glutamine was able to attenuate the adrenaline-induced inhibition of superoxide production in these cells (**Fig. 3**). In addition, Costa Rosa et al. (1995) demonstrated that glucose 6-phosphate dehydrogenase is inhibited in macrophages by adrenaline, but $NADP^+$-dependent malate dehydrogenase is activated under the same conditions, resulting in a situation in which a considerable proportion of NADPH generating capacity is provided via glutamine metabolism (see below). The adrenaline concentration in "stressed" postoperative patients (whose plasma glutamine concentration will be low) is likely to be high, thus inducing an inhibitory effect on neutrophil and monocyte superoxide generating capacity. Once incubated in 2 mmol glutamine in vitro, the superoxide generating capacity of the neutrophils is thus restored.

A rationale for high rates of glutamine utilization in lymphocytes, macrophages, and neutrophils

For many years, the question of the advantage of a high rate of glycolysis in tumor cells has been discussed (Newsholme, E. A. et al. 1987). A similar question can be raised concerning glutaminolysis, i.e., the pathway of incomplete glutamine oxidation that occurs at high rates in cells of the immune system. Glutamine carbon may be converted to glutamate and subsequently via the TCA cycle to malate. The action of $NADP^+$-dependent malate dehydrogenase [malic enzyme, which is present in lymphocytes, macrophages, monocytes and neutrophils (Newsholme et al. 1999)], converts malate and $NADP^+$ to pyruvate and NADPH, from which the "final" product of metabolism, lactate (in glutaminolysis) or CO_2, can be produced. I have outlined here that similar metabolic characteristics apply to various cells of the immune system despite the fact that their cell biology is different. Hence, any hypothesis must explain high rates of glutamine utilization in cells with widely different cell-biological characteristics. Glycolysis and glutaminolysis both provide metabolic intermediates for biosynthetic pathways [e.g., glycolysis provides glucose 6-phosphate for formation of ribose 5-phosphate and glycerol 3-phosphate for phospholipid synthesis; glutaminolysis provides glutamine (by increasing the availability of intracellular glutamine), ammonia and aspartate for purine and pyrimidine synthesis and ultimately for DNA and RNA synthesis]. Although the capacity for rapid cell division is retained by isolated lymphocytes, this does not apply to isolated neutrophils or macrophages, which are terminally differentiated cells with little capacity for cell division. However, neutrophils and macrophages have a large phagocytic capacity (requiring a high rate of lipid turnover and synthesis) and a large secretory activity, e.g., free radicals and cytokines. The mechanism by which glutamine can act to allow high rates of secretory product formation and release and sustain cell proliferation must account for the diverse nature of these secretory products and requirements for cell division and thus must contain at least one common metabolic product.

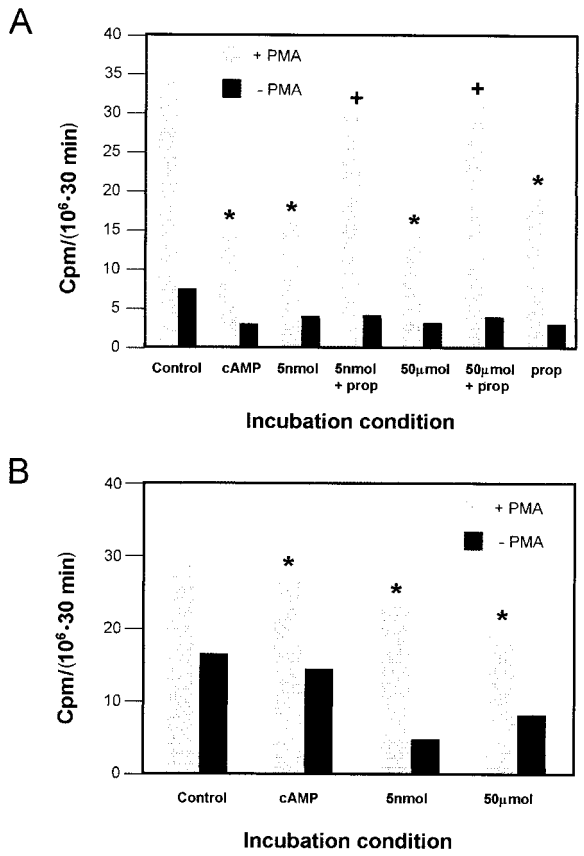

FIGURE 3 The inhibitory action of adrenaline on rat neutrophil superoxide production is attenuated by the presence of 2 mmol extracellular glutamine. Rat peritoneal neutrophils (3×10^6) were incubated in Krebs-Ringer buffer, pH 7.4, in the presence of 5 mmol glucose (*A*) or 2 mmol glutamine (*B*), lucigenin at 0.25 mmol, supplemented with adrenaline at 5 nmol/L or 50 μmol/L, dibutyryl cAMP at 100 μmol/L or propranolol (prop) at 200 μmol. Phorbol 12-myristate 13-acetate (PMA; 100 ng) was added as a stimulus. Chemiluminescence was directly proportional to superoxide production and was determined using a liquid scintillation counter. [Adapted from Garcia et al. (1999).]

