Branched-Chain Amino Acids: Metabolism, Physiological Function, and Application

Branched-Chain Amino Acids and Immunity[1,2]

Phillip C. Calder[3]

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

ABSTRACT Although there has been great interest in the effects of amino acids on immune function, little is known about the impact of changes in BCAA availability on the ability of the immune system to function. Human immune cells incorporate BCAA into proteins and are able to oxidize BCAA. The immune system exists to protect the host from pathogenic invaders and from other noxious insults. Upon infection, there is a marked increase in demand for substrates by the immune system; these substrates provide energy and are the precursors for the synthesis of new cells, effector molecules, and protective molecules. Cell culture studies show that BCAA are absolutely essential for lymphocytes to synthesize protein, RNA, and DNA and to divide in response to stimulation. In mice, dietary BCAA restriction impairs several aspects of the immune function and increases the susceptibility to pathogens. Postsurgical or septic patients given BCAA intravenously showed improved immunity and this may relate to improved outcome. BCAAs are therefore absolutely essential for lymphocyte responsiveness and are necessary to support other immune cell functions. However, many aspects of BCAA and its effects on immune function have been understudied or not studied at all. More research is needed to understand the extent of the immune system's requirement for BCAA. It is likely that the essentiality of BCAA for the function of immune cells relates to protein synthesis. J. Nutr. 136: 288S–293S, 2006.

KEY WORDS: • *branched-chain amino acids* • *isoleucine* • *leucine* • *valine* • *immunity* • *lymphocyte*

The immune system exists to protect the host from pathogenic (i.e., infectious) invaders and from other noxious insults. Upon infection there is a marked increase in demand for substrates by the immune system; these substrates provide energy and are the precursors for the synthesis of new cells, effector molecules (e.g., antibodies, cytokines, acute phase proteins), and protective molecules (e.g., glutathione). The physiology and biochemistry of an infected individual is fundamentally changed in a way that will ensure that the immune system receives nutrients from within the body. Muscle protein is catabolized to provide amino acids for synthesizing new cells, proteins, and peptides for the immune response. Furthermore, amino acids can be used as fuel by the immune system either directly, or following their conversion to other amino acids (e.g., glutamine) or to glucose. The extent of the catabolic process is highlighted by the significant increase in urinary nitrogen excretion from 9 g/d in mild infection to 20–30 g/d following major burns or severe traumatic injury (1). The loss of nitrogen from the body of an adult during a bacterial infection may be equivalent to 60 g of tissue protein, and in a period of persistent malarial infection, it may be equivalent to over 500 g of protein. Catabolic states are associated with increased susceptibility to infections. This may relate to a suboptimal supply of substrates, including amino acids, to the immune system. Thus, it is possible that an enhanced supply of key amino acids in certain clinical settings will improve patient outcomes. There has been great interest in the effect of amino acids on immune function (2). However, this interest has centered largely upon glutamine (3–6), arginine (7–9), and, to a lesser extent, sulfur-containing amino acids (10,11), whereas much less is known about the impact of changes in BCAA availability on the ability of the immune system to function. The aim of this review is to summarize current knowledge about the effect of altered BCAA availability on the capacity of the immune system to respond efficiently when challenged and on the subsequent ability of the host to deal with infectious agents. The article begins with a description of the immune system and its components and how they respond in an integrated manner when challenged. An extended description of the immune response may be found in any immunology textbook.

The immune system: An overview

The immune system acts to protect the host from infectious agents that exist in the environment (bacteria, viruses, fungi, parasites) and other noxious insults. The immune system has two functional divisions: the innate (or natural) and the acquired (also termed specific or adaptive). Both components

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the conference "Symposium on Branched-Chain Amino Acids," held May 23–24, 2005 in Versailles, France. The conference was sponsored by Ajinomoto USA, Inc. The organizing committee for the symposium and guest editors for the supplement were Luc Cynober, Robert A. Harris, Dennis M. Bier, John O. Holloszy, Sidney M. Morris, Jr., and Yoshiharu Shimomura. Guest editor disclosure: L. Cynober, R. A. Harris, D. M. Bier, J. O. Holloszy, S. M. Morris, Y. Shimomura: expenses for travel to BCAA meeting paid by Ajinomoto USA; D. M. Bier: consults for Ajinomoto USA; S. M. Morris: received compensation from Ajinomoto USA for organizing BCAA conference.

