0014-4754/96/050455-05$1.50 + 0.20

The possible role of glutamine in some cells of the immune system and the possible consequence for the whole animal

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Abstract. Glutamine is important for the function of lymphocytes and macrophages. A role for the high rate of glutamine utilisation by these cells is presented. Since muscle syntheses, stores and releases glutamine, this tissue may play a role in the immune response. Since the number of immune cells utilising glutamine may be large, the demand for glutamine from muscle, especially during trauma, sepsis or burns, may be very high. A speculative suggestion is put forward that this requirement for glutamine from muscle may play a role in cachexia under some of these conditions.

Key words. Glutamine; lymphocytes; macrophages, cachexia; trauma; burns; infection.

Introduction

Several different types of cell are involved directly in defending the body against invasion by a foreign organism. The fuels utilised by lymphocytes and macrophages have been studied in depth since the total number of such cells is very large – possibly more than 1.4 kg so that their fuel requirements could place severe demands on the body as a whole. Our current knowledge of the demands for fuel for these cells suggests that if excessive stress is placed on this system, which can occur in severe infection, major surgery, trauma or burns or in prolonged exhaustive exercise, the body may not be able to meet the fuel demands for all these cells satisfactorily. In normal animals, not subjected to a serious immune challenge, most of the lymphocytes will be in a quiescent state – known as resting lymphocytes. This has been taken to mean that such lymphocytes are also metabolically inactive. However, the role of these cells in the immune system means that they must be able, *at* any time, to respond rapidly, effectively and specifically to an immune challenge. This response involves, in part, rapid proliferation of particular clones of the cells and this requires high rates of utilisation of some fuels even in the resting state.

For many years, it had been considered that both lymphocytes and macrophages obtained most of their energy from the oxidation of glucose but it has now been shown that they also use glutamine and its rate of utilisation is either similar to or greater than that of glucose[1–3]. Since we will make important interpretations concerning this use of glutamine, we must be quite clear on the lines of evidence which support the view that glutamine is used at a very high rate by lymphocytes and by macrophages in vivo.

– The maximal catalytic activity of glutaminase, a key enzyme in the glutamine utilisation pathway, is high in lymphocytes and in macrophages freshly isolated from the intact animal[1–3].

Rates of utilisation of glutamine by freshly isolated lymphocytes and macrophages are also high[1–3].

– The rates of utilisation of glutamine by macrophages and by a B-lymphocyte hybridoma in culture are high[7,10].

– Although various lymphocyte subsets have not been studied, the available evidence suggests that B- and T-lymphocytes utilise glutamine at similar rates[5].

Surprisingly, little of the carbon of glucose ($< 10\%$) and only some of that of glutamine (10–30%) is oxidised completely by these cells: glucose is converted almost totally into lactate, glutamine into glutamate, aspartate, alanine and CO_2. The partial oxidation of these fuels are known as glycolysis and glutaminolysis, respectively.

From these simple metabolic characteristics several questions arise: what is the significance of these high rates, why is the oxidation only partial and what are the consequences for the whole organism? The answers to these questions provides the basis for the material presented in this paper.

The role of high rates of glycolysis and glutaminolysis

High rates of glycolysis and glutaminolysis will provide energy for these cells. However, if energy generation per se was the major reason for high rates, it would be expected that more of the carbon of glucose and glutamine would be completely oxidised since the enzymes of the complete oxidation system are present in these cells[2]. It has also been suggested that glutamine provides nitrogen for synthesis of several important compounds, e.g. purine and pyrimidine nucleotides, which are needed for the synthesis of new DNA and RNA

during proliferation of lymphocytes and for mRNA synthesis and DNA repair in macrophages. However, a problem is that the rate of glutaminolysis is markedly in excess of the rates of synthesis of these compounds. For example, the rate of utilisation of glutamine by lymphocytes is very much greater than the measured rate of synthesis of uridine nucleotides and much higher than the maximum activity of the rate limiting enzyme, carbamoyl phosphate synthase II[19]. The rates of the biosynthetic pathways for purine or pyrimidine nucleotide synthesis use the key amino acids glutamine and aspartate. If increased rates of synthesis of these nucleotides resulted in a decrease in concentrations of glutamine and/or aspartate in the cell, this would 'oppose' the stimulation of the biosynthetic pathway, and the increased rate may be less than required to provide enough nucleotide, for example, doubling the content of DNA precisely when required for proliferation. This would be expected to decrease the rate of proliferation. For macrophages, the need to synthesise large amounts of mRNA for the synthesis of the proteins such as cytokines, enzymes and key proteins, when the cells are stimulated, will also require specific and precise increases in the rate of synthesis of these nucleotides. This principle of control is known as branched-point sensitivity[9]. The important point to emerge from this is that glutamine (and glucose) must be used at a high rate for some of the cells of the immune system even when they are quiescent since an immune challenge can occur at any time so that cells must be 'primed' to respond whereas this is an invasion by a foreign organism.

