

WJG 20<sup>th</sup> Anniversary Special Issues (11): Cirrhosis**Impairment of innate immune responses in cirrhotic patients and treatment by branched-chain amino acids**

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**Abstract**

It has been reported that host defense responses, such as phagocytic function of neutrophils and natural killer (NK) cell activity of lymphocytes, are impaired in cirrhotic patients. This review will concentrate on the impairment of innate immune responses in decompensated cirrhotic patients and the effect of the treatment by branched-chain amino acids (BCAA) on innate immune responses. We already reported that phagocytic function of neutrophils was significantly improved by 3-mo BCAA supplementation. In addition, the changes of NK activity were also significant at 3 mo of supplementation compared with before supplementation. Also, Fisher's ratios were reported to be significantly increased at 3 mo of BCAA supplementation compared with those before oral supplementation. Therefore, administration of BCAA could reduce the risk of bacterial and viral infection in patients with decompensated cirrhosis by restoring impaired innate immune responses of the host. In addition, it was also revealed that BCAA

oral supplementation could reduce the risk of development of hepatocellular carcinoma in cirrhotic patients. The mechanisms of the effects will also be discussed in this review article.

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**Key words:** Branched-chain amino acids; Liver cirrhosis; Innate immunity; Natural killer cell activity of lymphocytes; Phagocytic function of neutrophils

**Core tip:** This review will discuss the recent research on impairment of innate immune responses in cirrhotic patients and the treatment by branched-chain amino acids (BCAA). It was revealed that BCAA oral supplementation could improve not only nutrition status but phagocytic function of neutrophils and natural killer activity of lymphocytes in cirrhotic patients. Therefore, BCAA supplementation might reduce the risk of bacterial and viral infection in patients with decompensated cirrhosis. Additionally, it was also revealed that BCAA oral supplementation could reduce the risk of development of hepatocellular carcinoma in cirrhotic patients. The mechanisms of the effects of BCAA described above will also be discussed.

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**INTRODUCTION**

Innate immunity is the first defense mechanism of host against pathogens such as bacteria and viruses. Patients with insufficient innate immunity may have increased

risk of bacterial and viral infection. Patients with liver cirrhosis, especially those with decompensated cirrhosis, are liable to suffer from bacterial infection such as bacteremia and spontaneous bacterial peritonitis (SBP), which could be lethal to the patients. The incidence of bacterial infections in cirrhotic patients is almost 4-7 times greater than that of general hospital population<sup>[1,2]</sup>. SBP occurs in 10%-25% of hospitalized cirrhotic patients and its mortality rate is 17%-50%<sup>[3]</sup>. The high incidence of bacterial infections in patients with cirrhosis has prompted an assessment of defects in their immune defense against microorganisms. The functional studies of peripheral blood neutrophils have shown defective phagocytic activity and intracellular killing activity in patients with cirrhosis<sup>[4-9]</sup>. In addition, natural killer (NK) cells also constitute the first line of host defense against invading pathogens. They usually become activated in an early phase of viral infection. Effective clearance of an acute viral infection requires the coordinated function of multiple arms of the immune systems, including innate immune systems mediated by NK cells and cytokines such as interferon, as well as adaptive immune responses. It was reported that not only phagocytic function of neutrophils but NK activity of lymphocytes were impaired in cirrhotic patients<sup>[10-14]</sup>.

So far, there are few therapies for restoration of activity of immune responses of innate immunity in patients with liver cirrhosis. Branched-chain amino acid (BCAA) supplementation has been previously shown to improve serum albumin levels and Fisher's ratios in those patients<sup>[15-17]</sup>. In addition, BCAA was also shown to improve impaired glucose tolerance of the cirrhotic patients with creatinine height index (CHI) greater than 80 by Urata *et al*<sup>[18]</sup>. It was reported that BCAA oral supplementation might reduce the incidence of events comprising the primary endpoint (which was a composite of death by any cause, development of liver cancer, rupture of esophageal varices, or progress of hepatic failure<sup>[19]</sup>). In addition, recent studies have revealed that BCAA promotes albumin synthesis in rat hepatocyte through activation of mammalian target of rapamycin (mTOR) signal transduction system<sup>[20]</sup>. Much is still unknown on the effects of BCAA supplementation on reactions of innate immunity. This review will concentrate on the impairment of innate immune responses in decompensated cirrhotic patients and the effect of the treatment by BCAA on innate immune responses.

