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Glutamine metabolism by lymphocytes, macrophages, and neutrophils: Its importance in health and disease

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Many aspects of the cell biology of lymphocytes, macrophages, and neutrophils have been studied extensively. Our recent work on these cells has investigated how fuel metabolism, especially glutamine metabolism, is related to the specific function of these cells in the inflammatory response. The high rate of glutamine utilization and its metabolism in such immune cells has raised the question of why glutamine is responsible for these functions. The macrophage has access to a variety of metabolic fuels both in vivo and in vitro. The quantitatively important role of glutamine in the processes of free radical and cytokine production has been established in our laboratories. Our current understanding 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, specifically the phagocytic and secretory capacities of cells of the defense system can be beneficially altered.
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Introduction

Lymphocytes, macrophages, and neutrophils play an important role in immune and inflammatory responses. 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. By contrast, macrophages are terminally differentiated end cells in which the ability to proliferate is gradually lost. Neutrophils constitute 60% of the circulating leukocytes. These cells act as the first line of defense in the plasma and carry out phagocytosis either alone or in cooperation with antigen-specific defenses.

In recent years, the molecular biology of lymphocytes, macrophages, and neutrophils and the process of chemical communication between them has attracted considerable interest, and much progress has been made in our understanding of some 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. Therefore, it is of importance in the clinical conditions of trauma, sepsis, burns, and recovery from surgery. The significance of the cellular immune system in overcoming infection has been highlighted recently by the high mortality rate associated with simple immune challenges in the disease of acquired immunodeficiency syndrome. Furthermore, selective heightened activity of the immune system may explain a number of pathologic conditions [e.g., diabetes mellitus (Type I), multiple sclerosis, osteoarthritis, rheumatoid arthritis, and ulcerative colitis], which usually are covered by the general term *autoimmunity*.

Despite the undoubted importance of the cells of the immune system, it is surprising that until recently, relatively little was known about the metabolism of these cells, the fuels they require to carry out their functions, the rates of utilization and fates of these fuels, and any implications for

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the overall metabolic homeostasis of the animal. Indeed, it was not until the pioneering work of Newsholme's laboratory in the early to mid-1980s that it was established that immune cells such as lymphocytes and macrophages could utilize glutamine at high rates in addition to glucose.[1–4] It was generally thought at that time that glutamine was only a quantitatively important fuel for cells the intestine, liver, and tumor cells. The importance of glutamine metabolism for immune cell function will be discussed later.

Cells of the immune system

Lymphocytes

Lymphocytes are small round cells with characteristic large nuclei and a thin rim of cytoplasm. Cells of similar morphology are found in the spleen, bone marrow, lymph nodes, thymus, and other areas such as Peyer's patches and tonsils. Lymphocytes in the tissue are in dynamic equilibrium with circulating blood. They may be subdivided according to their surface markers, reactions to stimuli, migratory patterns, and life span.

Differentiated lymphocytes first originate from primitive stem cells, which reside in the bone marrow in mammals. Further differentiation leads to the development of lymphoid stem cells that are directed to specific sites of the body for further differentiation. For example, in the thymus, these cells acquire certain characteristics by which they become known as T lymphocytes (i.e., thymus-derived lymphocytes); these cells take part in cell-mediated immune reactions. B lymphocytes mature in the bone marrow and they are the precursors of antibody-forming cells. On the other hand, T cells do not synthesize detectable amounts of immunoglobulin, but function as regulators of the immune response. This is achieved via interactions between various T-cell subsets (helper, suppressor, or cytotoxic T cells) and macrophages during the cell-mediated immune response. In normal animals not subjected to a serious immune challenge, most of the lymphocytes will be in a quiescent state and therefore are known as resting lymphocytes. However, these cells must respond promptly to an immune challenge. This might result in increased utilization of the some key amino acids, glucose, and fatty acids.