The reason for translocated pathways in immune cells

If pyruvate produced either from glucose of glutamine were fully oxidised via the Krebs cycle, the cells might produce too much ATP. Since the rate of production of ATP is controlled by demand for ATP rather than vice versa, too high a rate of production, in comparison to the rate of utilisation, would result in increases in the ATP/ADP concentration rates and this could lead to inhibition of the rates of glycolysis and glutaminolysis. Consequently, branched-point sensitivity would be lost. Indeed, it is possible that the rate of ATP production in these cells is normally too high and, in order to prevent loss of branched-point sensitivity, the mitochondria from lymphocytes may produce less ATP per NADH oxidised than mitochondria from other cells.

Importance of maintenance of the plasma glutamine level

Since the K_m for glutamine utilisation by these cells is higher than the normal plasma level, a direct consequence of branched-point sensitivity is that a decrease in the concentration of glutamine available to these cells would decrease the rate of glutamine utilisation and this would be expected to impair their function and hence decrease the effectiveness of the immune system. Indeed it has been shown that a decrease in the glutamine concentration in culture medium below that normally present in plasma does decrease the maximum rate of proliferation and slows the response to a mitogenic signal in both human and rat lymphocytes, even though they are provided with all other nutrients and growth factors in excess[5, 15, 19]. Furthermore, a decrease in glutamine concentration also decreased phagocytosis and the rate of cytokine production by macrophages[5].

Glutamine – a link between muscle and the immune system

Several tissues including liver, muscle, adipose and lung can synthesise and release glutamine into the bloodstream. This is important since most of the glutamine that enters the body via protein in the diet is utilised by the intestine. Of the tissues that release glutamine, muscle may be the most important, since not only can it synthesise glutamine but it also provides a store (as much glutamine is stored in muscle as glycogen is stored in the liver) and the rate of release across the plasma membrane, which occurs via a specific transporter, appears to be controlled by various hormones and it may be influenced by cytokines[14].

The use of the terms glutamine 'synthesis' (or 'production') and glutamine 'release' from muscle have not always been used systematically. For example, it has been assumed that the rate of glutamine synthesis is equivalent to that of glutamine release, and vice versa. This has led to a degree of confusion, since glutamine synthesis and release from muscle are independent processes. Thus, although glutamine synthesis is important to maintain the store of glutamine in muscle, the release from muscle, and not glutamine synthesis appears to be the key regulatory step for glutamine release into the bloodstream under normal conditions. Indeed glutamine release is the flux generating step for the release of glutamine by muscle[11]. This is because the intracellular glutamine concentration in muscle is well above the apparent K_m for release – hence any change in the intracellular glutamine level will not influence the rate of release unless it falls to very low levels. This suggests that there may be little clinical importance in the fall in the concentration of glutamine in muscle that has been observed in some conditions (e.g. trauma, surgery). If indeed muscle not only provides glutamine but helps to regulate the plasma level, via the transporter system in the membrane, and if much of this glutamine is used by some key cells of the immune system, muscle can be considered as part of the immune system. Consequently, it is suggested that immune cells may communicate with skeletal muscle in relation to the rate of glutamine release. This is why it is suggested that some cytokines,

Table 1. A list of some situations in which provision of glutamine is beneficial for the immune system.

Glutamine in total parenteral nutrition increases the biliary level of IgA, which is normally suppressed by this form of nutrition.
- The alanine-glutamine dipeptide given via total parenteral nutrition to tumour- bearing rats increases the rate of macrophage phagocytosis which had been suppressed[8].
- The alanine-glutamine dipeptide given via total parenteral nutrition to septic rats increases the rate of lymphocyte proliferation and increases the number of lymphocytes[20].
- Glutamine given via intravenous infusion to bone marrow transplant patients decreases the number of positive microbial cultures and decreases the number of clinical infections[18].