## IMMUNE RESPONSES IN CIRRHOTIC PATIENTS

### ***Increased risk of infection and death in cirrhotic patients***

It was reported that alterations of the immune system are common in patients with end-stage liver disease and associated with an increased risk of infection and death<sup>[1-3,21-24]</sup>. Bacterial infection involving such as urinary tract, ascites, blood, respiratory tract is a severe complication of decompensated cirrhosis. Additionally, it might induce longer hospital stay and increased mortality<sup>[21]</sup>. It

was previously reported that infections in patients with cirrhosis increase mortality 4-fold; 30% of patients die within 1 mo after infection and another 30% die by 1 year<sup>[22]</sup>. In patients with cirrhosis and severe sepsis, high production of proinflammatory cytokines seems to play a role in the worsening of liver function and the development of organ failures such as shock, renal failure, acute lung injury or acute respiratory distress syndrome, coagulopathy, or hepatic encephalopathy<sup>[23]</sup>.

### ***Impairment of innate immune response in cirrhotic patients***

Functional abnormalities of neutrophils and macrophages<sup>[4-9]</sup>, NK cells<sup>[10-14]</sup>, and the complement system<sup>[25]</sup> contribute to impaired innate immune responses. On Neutrophils, it was reported that there was a defect of neutrophil phagocytosis and a defect of intracellular killing of bacteria in cirrhotic patients. In addition, it was revealed that these neutrophil defects are caused by both reduced production of superoxide and defects of degranulation<sup>[4]</sup>. In addition, the function of macrophage Fc gamma receptors was reported to be impaired in patients with cirrhosis, and this impairment probably contributes to the high incidence of bacterial infections among such patients<sup>[6]</sup>. Neutrophil migration and phagocytosis were reported to be decreased in cirrhotic patients with previous episodes of bacterial infection compared with non-infected patients. In addition, expression of complement receptor type III (CR3) in circulating neutrophils was significantly higher in cirrhotic patients. These data suggest that deficient neutrophil recruitment to the infection site and impaired phagocytic activity may contribute to bacterial infections in cirrhotic patients with advanced liver disease<sup>[5]</sup>. Polymorphonuclear cells (PMNs) obtained from cirrhotic patients were reported to be less effective than those from controls in producing O<sub>2</sub><sup>-</sup> after stimulation with opsonized zymosan, while they were more effective in producing NO, NO synthase activity was higher in leukocytes from cirrhotic patients than in controls<sup>[8]</sup>. It was also reported that the plasma of cirrhotic patients induced neutrophil phagocytic dysfunction and the degree of the impairment was greater in cirrhotic patients with more severe disease<sup>[9]</sup>. The study also clarified that dysfunction of phagocytic function of neutrophils was associated with increased expression of toll-like receptors 2 and 4. Stable cirrhosis is characterized by neutrophil phagocytic dysfunction which may be subtle and only revealed in inflamed peripheral tissues where excessive inflammatory mediators continue to be released (Table 1).

NK cell activity was revealed to be significantly decreased in cirrhotic patients compared with normal controls and that in patients with other, non-malignant diseases, supporting the notion that immune-surveillance mechanisms may be affected in these patients<sup>[10-12]</sup>. Chuang *et al*<sup>[12]</sup> reported that cirrhotic patients with Child Pugh's C grade of severity of liver disease had lower NK cell activity. The depression of NK cell activity in cirrhotic patients was inversely correlated with prothrombin time ratios.

**Table 1 Impairment of innate immune responses in cirrhotic patients**

Impairment of phagocytic activity of neutrophils
Impairment of intracellular killing activity of neutrophils
Impairment of migration of neutrophils
Impairment of natural killer cell activity
Impairment of function of macrophage Fc gamma receptors
Impairment of opsonisation activity
Impairment of hemolytic complement function

And NK cell activity in cirrhotic patients with hepatic encephalopathy was lower than that in patients without hepatic encephalopathy. Thus, the diminished NK cell activity in cirrhotic patients might be related to the severity of liver damage<sup>[12]</sup>. Reduction of NK cell activity might occur partially due to lower frequency of NK cell in peripheral blood<sup>[13]</sup>. However, the mechanisms of diminished NK cell activity was not clarified so far.