Macrophages

Macrophages are mononuclear cells that are formed from circulating precursor cells (monocytes). Macrophages are terminally differentiated end cells in which the ability to proliferate is gradually lost.[5] Mature resident macrophages are found more widely distributed in hematopoietic, lymphoid, and other tissues, where they exist as biosynthetically active cells. After an inflammatory or immunologic stimulus, newly recruited macrophages with markedly different secretory and endocytic properties can accumulate in large numbers at specific sites. In spite of this functional heterogeneity, macrophages overall are characterized as motile, highly phagocytic cells that display marked plasma membrane activity. Their versatile secretory activities, which include enzymes and eicosanoids plus toxic nitrogen and oxygen products, can be altered by stimuli such as phagocytic particles, microbial products, and lymphokines acting on various macrophage plasma membrane receptors. Free radical production, which results in the formation of both superoxide and nitric oxide, is related to the function of the cell (i.e., efficient killing of foreign bacteria either extracellularly or inside phagocytic vesicles).

Neutrophils

Neutrophils contain a characteristic lobulated chromatin-dense nucleus that has given rise to the term *polymorphonuclear leukocyte*; they are 9 to 12 μm in diameter. At any time, more than 90% of the neutrophil population is located as newly differentiated cells within the bone marrow. The remainder are distributed between the circulation and the vascular endothelium, where they are attached to marginated pools or located within specific tissues.

Along with the onset of phagocytosis of bacteria or tissue fragments by neutrophils, a number of different cellular processes including motility, respiratory burst, and secretion of cytoplasmic (proteolytic) enzymes and immunomodulatory compounds are initiated. The combination of these processes assists in the killing and digestion of the engulfed bacteria and, if prolonged, the development of a local inflammation. An increase in the respiratory burst, which is characterized by activation of a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent membrane-associated oxidase, involves a sudden stimulus-induced increase in nonmitochondrial oxidative metabolism that results in the production of the superoxide anion and associated reactive oxygen species.[6] The mechanism and regulation of superoxide production is similar in both the neutrophil and macrophage. However, the neutrophil itself must be protected from these intracellular and extracellular reactive oxygen species. This self-protection is gained mostly from the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase.

The processes of endocytosis, secretion of active compounds, and generation of reactive oxygen species has been assumed to be dependent mostly on glucose metabolism in neutrophils[7] due to the large energy requirement of these processes. However, as we shall discuss later, this is not the case.

Metabolism in isolated cells

The question arises as to how is it possible to obtain, initially and relatively quickly, information on the metabolic properties of cells or tissues that are metabolically uncharacterized. One means of obtaining this information is from in vitro catalytic activities of a variety of enzymes. A comparison of activities within the tissue and between different tissues enables preliminary characteristics of the metabolism to be determined. These can than be tested or investigated by studying flux within the pathways. Which enzymes should be studied? The maximum in vitro catalytic activities of certain key enzymes in different pathways can provide information on the maximal capacities of certain pathways in metabolism. It is known that maximal catalytic activities of enzymes are often in considerable excess of the rate of metabolic flux (sometimes 100 to 10,000 times

Table 1 Activities of some enzymes of glucose and glutamine metabolism in rat macrophages, lymphocytes, and neutrophils*

Enzymes	Lymphocytes	Macrophages	Neutrophils
Hexokinase	17.3	76	33
Phosphorylase	3.8	3.0	—
6-Phosphofrutokinase	25.7	22.8	—
Pyruvate kinase	403.0	447.0	—
Lactate dehydrogenase	823.0	764.0	880.0
Glucose 6-phosphate dehydrogenase	17.5	34.5	68.0
6-Phosphogluconate dehydrogenase	21.1	23.8	—
Pyruvate dehydrogenase	3.3	3.2	
Citrate synthase	63.7	108.0	45.0
NAD^+-linked isocitrate dehydrogenase	6.3	5.8	19.0
$NADP^-$-linked isocitrate dehydrogenase	26.9	27.4	65.0
ATP citrate lyase	1.1	1.6	—
Oxoglutarate dehydrogenase	5.1	10.1	—
3-Oxoacid CoA-transferase	19.9	36.6	—
Acetoacetyl-CoA thiolase	27.4	15.5	—
3-Hydroxybutyrate dehydrogenase	4.8	0.17	—
Carnitine palmitoyltransferase	0.47	0.75	—
Phosphate-dependent glutaminase	39.4	152	56
Glutamate dehydrogenase	34.0	98.6	—
$NADP^+$-linked malate dehydrogenase	3.5	3.8	4.5
Phosphoenolpyruvate carboxykinase	4.9	7.6	
Pyruvate carboxylase	2.5	4.7	—
Aspartate aminotransferase	67.4	118	51
Alanine aminotransferase	10.3	4.4	26
NAD^+-linked malate dehydrogenase	683	452	—