[2] Author Disclosure: No relationships to disclose.

[3] To whom correspondence should be addressed. E-mail: pcc@soton.ac.uk.

of immunity involve various blood-borne factors and cells. All cells of the immune system originate in bone marrow. They are found circulating in the bloodstream, organized into lymphoid organs such as the thymus, spleen, and lymph nodes, or dispersed in other locations around the body.

Innate immunity is the first line of defense against infectious agents. It is present before exposure to pathogens and is concerned with preventing the entry of infectious agents into the body and, if they do enter, with their rapid elimination. The innate immune system includes physical barriers, soluble factors, and phagocytic cells. Innate immunity has no memory and is therefore not influenced by prior exposure to an organism. Phagocytic cells express surface receptors specific for bacterial antigens. Binding of antigen to the receptors triggers phagocytosis and subsequent destruction of the pathogenic microorganism by complement or by toxic chemicals, such as superoxide radicals and hydrogen peroxide. Natural killer cells also possess surface receptors and destroy pathogens by releasing cytotoxic proteins. In this way, innate immunity provides a first line of defense against invading pathogens. However, an immune response often requires the coordinated actions of both innate immunity and the more powerful and flexible acquired immunity (**Figure 1**).

The acquired immune response involves lymphocytes. It is highly specific, since each lymphocyte carries surface receptors for a single antigen. The acquired immune response becomes effective over several days after the initial activation, but it also persists for some time after the removal of the initiating antigen. This persistence gives rise to immunological memory, which is the basis for a stronger, more effective immune response upon re-exposure to an antigen (i.e., reinfection with the same pathogen).

B lymphocytes are characterized by their ability to produce antibodies (antigen-specific immunoglobulins; Ig), which are specific for an individual antigen. Immunity involving antibodies (humoral immunity) deals with extracellular pathogens. However, some pathogens, particularly viruses, but also some bacteria, infect individuals by entering cells. These pathogens will escape humoral immunity and are instead dealt with by cell mediated immunity, which is conferred by T lymphocytes. T lymphocytes express antigen-specific T-cell receptors on their surface. However, unlike B lymphocytes, they are only able to recognize antigens that are presented to them on a cell surface; this is the distinguishing feature between humoral and cell mediated immunity. Therefore, infection of a cell by an intracellular pathogen is signaled to T lymphocytes by cell surface