Also, acquired deficiencies of certain complement proteins and impaired opsonisation activity was reported to be implicated in the pathogenesis of the increased susceptibility to infections of patients with cirrhosis. Low serum C3 concentrations and decreased haemolytic complement function predispose to infection and increased mortality in patients with cirrhosis<sup>[25]</sup>.

#### **Impairment of adaptive immune response in cirrhotic patients**

On the adaptive immune response in cirrhotic patients, there was evidence to suggest that the response was defective<sup>[26,27]</sup>. Generally speaking, impairment of adaptive immune responses can be due to either impairment of antigen presentation of professional cells or to reduction of T cell responses. T cell such as cytotoxic T lymphocyte (CTL) and helper T cell (Th) recognize a peptide-MHC complex on antigen presenting cell (Table 2). In cirrhotic patients, levels of interleukin-10 (IL-10) in blood became high due to increase of endotoxin and tumor necrosis factor-alpha (TNF- $\alpha$ )<sup>[28-30]</sup>. IL-10 could reduce cytokine responses of T cells, reduction of MHC class II expression on antigen-presenting cells and suppression of co-stimulatory signals<sup>[31-34]</sup>.

In addition, there was a report on association of T cell responses *in vitro* and markers of bacterial translocation, serum IL-10, monocyte HLA-DR expression and T cell subsets in cirrhotic patients<sup>[24]</sup>. Advanced liver disease predisposes to bacterial translocation and endotoxaemia which can contribute to elevated circulating levels of IL-10 and down-regulation of MHC class II on antigen-presenting cells. Peter *et al*<sup>[24]</sup> evaluated antigen-specific T-cell responses toward common viral antigens in order to investigate defects in cellular immunity in cirrhosis. Compared to healthy controls, patients with cirrhosis had higher circulating levels of LBP and IL-10, an expansion of peripheral blood CD14<sup>+</sup> monocytes with low HLA-DR expression and an increased fraction of CD25-positive CD4<sup>+</sup> and CD8<sup>+</sup> T cells. These findings were reported to be most pronounced in cirrhotic patients with

**Table 2 Impairment of adaptive immune responses in cirrhotic patients**

Down regulation of major histocompatibility complex class II on monocyte/macrophages
Impairment of maturation and function of myeloid dendritic cells
Inhibition of T cell proliferation and T cell-mediated cytokine production
Inhibition of T-cell co stimulatory pathways
Impairment of tumor necrosis factor-alpha production of CD4 <sup>+</sup> and CD8 <sup>+</sup> cells
Increased fraction of CD4 <sup>+</sup> CD25 <sup>+</sup> cells
Increase in circulating anti-inflammatory cytokines

systemic inflammation. Furthermore, TNF- $\alpha$  production in responding T cells was attenuated in patients with a high frequency of CD14<sup>+</sup> HLA-DR- monocytes. The results of the study suggested that bacterial translocation, endotoxaemia, inflammation and T cell activation in cirrhosis are accompanied by an increase in circulating anti-inflammatory cytokines, reduced monocytic MHC class II expression and attenuated cytokine production in T cells.