Note: Lymphocytes and macrophages were prepared and extracted and enzymes were assayed as described previously.[3,51] Activities were measured at 25°C, except for glutaminase and phosphoenolpyruvate carboxykinase (37°C). The results are from Curi et al.,[2] Newsholme et al.,[3,33] and Pithon Curi et al.[51]
*Measured in nmol/min/mg protein.

higher). Some enzymes do not appear to be present in such excessive amounts and it is often found that these enzymes are regulatory.[8] The quantitative value of the approach depends on the care with which the enzymes are chosen. They must function only in the pathway being assessed and must catalyze nonequilibrium reactions. It should be emphasized that maximum enzyme activities indicate only the maximum potential for the utilization of a particular fuel and cannot determine whether a fuel is actually used in a given situation, although fuel utilization rates may be independently assessed.[9–11] This "simple" approach can quickly lead to an overall impression of metabolism of the cell under investigation, but it cannot provide accurate quantitative information on the regulatory enzymes. This information can be obtained by detailed analysis of flux control coefficients, elasticities, and response coefficients (net sensitivities) as described and defined elsewhere.[12]

The "simple" approach that we have taken in the past has lead to some remarkable discoveries. We will begin with the description of our work on immune cell metabolism at the time when maximal catalytic activities of some key enzymes in the metabolism of rat lymphocytes, macrophages, and neutrophils were under investigation in the laboratory of Newsholme in the early to mid-1980s. The activities are reproduced in *Table 1*. These activities indicated the possibility that the cells were able to utilize glucose and glutamine. They also suggested that the capacities for the utilization of glucose and glutamine were very high. The activity of hexokinase was high in comparison to phosphorylase, suggesting the rate of glucose utilization was much higher than glycogenolysis in vivo. In addition, hexokinase activity was substantially in excess of 6-phosphofructokinase, whereas the activity of glucose 6-phosphate dehydrogenase was relatively high, indicating the importance of the pentose phosphate pathway in these cells. The high activities of glutaminase and glutamate dehydrogenase would indicate that glutamine utilization and metabolism occurred at a significantly high rate. The fact that pyruvate dehydrogenase activity was low and lactate dehydrogenase was exeptionally high would suggest that oxidation of glucose and glutamine was not a major fate of these fuels but conversion to lactate was the predominant pathway. The actual rates of utilization and oxidation of glucose and glutamine by isolated and incubated cells (in vitro) are reproduced in *Table 2*. Furthermore, on the basis of rates of glucose and glutamine utilization and the rate of end product formation, it appears that oxidation via acetyl-coenzyme A (CoA) and the classic citric acid cycle play a minor role in their metabolism. Glucose is converted almost totally into lactate and glutamine into glutamate, aspartate, and lactate (*Table 2*).[1,4,13,14]

Fate of utilized glutamine in lymphocytes, macrophages, and neutrophils

Up until the early 1980s, it was believed that lymphocytes, macrophages, and neutrophils obtained most of their energy by metabolism and oxidation of glucose and that lymphocytes, which had not been subjected to an immune response

Table 2 Rates of utilization of glucose or glutamine and of production of lactate, glutamate, and aspartate and $^{14}CO_2$ production by isolated incubated mouse macrophages, rat lymphocytes or rat neutrophils

Addition incubation medium	Glucose	Glutamine	Lactate	Glutamate	Aspartate	$^{14}CO_2$ Production
Mouse macrophages						
Glucose	−355	—	632	—	—	11
Glutamine	—	−186	33	137	25	9
Mouse 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

Note: *Units:* nmol/hr/mg protein. Negative sign (−) indicates utilization.
Data from Ardawi and Newsholme,[1] Newsholme et al.,[4] and Pithon Curi et al.[51]

(resting lymphocytes), were metabolically quiescent. Recent evidence has shown that the rate of glutamine utilization by these cells is either similar to or greater than that of glucose and that neither glutamine nor glucose is fully oxidized (almost all of the glucose used is converted to lactate and almost all of the glutamine to glutamate, lactate, and aspartate; *Table 2*). A high rate of glutamine utilization, but only partial oxidation, is characteristic of other cells (e.g., enterocytes, thymocytes, colonocytes, fibroblasts, and tumor cells).[15–17] The pathway of partial glutamine oxidation was reported previously for tumor cells, and the process was termed *glutaminolysis* by McKeehan.[18] On the basis of end products of metabolism (*Table 2*) and maximal activities of some enzymes (*Table 1*), it is thought that glutamine is utilized by a similar if not identical pathway in macrophages, lymphocytes, and neutrophils.