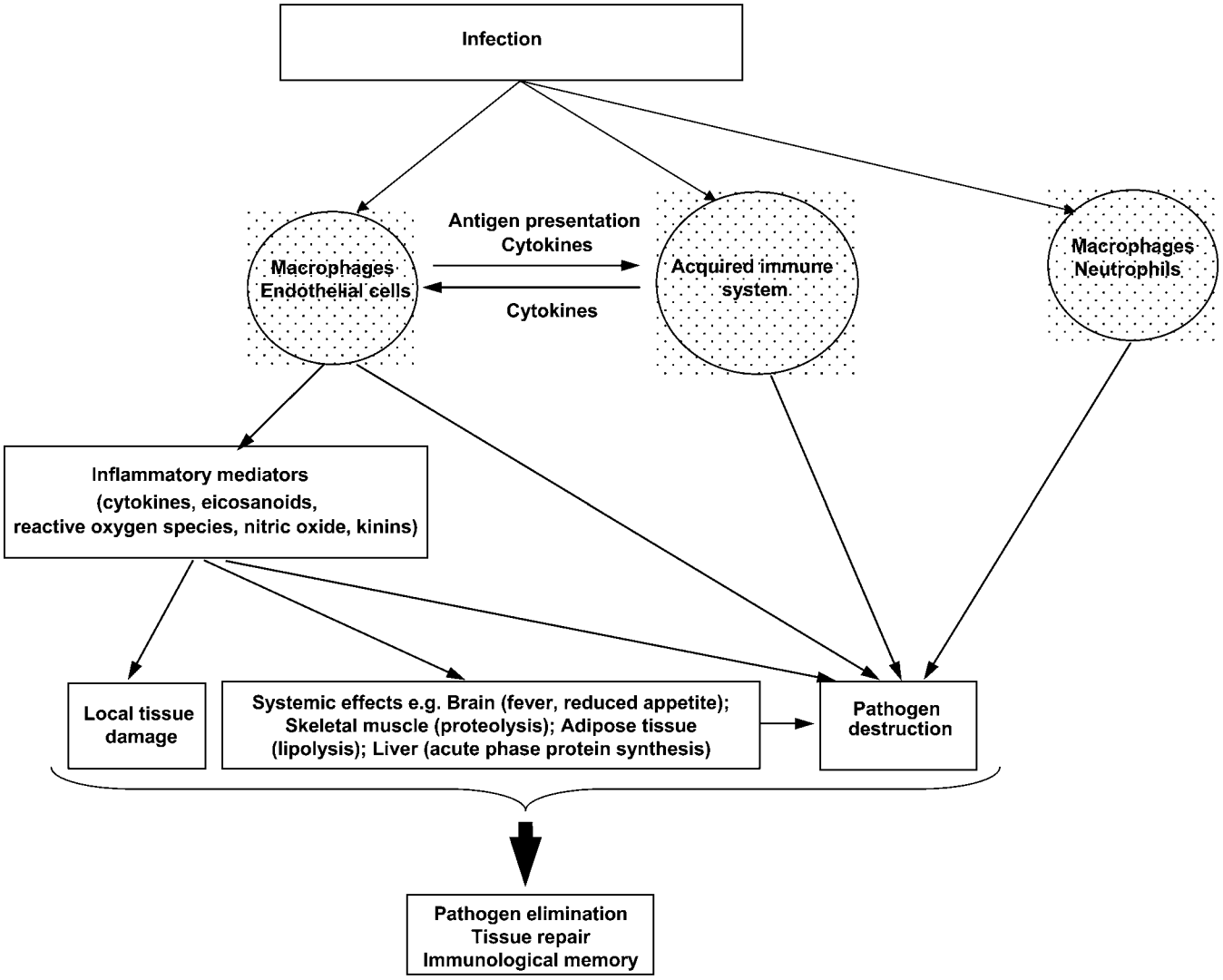

FIGURE 1 Overview of the immune system showing interactions among various components.

expression of peptide fragments derived from the pathogen. These fragments are transported to the surface of the infected cell and expressed there in conjunction with proteins called the major histocompatibility complex (MHC);[4] in humans, MHC is referred to as the human leukocyte antigen. It is the combination of the pathogen-derived peptide fragment bound to MHC that is recognized by T lymphocytes. Intracellular pathogens stimulate cytotoxic T lymphocytes to destroy the infected cell, while extracellular pathogens stimulate a helper T-cell–mediated response.

Communication within the acquired immune system and between the innate and acquired systems is brought about by direct cell to cell contact and by the production of chemical messengers. Chief among these chemical messengers are cytokines, which can have multiple activities on different cell types. Tumor necrosis factor-α, interleukin (IL)-1 and IL-6 are among the most important cytokines produced by monocytes and macrophages. Helper T lymphocytes produce IL-2 and interferon-γ among other cytokines.

Utilization of BCAA by cells of the immune system

Human immune cells incorporate BCAA into proteins; incorporation of isoleucine is greatest into lymphocytes, followed by eosinophils, followed by neutrophils (12), and perhaps reflecting cell specific differences in protein-synthetic rates and in the types of proteins made. Furthermore, human immune cells express branched-chain alpha keto acid dehydrogenase and decarboxylase activities (13,14) and so can oxidize BCAA. Indeed, human lymphocytes take up and oxidize leucine in vitro (15–18). Isoleucine is oxidized by human neutrophils and lymphocytes (12). Mitogen stimulation of lymphocytes increases leucine transport by 270%, leucine transamination by 195% and leucine oxidation by 122% (18). α-Keto–isocaproic acid increases the activity of lymphocyte branched-chain keto acid dehydrogenase (14) suggesting that leucine can promote its own degradation. Glutamine, valine, and isoleucine all decrease leucine oxidation by human lymphocytes (14,17). The uptake of BCAA by a B cell line was studied as a function of progress through the cell cycle (19). The pattern of uptake of all three BCAAs through the cell cycle is the same, although the order of the rate of uptake is leucine $>$ isoleucine $>>$ valine. The highest rate of uptake of BCAA is during the S phase, with a progressive decline in uptake through the G2 and M phases, the nadir coinciding with the time of mitosis, followed by an increase in uptake through the G1 phase. The rate for BCAA uptake most likely reflects the timing of protein synthetic activity.