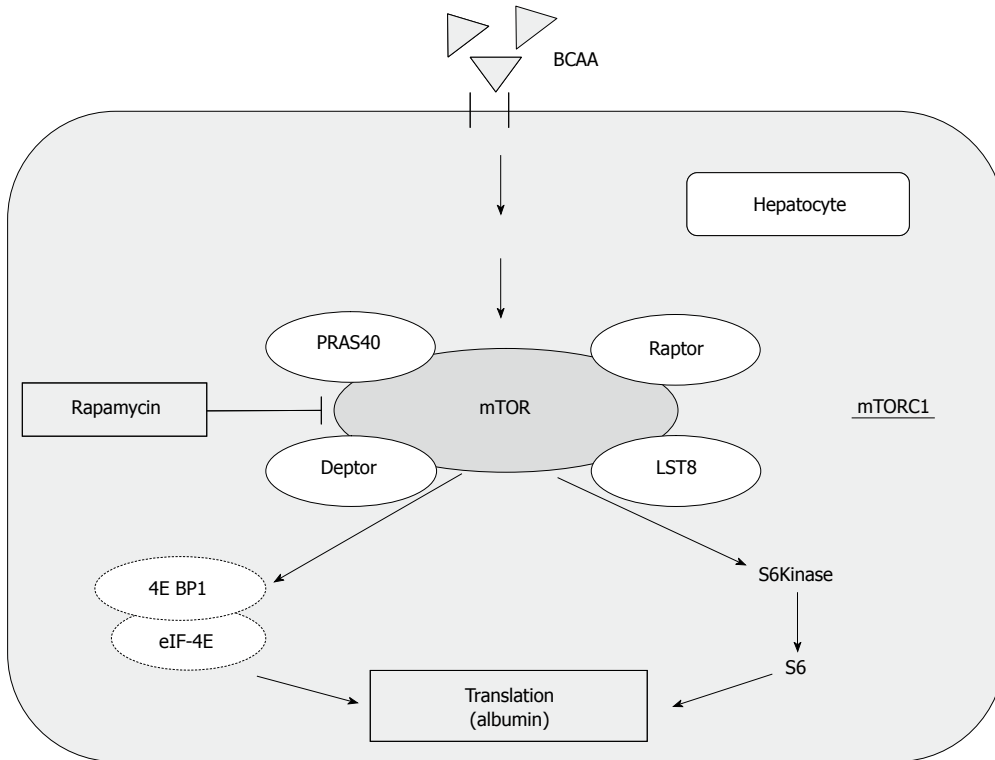
## **TREATMENT BY BCAA AND THE EFFECT ON INNATE IMMUNE RESPONSES IN CIRRHOTIC PATIENT**

### **BCAA and mTOR signaling pathway**

BCAA comprise three essential amino acids: leucine (Leu), isoleucine (Ile), and valine (Val). And chemical formulas of Leu, Ile, and Val are HO<sub>2</sub>CCH(NH<sub>2</sub>)CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, HO<sub>2</sub>CCH(NH<sub>2</sub>)CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>, and HO<sub>2</sub>CCH(NH<sub>2</sub>)CH(CH<sub>3</sub>)<sub>2</sub> respectively.

They are often used as supplemental therapy to improve protein malnutrition in patients with liver cirrhosis. Long-term oral supplementation with BCAA granules to cirrhotic patients improved their nutrition status (*i.e.*, hypoalbuminemia)<sup>[19,35]</sup>. In addition to the role of acting as nutrient substrates, recent studies have demonstrated that BCAA also serve as physiologically active substances. BCAA have been shown to have pharmacological effects, such as induction of protein synthesis<sup>[36]</sup> and promotion of glucose uptake in skeletal muscle<sup>[37]</sup>. In rat primary hepatocytes, albumin synthesis is significantly increased by BCAA administration, which is dependent on activation of the mammalian target of rapamycin (mTOR), mainly induced by leucine (Figure 1)<sup>[20]</sup>.

mTOR integrates the input from upstream pathways, including insulin, growth factors (such as IGF-1 and IGF-2), and amino acids<sup>[38]</sup>. In addition, mTOR also senses cellular nutrient, oxygen, and energy levels<sup>[39]</sup>. The mTOR pathway is dysregulated in human diseases, such as diabetes, obesity, depression, and certain cancers<sup>[40]</sup>. mTOR is the catalytic subunit of two molecular complexes: mTORC1 and mTORC2<sup>[41]</sup>. mTOR complex 1 (mTORC1) is composed of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal



**Figure 1 Mammalian target of rapamycin signaling pathway.** Branched-chain amino acids (BCAA) promotes albumin synthesis through activation of the mammalian target of rapamycin (mTOR) signal transduction system. mTOR complex 1 (mTORC1) is composed of mTOR, Raptor, LST8, PRAS40 and Deptor. Raptor: Regulatory-associated protein of mTOR; LST8: Mammalian lethal with SEC13 protein 8; PRAS40: Protein-rich Akt substrate of 40 kDa; Deptor: DEP domain-containing mTOR-interacting protein; eIF-4E: Eukaryotic translation initiation factor 4E; 4E BP1: Eukaryotic translation initiation factor 4E binding protein 1.

with SEC13 protein 8 (LST8) and the recently identified partners PRAS40 and DEPTOR (Figure 1)<sup>[42,43]</sup>. This complex is characterized by the classic features of mTOR by functioning as a nutrient/energy/redox sensor and controlling protein synthesis<sup>[38,42]</sup>. The activity of this complex is stimulated by insulin, growth factors, serum, phosphatidic acid, amino acids (particularly leucine), and oxidative stress<sup>[42,44]</sup>.