Macrophage adenosine triphosphate generation and oxygen consumption rates

Macrophages are known to have a large oxidative capacity and their oxygen (O_2) consumption rates (515 nmol/hr/mg protein) are similar to those of sheep heart (696 nmol/hr/mg protein) and rat liver (520 nmol/hr/mg protein) in vitro as calculated by Newsholme[19] using original published data from Krebs and Johnson and Karnovsky et al. Newsholme calculated adenosine triphosphate (ATP) generation rates for isolated and incubated macrophages in in vitro and cultured macrophages taking into account oxygen utilized by the NADPH oxidase of these cells.[19] The ATP generation rate in the presence of both glucose and glutamine was 930 nmol/hr/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 approximately 7 nmol/mg protein,[4] the total ATP concentration of the cell must have been turned over at least two times per minute. The macrophage, when studied over longer periods (82 hours of culture), had a similar dependency on these fuels, with glucose contributing 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/hr/mg protein over 82 hours).

Macrophage glutamine utilization in different culture conditions

Of the many amino acids available in culture, macrophages utilize only glutamine and arginine in significant quantities (approximately 6 μmol glutamine and 3 μmol arginine per milligram of protein over 96 hours of culture for thioglycolate elicited macrophages).[19] Cultured macrophages also appear to utilize glutamine at different rates, depending on the state of macrophage activation and the availability of glucose and arginine (*Table 3*). We suggest that the rate of utilization of glutamine may be related to the requirement for different metabolic end products depending on the activating stimulus and extracellular availability of metabolites (see below for further discussion).

The role of high rates of glutaminolysis (and glycolysis) 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.[20] A similar question can be raised concerning glutaminolysis. We have outlined here that similar metabolic characteristics apply to various cells of the immune system despite the fact that their cell biology is different. The lymphocytes under study are not rapidly dividing but possess the potential for cell division, macrophages are terminally differentiated cells, and neutrophils have a life span of approximately 10 hours in vitro. Hence any hypothesis must explain high rates of fuel utilization in cells with widely different cell biological characteristics. Glycolysis and glutaminolysis both provide metabolic intermediates for biosynthetic pathways [glycolysis provides glucose 6-phosphate for formation of ribose 5-phosphate for DNA and RNA synthesis 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

Table 3 Glutamine utilization rates in resident and activated murine macrophages

Conditions	Hours of Pre-incubation			
	Resident macrophage		Activated macrophage	
	24	48	24	48
5 mM glucose + arginine	20.7 ± 0.3	37.9 ± 4.2	73.8 ± 9.4	95.5 ± 12.7
+ LPS	26.7 ± 3.0[b]	71.0 ± 9.3[b]	100.4 ± 15.0	118.1 ± 23.5
5 mM glucose − arginine	56.1 ± 6.1	104.6 ± 10.2	126.8 ± 18.6	121.1 ± 4.0
+ LPS	74.1 ± 6.4[a]	127.5 ± 15.9	168.7 ± 26.4	136.6 ± 5.1[a]
w/o Glucose + arginine	36.1 ± 4.8	92.6 ± 10.7	57.8 ± 16.1	54.6 ± 11.0
+ LPS	76.7 ± 9.3[b]	102.4 ± 14.1	88.8 ± 7.2[a]	78.7 ± 12.9[a]
w/o Glucose − arginine	39.8 ± 4.3	63.8 ± 8.9	125.0 ± 29.0	76.8 ± 7.9
+ LPS	83.2 ± 9.9[b]	93.4 ± 13.1[a]	124.7 ± 1.2	113.2 ± 4.2[b]