In the formation of the reactive species, nitric oxide and superoxide, NADPH is required by the enzymes responsible for free radical production, iNOS and NADPH oxidase, respectively. NADPH is also required for the formation of new proteins, DNA or RNA. Glutamine, via catabolic metabolism involving $NADP^+$-dependent malate dehydrogenase [glutamine → glutamate → 2-oxoglutarate → malate → pyruvate; (Newsholme et al. 1996)] can thus generate considerable NADPH for cell requirements. The $NADP^+$-dependent malate dehydrogenase step will result in the formation of pyruvate, which can be converted either to lactate (ending the pathway of glutaminolysis) or to acetyl-CoA and thus CO_2. Thus, depending upon the energy demands placed on the cell, glutamine may be partially oxidized in the pathway of glutaminolysis or may be fully oxidized (at least in macrophages), but the outcome of metabolism in either case is NADPH production. Glucose may also generate NADPH, via metabolism through the pentose phosphate pathway. However, during periods of active pinocytosis and phagocytosis, glucose carbon may be diverted toward lipid synthesis, and therefore the pentose-phosphate pathway may be compromised (Newsholme et al. 1996). Additionally, glutamine carbon may be used for new amino acid synthesis in periods of active synthesis and secretion.

I am therefore making a case for NADPH as the "common factor" that links the diverse effects for which glutamine is responsible in cells of the immune system. Evidence in support of my hypothesis is provided by the beneficial effect of glutamine on superoxide generation in neutrophils and monocytes (Furukawa et al. 2000, Garcia et al. 1998, Saito et al. 1999) and recent in vitro data that cell proliferation in response to growth factors is positively related to the level of

superoxide produced intracellularly (Suh et al. 1999). Superoxide generation in cells requires the electron donating ability of NADPH if generated via the enzyme NADPH oxidase, which directly reduces molecular oxygen. The latter enzyme is quantitatively the most significant source of superoxide in immune cells.

The concept of "conditional deficiency"—muscle and glutamine production in stress

Under normal dietary conditions, very little of the glutamine derived from dietary protein enters the blood stream. The epithelial cells of the intestine will consume much of the dietary-derived glutamine and utilize it as a respiratory fuel. There are a number of tissues that may serve the role of glutamine producers for other organs and tissues of the body. The lung may produce glutamine in acute situations (Souba et al. 1990a) and the brain will synthesize glutamine for conversion to the neurotransmitter glutamate when required. However, skeletal muscle is able to produce glutamine in large amounts; it contains 90% of the whole-body glutamine pool (Darmaun et al. 1986) and can release glutamine in significant quantities [in catabolic stress in humans, glutamine comprised 26% of the released amino acids, (Wilmore and Shabert 1998)]. Glutamine synthetase in skeletal muscle is sensitive to regulation by glucocorticoids (Calder and Yaqoob 1999, Max et al. 1988), and the stress response will result in an increase in activity of this enzyme and the release of glutamine into the blood stream [also sensitive to glucocorticoid regulation (Parry-Billings et al. 1990b)]. TNF-α has also been shown to induce glutamine synthetase gene expression in cultured skeletal muscle cells (Chakrabarti 1998), thus providing a possible link between increased macrophage activity and glutamine utilization at sites of inflammation and increased amino acid metabolism in muscle. Release of glutamine exceeds synthesis in skeletal muscle in conditions of stress, resulting in the lowering of intracellular glutamine concentration, leading to enhanced rates of protein breakdown. However, plasma glutamine concentrations are also decreased in stress situations such as burns (Parry-Billings et al. 1990a) trauma (Jensen et al. 1996, Long et al. 1995), premature birth (Lacey et al. 1996) and sepsis (Ardawi 1991).

As has been described elsewhere in this review, glutamine is utilized at high rates by various immunologic tissues and cells. During inflammatory states, which occur in conditions such as sepsis and injury, the glutamine consumption in immunologic tissues and cells increases. This increase in glutamine consumption, coupled with enhanced utilization by other tissues, results in a demand for glutamine that outstrips supply. As a result, blood, immunologic tissue and muscle glutamine levels fall. The low concentrations of glutamine limit the function of key tissues and cells, especially cells of the immune system. It has been estimated that when plasma glutamine levels fall in a "glutamine-deficient" state, e.g., <0.4 mmol compared with "normal" levels of plasma glutamine, e.g., >0.6 mmol, then the immune system is compromised (Wilmore and Shabert 1998). If this hypothesis is correct, then providing exogenous glutamine to infected or stressed animals or humans and thus raising plasma concentrations to normal should enhance immunologic responses and improve outcome.