In this paragraph, several reports on promotion of albumin synthesis by BCAA through activation of the mammalian target of rapamycin (mTOR) signal transduction system were introduced (Figure 1)<sup>[20]</sup>.

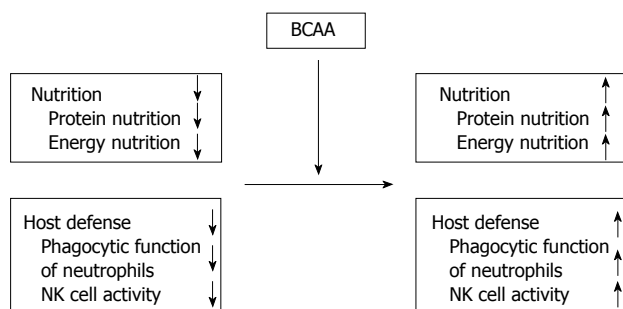
### **Restoration of innate immune responses by oral supplementation of BCAA in cirrhotic patients**

We reported on restoration of innate immune responses such as phagocytic function of neutrophils and NK cell activity<sup>[45,46]</sup>. In the reports, patients with decompensated cirrhosis received 12 g BCAA daily for 3 mo. Phagocytic function of neutrophils and NK cell activity of lymphocytes as well as Fisher's ratio were determined before and at 1 and 3 mo of BCAA supplementation. For quantification of phagocytic function, fluorescent intensities of cells in the neutrophil region in the cytogram were determined by flow cytometry after incubation of whole blood with fluorescent microspheres. NK cell activity was estimated by <sup>51</sup>Cr release assay using K-562 cell line as target cells. Fisher's ratio was reported to be significantly increased at 1 mo of BCAA supplementation compared

with that before oral supplementation and also at 3 mo of BCAA supplementation compared with that before oral supplementation. In addition, the phagocytic function of neutrophils was reported to be significantly increased at 3 mo of supplementation compared with that before BCAA supplementation. It was also reported on the effects of BCAA supplementation on NK activity of lymphocytes. NK cell activity were also significantly improved at 3 mo of supplementation compared with that before BCAA supplementation.

In the chapter of discussion in our report, the several points were discussed as follows<sup>[46]</sup>. There were few therapies to restore the reactions of innate immunity such as phagocytic function of neutrophils and NK activity of lymphocytes. The mechanisms of the improvement by BCAA supplementation were not completely elucidated. It has been reported that BCAA supplementation promotes albumin synthesis through activation of the mTOR signal transduction system<sup>[20]</sup>. In addition, not only synthesis of albumin but HGF synthesis was enhanced by BCAA supplementation. Therefore, production of a factor which could stimulate neutrophil function of phagocytosis such as tuftsin<sup>[47,48]</sup> might be increased by BCAA by mTOR signal transduction system. Alternatively, improvement of poor nutritional status in cirrhotic patient by BCAA might restore phagocytic function of neutrophils and NK activity of lymphocytes by indirect mechanisms. In the study, phagocytic function of neutrophils and NK activity of lymphocytes were





**Figure 2 Restoration of nutrition state and host immune responses by branched-chain amino acids in decompensated cirrhotic patients.** Branched-chain amino acids (BCAA) oral supplementation could not only improve nutrition state (protein nutrition, energy nutrition) but restore host defense mechanisms [phagocytic function of neutrophils and natural killer (NK) cell activity] in cirrhotic patients.

significantly restored before serum albumin level became statistically improved. That might imply a clue to clarify the mechanisms of effect on improvement of reactions of innate immunity by BCAA oral supplementation. To estimate the effects of BCAA supplementation on the innate host responses in cirrhotic patients, further studies on intrahepatic neutrophils, Kupffer cells and pit cells might be valuable.