Note: Cells were isolated and purified via adherence to tissue culture plastic as previously described. Following purification cells were preincubated for 24 or 48 hours at 37°C, in 95% air and 5% CO_2, in selected tissue culture media supplemented with a combination of 2 mM glutamine with or without 5 mM glucose or 0.4 mM L-arginine. Following preincubation the media was replaced and the cells were incubated for 1 hour under the conditions described above in fresh media and glutamine utilization was determined. Glutamine utilization was linear over this period. *P*-values, where indicated, refer to statistical difference between glutamine utilization in the presence of 15 μg/mL lipopolysaccharide (LPS) compared with its absence. *Units:* nmol glutamine consumed/hr/mg protein.
Values are expressed as means ± SEM values of three or more incubations. [a]$P < 0.05$. [b]$P < 0.01$.
Data from Murphy and Newsholme.[26]

synthesis]. However, the rates of both glycolysis and glutaminolysis—at least in lymphocytes—are greatly in excess (>100-fold) of the requirements for these biosynthetic processes.[1,17] Application of the quantitative theory of metabolic control to branched pathways[21] provides a hypothesis to account for the high rate of both glycolysis and glutaminolysis in all cells discussed above [i.e., rapidly dividing cells (e.g., tumor cells), those with the potential for cell division (e.g., lymphocytes, some enterocytes, endothelial cells), and terminally differentiated cells such as macrophages, some enterocytes, colonocytes, and neutrophils]. High rates of glycolysis and glutaminolysis provide precursors for biosynthetic pathways, but the fluxes through these biosynthetic pathways are normally very small in comparison to the fluxes through glycolysis and glutaminolysis. However, the fluxes through the biosynthetic pathways will increase considerably during the synthesis of DNA and RNA, and also for phospholipid synthesis for new cell membranes. Because provision of these intermediates at a precise time of the cell cycle is very important, the sensitivity of the biosynthetic processes to their specific regulators would need to be very high.[22]

Another suggestion is that these processes provide energy for the cell: In fact, energy provision is considered to be a major role of glutaminolysis in tumor cells and enterocytes.[20,23] Under conditions of extended cell culture, glutamine can be fully oxidized by the macrophage,[24] and thus may become a major oxidative fuel under these conditions. However, due to limited survival of lymphocytes and neutrophils in vitro, information on glutamine oxidation rates over several days is not available. Although the capacity for rapid cell division is retained by isolated lymphocytes, this does not apply to isolated neutrophils or macrophages that 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 secretory activity. This secretory activity may be subdivided into many classes of product,[25] but for the purpose of this review we will concentrate our discussion on two major types of product: (1) free radicals and (2) cytokines.

The role of glutamine in the process of nitric oxide production from macrophages,[26] superoxide production from neutrophils,[27] tumor necrosis factor α (TNFα) production from macrophages,[28] interleukin-8 (IL-8) production from monocytes,[26] and IL-1[29] and IL-6[30] production from macrophages has been addressed recently. Macrophages appear to utilize only the nitrogen-rich amino acids glutamine and arginine at high rates in culture.[19] This may indicate that nitrogen provision for new amino acids, proteins, and nitric oxide may come from extracellular glutamine and arginine. However, immunostimulated macrophages secrete the enzyme arginase, thus depleting their extracellular supply of arginine.[26,31] Therefore, macrophages may be dependent absolutely on extracellular glutamine for optimal secretory activity. Evidence to support this hypothesis has been provided by several recently published studies. (1) In the absence of extracellular arginine, but in the presence of 2 mM glutamine, mouse bacillus Calmette-Guerin (BCG)-activated macrophages could produce nitric oxide at high rates (*Table 4*). (2) In the absence of extracellular arginine, but in the presence of 2mM glutamine, mouse BCG-activated macrophages could produce TNFα at high rates in the presence of a lipopolysaccharide (LPS) stimulus; in addition, human monocytes could produce IL-8 at high rates under similar conditions of culture (*Figure 1*). (3) Rat neutrophils could be protected from adrenaline-induced inhibition of superoxide production by the addition of 2 mM glutamine.[32] Thus, a high rate of glutamine utilization and metabolism via a number of alternative routes may provide a metabolic basis for the sustained production of many secretory products when required, as originally proposed by Newsholme et al.[22] However, the mechanism by which glutamine can act to allow high rates of secretory product formation and release must account for the diverse nature of these secretory

Table 4 Nitrite production in resident and bacillus Calmette-Guerin-activated murine macrophages