BCAA and immune cell function

Cell culture studies. Early work by Eagle and co-workers (20–24) established that leucine, isoleucine, and valine are among the 13 amino acids absolutely required by cultured mammalian cells including lymphocytes. The concentrations of leucine, isoleucine, and valine in Roswell Park Memorial Institute medium, a typical commercial cell culture medium, are 0.38, 0.38, and 0.17 mmol/L, respectively. These concentrations are 2- to 3-fold higher than those typically found in the plasma of healthy adult humans (25–27). Observations that the omission of a single BCAA from the medium of cultured lymphocytes results in complete abolition of protein synthesis (28,29) simply reflect the essentiality of these amino acids. Omission of leucine, isoleucine, or valine from the medium of cultured lymphocytes also abolishes the ability of lymphocytes to proliferate in response to phytohemagglutinin by 82%, 90%, and 100%, respectively (30). However, this most likely reflects an inability to synthesize proteins required for cellular proliferation to occur. There is little information from cell-culture studies about the immunologic effect in variations in BCAA concentrations over the range that might occur physiologically or pathophysiologically. Skaper at al. (13) established human lymphocytes in long-term culture and investigated the effects of altering the concentration of BCAA on their growth. Changing total BCAA concentration from 0.4 to 0.2 mmol/L had little impact on lymphocyte growth, although decreasing total BCAA concentration below 0.2 mmol/L slowed the increase in cell number over time (13). Increasing total BCAA concentration above 1 mmol/L did not enhance cell number, and in fact tended to decrease it slightly (13). In agreement with this finding, Espositio et al. (31) showed that increasing individual BCAA concentrations by 300% and 1000% above those normally found in culture medium did not alter proliferation of human T lymphocytes in response to phytohemagglutinin. These cell culture studies indicate that lymphocyte proliferative responses are impaired by a substantial reduction in BCAA availability, but are little affected by increases in BCAA concentrations above those that are normally observed in plasma.

Animal feeding studies. Dietary leucine or valine restriction (to either 25% or 50% of the standard amount) results in significant impairment of spleen lymphocyte-mediated tumor-cell lysis in tumor-bearing mice (32). The effects are dose dependent with a 78% or 90% decrease in cell killing after the lowest dietary leucine or valine intake, respectively (32). In another study, free BCAAs were added to a rat diet containing 14% casein, and the impact on liver-associated lymphocytes in a model of liver cirrhosis was examined (33); control rats consumed a standard diet containing 24% casein. Addition of BCAA results in a greater number of liver-associated lymphocytes than in control rats, and in a greater number of cytotoxic T lymphocytes and natural killer cells. Liver natural killer- and lectin-dependent cytotoxic cell activities tend to be higher with BCAA addition, and the total liver capacity of target cell killing is higher (33). These studies indicate that the amount of BCAA in the diet of laboratory rodents can affect killer-cell activities; typically these are involved in the elimination of virally infected or tumor cells.

Petro and Bhattacharjee (34) fed mice diets containing the normal levels of protein and of individual amino acids, except they limited BCAA (by 50%) in one, and then examined a number of immune outcomes and the susceptibility to infection with *Salmonella typhimurium*. Dietary restriction of any of the BCAAs results in higher mortality to *S. typhimurium* SR11 (or SL3770) and a greater number of viable *S. typhimurium* in the liver and spleen (**Table 1**). The ability of peritoneal macrophages to phagocytose and to kill *S. typhimurium* is not affected by BCAA restriction. Vaccination of BCAA-restricted mice with heat-killed *S. typhimurium* SL3770 results in lower serum antibody titers and decreases protection against the live bacteria (Table 1). These findings indicate that limiting the amount of any of the BCAA in the diet results in impaired host defense against live *S. typhimurium* (and perhaps other microorganisms) and in an impaired response to vaccination. The impaired host defense does not appear to involve macrophages, but may involve lymphocytes. Indeed, BCAA restriction results in a lower number of spleen cells (Table 1), perhaps indicative of decreased lymphocyte proliferation in vivo. Although the absolute proliferative response of spleen lymphocytes to phytohemagglutinin is not significantly affected by BCAA restriction

[4] Abbreviations used: IL, interleukin; MHC, major histocompatibility complex.