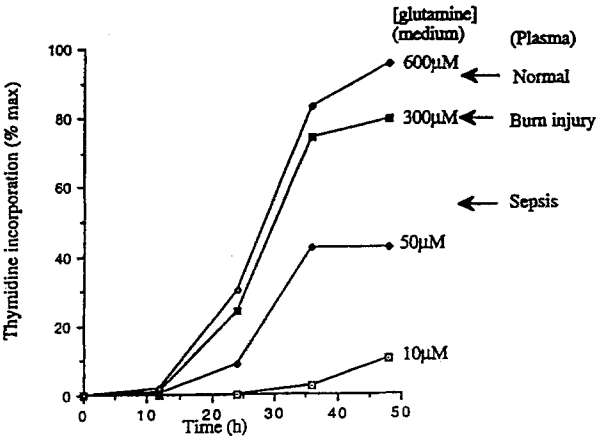

Figure 1. The effect of lowering the concentration of glutamine below the physiological plasma level in the culture medium of lymphocytes stimulated to proliferate by a polyclonal mitogen (concanavalin-A) and the approximate plasma level of glutamine in burns and sepsis. Data taken from reference 15 and from some unpublished work of the author.

released from cells of the immune system, may mediate changes in the rate of muscle glutamine release. Such communication between immune cells and muscle would be physiologically very important. It is thus tempting to speculate that cytokines will influence the activity of the glutamine transporter in muscle.

The plasma concentration of glutamine is decreased in a number of conditions: these include trauma, burns, major surgery, sepsis and prolonged exhaustive exercise[12,15–17]. And there is evidence that the immune system is suppressed under these conditions[6]. Such decreases in the plasma glutamine level may provide, at least in part, a possible explanation for the immunosuppression that characterises these conditions. Further work is needed to determine whether in other clinical conditions (e.g. viral infections) the plasma glutamine level is also decreased.

The role of glutamine in immune cells – its clinical importance

Over many years, there has been considerable clinical interest in the phenomenon of hypoglycaemia since this can cause abnormal function of the brain, which is normally dependent upon glucose as a fuel[13]. Similar considerations should be applied to the maintenance of the plasma glutamine level which can be considered to be as important a plasma fuel as that of glucose, but for different cells. Furthermore, the requirement for glutamine, synthesised within muscle and other cells, will increase after trauma, major surgery, infection and burns, since there will be increased activity of the immune system, an increased number of cells involved in proliferation and repair and, if the patient is not fed, intestinal cells will also use endogenously-produced glutamine.

The question therefore arises as to whether the plasma concentration of glutamine could be maintained by dietary, or other means, under these conditions which could be very beneficial for the patient. The administration of glutamine to patients via the enteral route may have several advantages since it can be easily provided and will 'feed' the intestine as well as the immune system[4]. Parenteral administration has the disadvantage that heat sterilisation and storage may increase the rate of degradation of glutamine to glutamate and ammonia or to pyroglutamate, so that glutamine-containing depeptides (e.g. alanylglutamine) have been developed to provide a more stable form of this amino acid that are stable to heat sterilisation.

Given the proposed importance of the release of glutamine from skeletal muscle, it is suggested that dietary or pharmacological treatments, which result in an increased rate of glutamine release from muscle, together with provision of precursors of glutamine (e.g. branched-chain amino acids) may represent another avenue for therapy in a number of pathological states. Some situations in which the provision of glutamine appears to have been beneficial for the immune system are given in table 1.

Cachexia and glutamine: a speculative proposal

It has been known for some time that the net rate of protein breakdown especially in muscle is increased after injury, major surgery, sepsis and that the resultant amino acids are released: this can result in severe loss of body protein. This process is considered to be important for several reasons *a)* the provision of precursors for gluconeogenesis; *b)* for the synthesis of important proteins by the liver (acute phase proteins); and *c)* for the synthesis of important peptides such as glutathione.