Conditions	Resident macropage		Activated macrophage	
	24 Hours	48 Hours	24 Hours	48 Hours
0 mM 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[b]	0.0 ± 0.0	11.4 ± 1.1[a]
0.06 mM L-Arginine				
Control	8.1 ± 0.8	8.8 ± 1.4	16.3 ± 1.2	16.5 ± 1.2
+ LPS	11.0 ± 1.1[a]	10.4 ± 0.9	21.9 ± 2.9[a]	18.7 ± 2.8
0.4 mM L-Arginine				
Control	14.2 ± 3.5	13.8 ± 1.2	36.5 ± 3.1	46.3 ± 2.3
+ LPS	22.3 ± 1.7[b]	15.1 ± 0.7[a]	42.5 ± 0.7[b]	47.4 ± 0.9

Note: Peritoneal murine macrophages were isolated and purified as previously described. Following purification via adherence to tissue culture plastic cells were cultured for 4, 24, or 48 hours at 37°C, in 95% air and 5% CO_2 in tissue culture media supplemented with 2 mM glutamine and 5 mM glucose in the absence or presence of varying concentrations of L-arginine. Following preincubation, the media was replaced and the cells were incubated for 1 hour under the conditions described above, after which nitrite production was determined. Nitrite production was linear over this period P-values, where indicated, refer to statistical difference between glutamine ultilization in the presence of 15 μg/mL lipopolysaccharide (LPS) compared with its absence. *Units:* nmol nitrite/hr/mg protein.
Values are expressed as means ± SEM values of three or more incubations. [a] $P < 0.05$. [b] $P < 0.01$.
Data from Murphy and Newsholme.[26]

products and must contain at least one common metabolic product.

In the formation of the reactive species nitric oxide and superoxide, NADPH is required by the enzymes responsible for free radical production, inducible nitric oxide synthase (iNOS) and NADPH oxidase, respectively. During formation of new proteins, NADPH also is required. Via catabolic metabolism involving $NADP^+$-dependent malate dehydrogenase,[33] glutamine can thus generate considerable NADPH for cell requirements. The NADP+-dependent malate dehydrogenase step will result in the formation of pyruvate, which either can be converted to lactate (ending the path-