TABLE 1

Effect of limiting dietary BCAA on immune functions and infectious mortality in mice[1]

Dietary limitation	Mortality of CF1 mice injected with SR11 (at 14 d)	Mortality of CF1 mice injected with SL3770 (at 14 d)	Viable SR11 in spleen and liver 3 d after injection (log 10)[2]	Mortality of CF1 mice injected with SL3770 5 d post immunization (number at 14 d)	Total spleen cells ($\times 10^{-7}$)[2]	Proliferative response of spleen cells to PHA (cpm $\times 10^{-3}$)[2]	Spleen cell stimulation index to PHA[3]
None	12/21	1/23	5.5 ± 0.9	0/17	9.5 ± 1.6	22 ± 18	22
Leucine	19/20*	3/22	6.3 ± 0.9	4/17*	8.4 ± 1.4	27 ± 26	12
Isoleucine	18/18*	4/19*	6.5 ± 0.9*	5/15*	3.1 ± 1.9*	44 ± 22*	15
Valine	16/19*	12/17*	6.5 ± 0.9*	4/15*	3.6 ± 1.0*	44 ± 29	13

[1] Data are taken from Petro and Bhattacharjee (34).
[2] Data are mean ± SD ($n \geq 5$ per group).
[3] Results estimated from data available and statistical analysis not possible.
* Different from control group (i.e., no dietary limitation) ($P < 0.05$).

(34), the stimulation index (i.e., proliferation in the presence or absence of mitogen) is lower (Table 1). This observation of decreased ex vivo proliferation of spleen lymphocytes taken from animals fed diets with restricted amounts of BCAA is in accordance with the smaller size of the spleen in those animals.

Although it is clear that insufficient availability of BCAA impairs some aspects of immune function, including killer-cell activity and lymphocyte proliferation (see above), an excess of leucine in a rat diet containing a lower than normal amount of protein also results in immune impairment. Feeding young growing rats a diet containing 3% of dry weight as leucine for 2 wk, and then 7% of dry weight as leucine for 6 wk, results in a marked decrease in the number of rosette-forming and plaque-forming cells in the spleen if the diet contains 4% by weight of casein, but not if it contains 18% by weight of casein (35). The decrease is also evident in the serum antibody response to immunization with sheep red blood cells if the diet contains 4% by weight of casein (35). The detrimental immunologic effects of excess leucine seen against a low protein background are avoided by adding leucine and valine to the low protein diet (**Table 2**), suggesting that the effects are due to an imbalance among the BCAAs (36).

Vitamin B-2, but not an amino acid solution, enhances survival of mice to a normally lethal dose of endotoxin (37). However, the combination of vitamin B-2 and the amino acid solution, which included all three BCAAs, enhances survival further. Isoleucine or valine, but not leucine, improve the effect of vitamin B-2, although the dose of amino acid used is very important in determining its effect.

Human studies. Early work focused on the use of parenteral BCAA in patients postsurgery (38,39). Patients received a solution containing 45% BCAA (leucine:isoleucine:valine; 1:4:7 by vol) for 7 d postsurgery; controls received a standard solution that included 24% BCAA. The two solutions were isonitrogenous and isocaloric. Patients receiving the high BCAA solution had higher blood lymphocyte counts at d 7 postsurgery (225% higher than presurgery and 50% higher than the control group at d 7). All patients were anergic to intradermal application of tuberculin, mumps, and Candida preoperatively, indicating marked impairment of cell mediated immunity. However, at d 7, 60% of patients in the high-BCAA group were responsive compared with only 10% of patients in the control group. Details of what antigens caused a response and the extent of the response were not given. The improved immune response with administering BCAA may be relevant to the findings of another early study. Freund et al. (40) demonstrated that patients who survived sepsis had higher total plasma BCAA concentrations (mean 0.34 mmol/L) than septic patients who died (mean 0.232 mmol/L). This may be related to better immune function in the survivors, but immune parameters were not reported. One more-recent study reported that septic patients receiving the high-BCAA preparation showed decreased mortality (41). However, another study failed to show that parenteral BCAA effects mortality related to sepsis or stress (42).