On the basis of the discussion on the important role of glutamine for cells of the immune system, it is tempting to suggest that the prime role for net muscle protein breakdown is to provide precursors for the synthesis of glutamine. This is achieved by maintaining a pool of

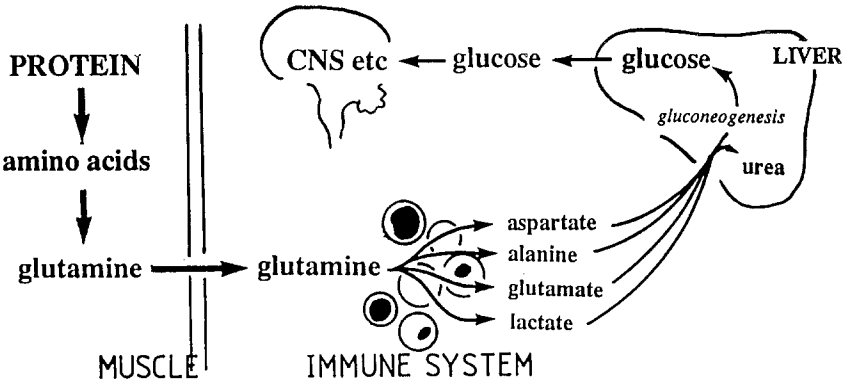

Figure 2. Provision and fate of glutamine during trauma, sepsis or burns. If glutamine is not provided exogenously, it will be synthesised de novo in several tissues but muscle may be the most important. Some of the nitrogen in the amino acids derived from protein in the muscle is used for synthesis of glutamine. The 'inefficient' metabolism in immune cells, which is designed to provide branched-point sensitivity, produces aspartate, laanine, glutamate and lactate (much of the latter will be derived from glucose) all of which are excellent gluconeogenic substrates. The glucose will then be used by the brain.

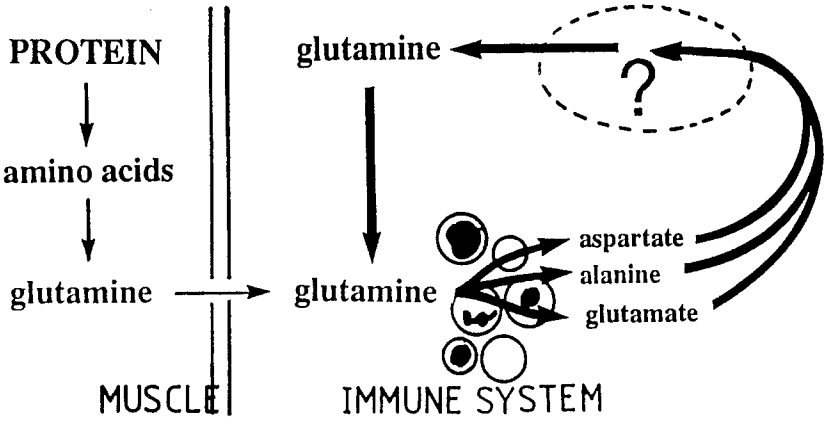

Figure 3. The glutamine cycle: a speculative fate of some of the amino acids produced from glutamine by cells of the immune system. There is no indication which tissues might be involved in the synthesis and hence in the salvage of glutamine but suggestions include adipose tissue and intestine. The proposed glutamine cycle is analogous to the Cori cycle for glucose and lactate.

available amino acids (e.g. valine, leucine, isoleucine, glutamate, aspartate) in muscle that can be transaminated (and then metabolised) to provide nitrogen for the synthesis of glutamine. If provision of glutamine for cells of the immune system is the major reason for increased protein breakdown then it can be argued that the increased rate of gluconeogenesis, which occurs under these conditions, is a *consequence* and *not a cause* of the increased rate of protein degradation (fig. 2). This is not just of academic interest since some important consequences stem from this interpretation. For example, improving nitrogen balance (i.e. decreasing protein degradation) in a patient *without* maintaining the plasma levels of glutamine could be of no value or may even be detrimental to the immune system and hence to recovery of the patient.

This speculative proposal raises a further interesting point: if net protein degradation in muscle occurs primarily to provide nitrogen for the formation of glutamine – and the use of glutamine by immune cells is to provide branched-point sensitivity, then the amounts of nitrogen actually incorporated into new protein, DNA and RNA is very small – and the majority of the nitrogen is released in the form of glutamate, aspartate and alanine (plus, of course, ammonia). Why then does the body not attempt to 'salvage' this nitrogen – that is, convert the products of the immune cell metabolism, back into glutamine rather than to urea (fig. 3)? This would then be similar to the Cori cycle in which glucose that is converted to 3-carbon intermediates such as lactate and alanine can be converted back to glucose via hepatic gluconeogenesis. Could it be that a nitrogen 'salvage process' has been adaptively lost in Western humans due to continuous availability of a diet rich in protein?

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