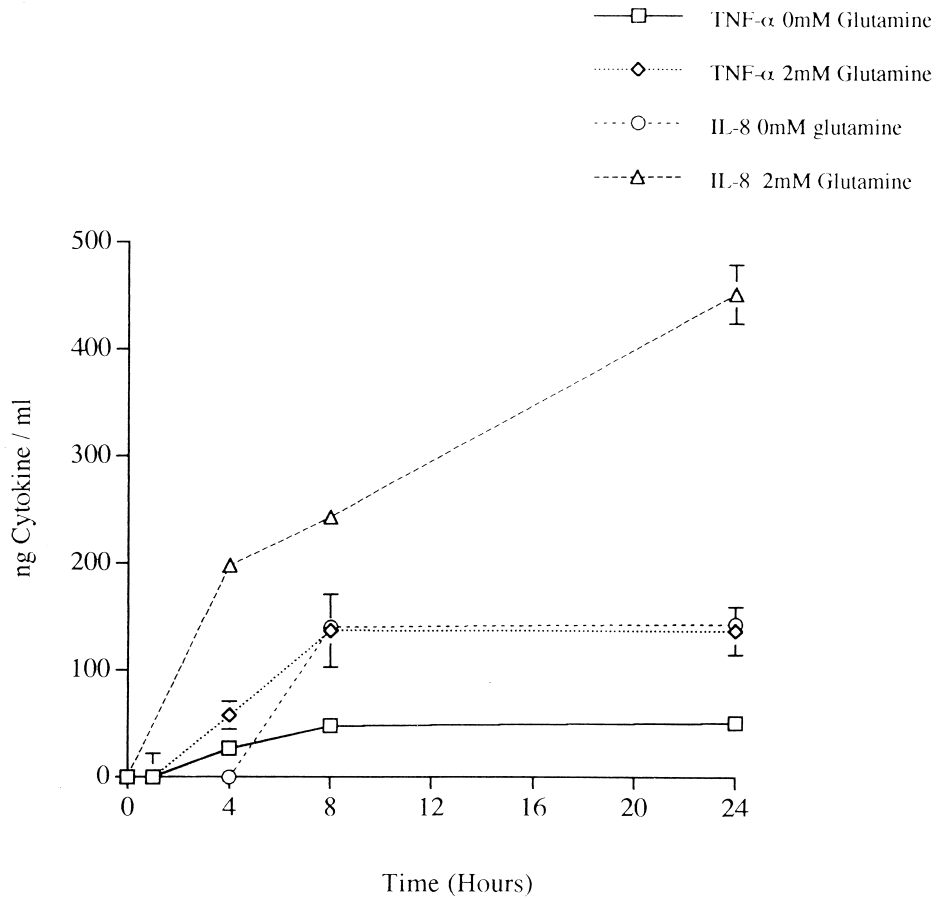

Figure 1 Tumor necrosis factor (TNF-α) and interleukin 8 (IL-8) production from murine macrophages and human monocytes, respectively, following incubation in the presence or absence of L-glutamine and lipopolysaccharide (LPS). Bacillus Calmette-Guerin (BCG)-activated murine macrophages were isolated and purified as previously described.[28] Following purification via adherence the cells were incubated for up to 24 hours in MEM tissue culture media in the presence or absence of 2 mM glutamine and presence or absence of 15 μg/mL bacterial LPS. TNF-α production was determined using a Duo-Set immunosorbant enzyme-linked immunoassay (ELISA) test kit as previously described.[28] Cytokine concentration is expressed as nanograms per milliliter, which has been adjusted to reflect the amount produced from 1 mg cell protein (after cell protein assay). Human monocytes were isolated and purified as prevoiusly described.[28] Following purification the adherent monocytes were incubated for up to 48 hours in MEM tissue culture media in the presence of 15 μg/mL bacterial LPS and the presence or absence of 2 mM glutamine. At specified time intervals supernatant aliquots were removed and IL-8 concentration was determined as previously described.[28] Cytokine concentration is expressed as ng/mL, which has been adjusted to reflect the amount produced from 1 mg cell protein (after cell protein assay). Data from Murphy and Newsholme.[28]