Glutamine supplementation, immune function and survival in animal models of infection and trauma

There have been several well-designed animal studies that have reported that glutamine can improve survival rates after infection. These studies were reviewed in detail previously (Calder and Yaqoob 1999, Wilmore and Shabert 1998); thus, I will provide only a brief summary of the findings here. Glutamine-supplemented parenteral nutrition improved survival (75 vs. 45% in the control, standard parenteral nutrition group) in rats after cecal ligation and puncture (Ardawi 1991). Glutamine improved nitrogen balance, attenuated the loss of glutamine from the skeletal muscle intracellular pool, enhanced muscle protein synthesis and decreased protein degradation. Delivery of glutamine-supplemented parenteral nutrition improved survival (92 vs. 55% in the control group) after intraperitoneal injection of live *E. coli* into rats (Inoue et al. 1993). In a different model of infection and glutamine supplementation, mice were fed diets containing casein or casein supplemented with 20 or 40 g glutamine/kg for 10 d and then inoculated intravenously with live *Staphylococcus aureus* (Suzuki et al. 1993). Mortality was assessed over the following 20 d in which the mice consumed the same diets. During this period, 20% of the mice consuming control diets survived, whereas survival increased to 40 and 70% of the mice in the 20 and 40 g/kg groups, respectively. However, immune function was not assessed in any of these studies.

There are a limited number of studies that have addressed the question of whether dietary glutamine enhances immune cell function in infected animals. Yoo et al. (1997) reported that proliferation of blood lymphocytes from *E. coli*–infected piglets was significantly higher if the piglets consumed a diet containing 40 g glutamine/kg compared with a diet that was not supplemented with glutamine. Infusion of the dipeptide alanyl-glutamine into septic rats increased in vitro proliferation of mitogen-stimulated blood lymphocytes (Yoshida et al. 1992). Additionally, glutamine or alanyl-glutamine, provided parenterally, maintained the lymphocyte yield from Peyer's patches and intestinal integrity in influenza virus–inoculated mice (Li et al. 1998). It is likely that glutamine, via supply of NADPH and possibly other key metabolites, is able to sustain lymphocyte proliferation and viability in these animals.

Provision of glutamine in trauma in humans

At least 16 randomized, blind, controlled clinical trials have demonstrated beneficial effects of glutamine-supplemented parenteral and/or enteral nutrition in catabolic patient subgroups, including those with inflammatory bowel disease [recently reviewed by Jonas and Ziegler (1999)] and those patients receiving abdominal radiation treatment (Souba et al. 1990b). These studies reported improved nitrogen balance, muscle mass and/or gut integrity. In contrast, a number of other studies have not demonstrated improved clinical outcomes e.g., chemotherapy-induced toxicity (van Zaanen et al. 1994), bone-marrow transplantation (Schloerb and Skikne 1999) and Crohn's disease (Den Hond et al. 1999). However, reduced hospital infection rates with glutamine-enriched nutrition have been shown in adult patients after bone-marrow transplantation (Ziegler et al. 1992), major trauma (Houdijk et al. 1998) and in premature infants requiring intensive care (Neu et al. 1997). In addition to these reports, in vitro studies and clinical results have demonstrated a potent effect of glutamine supplementation on human immune cell number and function [reviewed by Ziegler (2000)].

Role of glutamine in the pathogenesis of Type-1 diabetes

It has been proposed that the availability of extracellular glutamine, as an essential amino acid for lymphocyte function, could play a role in the pathogenesis of some autoimmune

conditions such as Type-1 diabetes (Wu et al. 1991). Indeed, the administration of the antiglutamine utilization drug acivicin delayed or stopped the progression of the disease in diabetes-prone rats (Misra et al. 1996). We demonstrated recently that the addition of the glutaminase inhibitor 6-diazo 5-oxo norleucine to macrophages before exposure to clonal rat pancreatic β-cells in vitro virtually abolished the lytic capacity of the macrophage toward the target β-cells (Murphy and Newsholme 1999). We speculated that this inhibition of destructive capability is achieved via inhibition of secretion of TNF-α, a cytokine to which the β-cell is particularly sensitive (Dunger et al. 1996, Mandrup-Paulsen et al. 1986, Pukel et al. 1988). We recently obtained evidence that glutamine utilization is increased in macrophages exposed to Type-1 diabetic patient serum (unpublished work) and that the glutamine concentration in the plasma of moderately ketoacidotic patients at diagnosis is significantly elevated, thus adding further weight to the argument that this amino acid is important to the pathogenic process.

CONCLUSIONS

From the initial observations made in Eric Newsholme's laboratory in the 1980s, it has now become clear that many cells of the immune system utilize glutamine at high rates. More recently, glutamine utilization has been linked to functional activities of cells of the immune system such as proliferation, antigen presentation, cytokine production, nitric oxide production, superoxide production and phagocytosis. Many of these functional parameters appear to be directly or indirectly dependent upon the intracellular supply of NADPH. The initial pathway of glutamine metabolism, which is common to all cells of the immune system, can generate NADPH from $NADP^+$, thus providing a possible link between high rates of glutamine utilization and the beneficial effect on the many diverse functions of immune cells. Glutamine supplementation of diet or parenteral nutrition has resulted in beneficial clinical outcomes. In the future, it may be possible to manipulate glutamine metabolism in vivo. This approach may provide novel treatment for a growing list of diseases in which glutamine utilization may directly or indirectly contribute to disease pathogenesis such as acquired immunodeficiency syndrome, sickle cell anemia, obesity and diabetes.

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