### Reduction of the risk of hepatocellular carcinoma development by BCAA in cirrhotic patients

A multicenter, randomized, and nutrient intake-controlled trial on the comparative effects of BCAA, conducted in 646 patients with decompensated cirrhosis, showed that the incidence of events comprising the primary endpoint (which was a composite of death by any cause, development of liver cancer, rupture of esophageal varices, or progress of hepatic failure) significantly decreased in the BCAA supplementation group as compared with the control group<sup>[19]</sup>. Marchesini *et al*<sup>[35]</sup> also reported that long-term oral supplementation of BCAA granules to cirrhotic patients were reported to improve not only their nutrition status (*i.e.*, hypoalbuminemia) but their event-free survival. In addition, Muto *et al*<sup>[49]</sup> reported that close association exists between insulin resistance due to hyperinsulinemia and BCAA, and that this association contributes to the progression of hepatocellular carcinoma (HCC) in cirrhotic patients. It was also reported that the risk for liver cancer was significantly reduced by oral BCAA supplementation in the patients with a BMI of 25 or higher. Oral supplemental treatment with BCAA might reduce the risk of liver cancer in cirrhotic patients<sup>[49]</sup>. Recent studies have revealed that BCAA supplemental therapy to patients with liver cirrhosis improves their insulin resistance and hyperinsulinemia<sup>[50,51]</sup>, which can account for the reduced risk of HCC. BCAA is supposed to prevent insulin resistance through improving glucose tolerance by promoting insulin-independent glucose uptake by skeletal muscle<sup>[52]</sup>.

There have been only a few reports to date regarding the suppression of liver cancer progression by BCAA.

Murata *et al*<sup>[53]</sup> showed that isoleucine prevents tumor growth in a mouse liver metastatic model of colon cancer through inhibition of vascular endothelial growth factor (VEGF). Yoshiji *et al*<sup>[54]</sup> reported that BCAA exerts a chemopreventive effect against HCC, which is associated with the suppression of VEGF expression and hepatic neovascularization in obese diabetic rats. Both of these reports suggest an anti-angiogenesis activity of BCAA or Isoleucine through suppression of VEGF expression.

In the study by Miura *et al*<sup>[55]</sup>, they analyzed the expression of vascular VEGF in HepG2 cells under high-insulin culture conditions, and examined the effect of BCAA on VEGF expression. VEGF secretion was significantly increased by 200 nmol/L of insulin under BCAA deficient conditions, but it was repressed by the addition of BCAA. BCAA activated the mTOR pathway and increase HIF-1 $\alpha$  expression under high-insulin culture conditions, however quantitative PCR analysis showed that insulin-induced expression of VEGF mRNAs decreased 2 h after the addition of BCAA. The half-lives of VEGF mRNAs were shortened in the presence of BCAA compared to the absence of BCAA. Therefore, the results of the study suggested that BCAA regulate VEGF expression mainly at the post-transcriptional level in patients who have hyperinsulinemia and are in the process of developing HCC. They also examined which of the Valine, Leucine, and Isoleucine components of BCAA were essential for VEGF mRNA degradation. All three BCAA components were revealed to be required for acceleration of insulin-induced VEGF mRNA degradation.

Recently, another study on the mechanisms of reduction of the risk of HCC development by BCAA was reported<sup>[56]</sup>. Hagiwara *et al*<sup>[56]</sup> reported the result of the study to investigate the effects of BCAA on insulin-induced proliferation of hepatic tumor cells and determine the underlying mechanisms. BCAA was reported to suppress insulin-induced cell proliferation of H4 II E, HepG2 cells. They demonstrated that BCAA inhibited PI3K/Akt pathway not only by promoting negative feedback loop from mammalian target of rapamycin complex 1 (mTORC1)/S6K1 to PI3K/Akt pathway, but also by suppressing mTORC2 kinase activity toward Akt. Their findings suggested that BCAA supplementation may be useful to suppress liver cancer progression by inhibiting insulin-induced PI3K/Akt and subsequent anti-apoptotic pathway, indicating the importance of BCAA supplementation to the obese patients with advanced liver disease.

Furthermore, NK cells were previously revealed to play important roles not only in the defense against viral infection but in tumor surveillance<sup>[57,58]</sup>. Therefore, the restoration of NK cell activity by BCAA supplementation might partly contribute to the reduction of the risk of HCC in cirrhotic patients.

## CONCLUSION

BCAA oral supplementation could improve not only nutrition status (both protein nutrition and energy nutrition

) but phagocytic function of neutrophils and NK activity of lymphocytes in cirrhotic patients (Figure 2). BCAA supplementation might reduce the risk of bacterial and viral infection in patients with decompensated cirrhosis. In addition, administration of BCAA could improve glucose intolerance and hyperinsulinemia in cirrhotic patients. It has also been reported that the risk of developing HCC could be significantly reduced following long-term administration of BCAA in obese cirrhotic patients with diabetes mellitus.

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