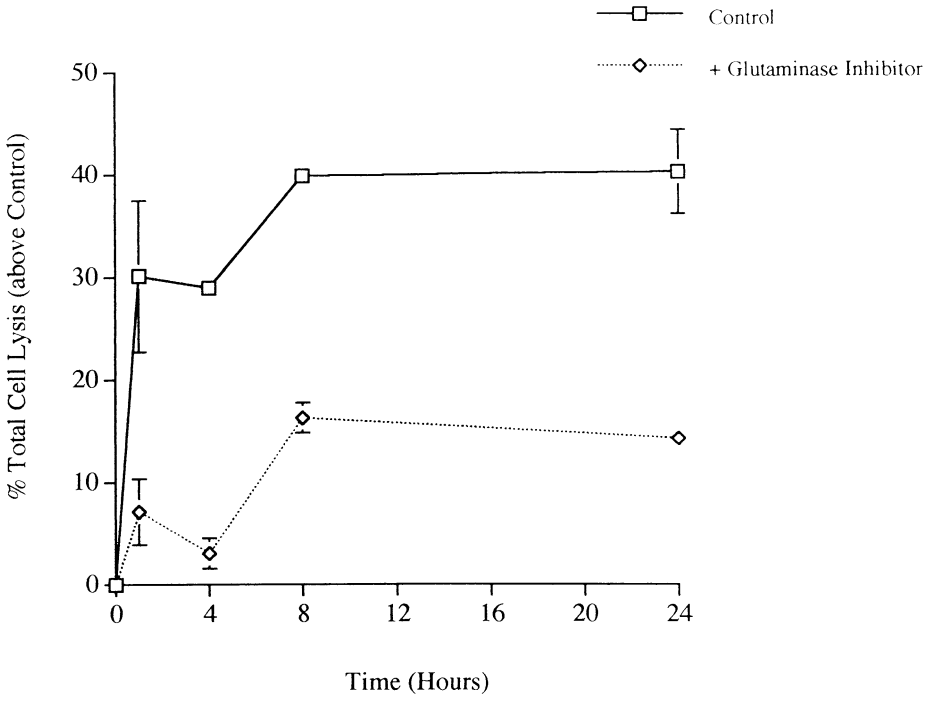

Figure 2 Effect of co-culture of bacillus Calmette-Guerin (BCG)-activated macrophages and Brin-BD11 β cells on rates of β-cell lysis following 48 hours of preincubation. BCG-activated macrophages were isolated and purified as previously described.[28] Following purification the adherent macrophages were incubated for 48 hours in MEM tissue culture media supplemented with 2 mM glutamine and 15 μg/mL bacterial lipopolysaccharade (LPS) in the absence of L-arginine. Additional macrophage incubations were performed in the absence of L-arginine but in the presence of 200 μM 6-Diazo 5-oxo norleucine (a glutaminase inhibitor) and 15 μg/mL LPS. Following incubation in these pre-coculture conditions the cells were removed from tissue culture plates and seeded at a density of 4×10^4 macrophages per 2×10^5 Brin-BD11 β cells in new culture plates. Subsequent β cell lysis was determined as previously described.[28] Data from Murphy and Newsholme.[28]

way of glutaminolysis) or to acetyl-CoA and thus carbon dioxide (CO_2). Thus, depending on 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. Cellular NADPH concentration and $NADP^+$-dependent malate dehydrogenase levels in the presence and absence of extracellular glutamine are currently under investigation in our laboratories. Glucose also may 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.[33] In addition, glutamine carbon may be used for new amino acid synthesis in periods of active synthesis and secretion.

Muscle and glutamine production

In the 1960s, it was established that the liver plays a quantitatively major role in the uptake and utilization of most of the dietary amino acids with exception of the branched-chain amino acids, which are taken up primarily by muscle,[34] and glutamate, aspartate, and especially glutamine (plus asparagine), which are utilized by the absorptive cells of small intestine.[23,35]

Although muscle takes up branched-chain amino acids, it releases alanine and glutamine. Muscle tissue is a major site for glutamine synthesis in the human body[36] and it contains over 90% of the whole-body glutamine pool.[37] Quantitative studies in humans demonstrated that, in the postabsorptive state, 60% of the amino acids released comprised alanine plus glutamine.[38] Furthermore, the addition of branched-chain amino acids to the incubation medium of an isolated rat muscle increased the rate of formation and release of alanine and glutamine.[39] These observations led to the suggestion that the nitrogen and some of the carbon of the branched-chain amino acids could be used to synthesize alanine and glutamine in muscle[40,41] and that muscle was the major source of these two amino acids in the blood.[42] However, it has been shown, at least in the rat, that branched-chain amino acids are transaminated, but not further metabolized because the key enzyme controlling their rate of oxidation (2-oxoisovalerate dehydrogenase) is almost totally in the inactive form in skeletal muscle.[43,44] Hence, rat muscle takes up branched-chain amino acids primarily to use their nitrogen for the formation of glutamine and alanine. Therefore, once the high rate of glutamine utilization was discovered and the importance of glutamine metabolism established for lymphocytes, macrophages, and neutrophils, the question arose as to the source of the glutamine. As independently proposed by Newsholme and Marliss et al.,[42] it is likely to be skeletal muscle.

Thus, in most physiologic conditions, muscle will release alanine and glutamine. Therefore, the supply of glutamine for cells of the immune system is not normally compromised. However, in some pathologic conditions muscle tissue may reduce glutamine output, perhaps resulting in immunosuppression.[45]

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.[46] Indeed,

the administration of the anti-glutamine utilization drug acivicin delayed or stopped the progression of the disease in diabetes-prone rats.[47] We recently demonstrated 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 cell (*Figure 2*). We have speculated that this inhibition of destructive capability is achieved via inhibition of secretion of TNF-α,[24] a cytokine to which the β cell is particularly sensitive.[48–50] We have recently obtained evidence that glutamine utilization is increased in macrophages exposed to serum of type 1 diabetic patients, adding further weight to the argument that this amino acid is important to the pathogenic process.

Concluding remarks

From the initial observations made in Newsholme's laboratory in the 1980s, it has now become clear that many of cells of the immune system utilize glutamine at high rates. More recently, glutamine utilization has been linked to functional activities of immune cell function such as cytokine production, nitric oxide production, superoxide production, and phagocytosis. In the future it may be possible to manipulate glutamine metabolism in vivo. This approach may provide novel treatment of diseases in which glutamine utilization, by inappropriately activated cells of the immune system, is essential for the pathogenic process.

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