Bassit et al. (43) reported the effect of BCAA-supplemented diets on some immune parameters of young male elite athletes; however, the study design was complicated and differed between triathletes and runners, making the interpretation of the

TABLE 2

Effect of BCAA in a low-protein diet upon immune function in young rats[1]

Dietary casein %:	18	4	4	4
Leucine added %:	0	0	7	7
Isoleucine + valine added % :	0	0	0	0.2 + 0.2
Thymus weight *mg*	533 ± 52[a]	119 ± 14[b]	48 ± 6[c]	159 ± 10[d]
Spleen weight *mg*	659 ± 48[a]	198 ± 16[bc]	158 ± 12[b]	218 ± 15[c]
Cervical lymph node weight *mg*	24 ± 4[a]	9 ± 1[b]	6 ± 1[b]	11 ± 1[c]
Lymphocytes *per mg spleen*	1263 ± 58[a]	1005 ± 17[b]	863 ± 59[b]	990 ± 45[b]
Rosette forming cells *per* 10^6	7322 ± 3777[a]	5250 ± 2665[a]	117 ± 57[b]	2441 ± 587[a]
Plaque forming cells *per* 10^6	131 ± 17[a]	122 ± 28[ab]	8 ± 2[c]	55 ± 6[bc]
IgG *log10*	2.22 ± 0.14[a]	2.11 ± 0.17[a]	0[b]	1.85 ± 0.05[a]

[1] Data are from Aschkenasy (36). Data are mean ± SEM ($n = 7$ or 5 per group). Values across a row not sharing a common superscript letter are significantly different from one another ($P < 0.05$).

findings difficult. BCAA supplementation resulted in higher lymphocyte proliferation in response to concanavalin A or endotoxin compared with the placebo group. BCAA also prevented exercise-induced decreases in the production of tumor necrosis factor-α and IL-1 by stimulated mononuclear cells, increased the production of interferon-γ and decreased the production of IL-4.

Discussion and conclusion

Cell culture and animal feeding studies indicate that an adequate supply of BCAA is necessary to support efficient immune function. However, many aspects of BCAAs and their effect on immune function have been understudied or not studied at all. For example, the importance of BCAAs in supporting the production of different immunoglobulins and cytokines and in permitting antigen processing and presentation to occur is not known. Clearly more research is needed before the extent of the immune system's requirement for BCAA is fully clarified.

Although BCAAs are oxidized by immune cells, at least in vitro, it seems unlikely that their role as substrates for generating energy is the mechanism underlying their essentiality. This is because cells in culture have many other energy sources available to them, including glucose and glutamine, and the relative contribution of BCAAs to energy generation is small. Thus, a total lack of any single BCAA is not likely to impair the ability of cells to generate energy. BCAAs also act as donors of nitrogen and of carbon skeleton for the synthesis of other amino acids, like glutamine, that are important in supporting immune cell function (3–6). Undoubtedly, this is important at the whole-body level and may account for some of the immunologic benefits of enhanced-BCAA supply to athletes (43) and to postsurgical and septic patients (38–41) who frequently show partial depletion of muscle and plasma glutamine (25,26,44–50). However, cell culture studies conducted in the presence of optimal glutamine concentrations reveal an absolute requirement for BCAAs (28–30), suggesting that the BCAAs themselves are needed, at least by isolated immune cells. It seems most likely that the essentiality of BCAAs for immune cells relates to protein synthesis. The immune system has a high dependence upon protein synthesis, since mounting an immune response requires generation of new cells and synthesis of antigen-presenting machinery, immunoglobulins, cytokines, cytokine receptors, acute phase proteins etc. Clearly insufficient availability of BCAAs does not allow the synthesis of these proteins to occur optimally and so will prevent an optimal immune response from being mounted. In the cell culture situation where one BCAA is absent, the inability to synthesize protein abolishes the ability of lymphocytes to respond to stimulation. Cell culture and animal feeding studies, where the amounts of BCAA available have been limited, clearly demonstrate that there is a dose-response relationship between BCAA supply and immune response. However this relationship is poorly defined.

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