

## Review Article

# Amino acids and immune function

Peng Li<sup>1</sup>, Yu-Long Yin<sup>1,2</sup>, Defa Li<sup>3</sup>, Sung Woo Kim<sup>1,4</sup> and Guoyao Wu<sup>1,2,4\*</sup>

<sup>1</sup>Faculty of Nutrition and Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

<sup>2</sup>Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, Hunan, China 41012

<sup>3</sup>National Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100094

<sup>4</sup>Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA

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A deficiency of dietary protein or amino acids has long been known to impair immune function and increase the susceptibility of animals and humans to infectious disease. However, only in the past 15 years have the underlying cellular and molecular mechanisms begun to unfold. Protein malnutrition reduces concentrations of most amino acids in plasma. Findings from recent studies indicate an important role for amino acids in immune responses by regulating: (1) the activation of T lymphocytes, B lymphocytes, natural killer cells and macrophages; (2) cellular redox state, gene expression and lymphocyte proliferation; and (3) the production of antibodies, cytokines and other cytotoxic substances. Increasing evidence shows that dietary supplementation of specific amino acids to animals and humans with malnutrition and infectious disease enhances the immune status, thereby reducing morbidity and mortality. Arginine, glutamine and cysteine precursors are the best prototypes. Because of a negative impact of imbalance and antagonism among amino acids on nutrient intake and utilisation, care should be exercised in developing effective strategies of enteral or parenteral provision for maximum health benefits. Such measures should be based on knowledge about the biochemistry and physiology of amino acids, their roles in immune responses, nutritional and pathological states of individuals and expected treatment outcomes. New knowledge about the metabolism of amino acids in leucocytes is critical for the development of effective means to prevent and treat immunodeficient diseases. These nutrients hold great promise in improving health and preventing infectious diseases in animals and humans.

### Diet: Immunity: Lymphocyte: Macrophage: Protein: Health

Malnutrition and infection are major obstacles to survival, health, growth and reproduction of animals and humans worldwide (Field *et al.* 2002; Calder & Yaqoob, 2004). This global concern has led to the development of nutritional immunology as a new scientific discipline that integrates nutrition and immunology research methodologies to define a role for nutrients in the metabolism and function of cells of the immune system at molecular, cellular, tissue and whole-body levels. Recent studies indicate that dietary protein deficiency, which reduces concentrations of most amino acids in plasma (Wu *et al.* 1999) and compromises the immune system, remains a significant nutritional problem in developing countries and also occurs in subpopulations (e.g. the elderly or hospitalised patients) of developed nations (Woodward, 1998; Dasgupta *et al.* 2005). Thus, there is growing interest in the role of amino acids in the immune function of mammals, birds, fish and other species (Roch, 1999; Calder, 2006; Grimble, 2006; Kim *et al.* 2007).

Although dietary supplementation with high-quality protein may be effective in improving protein nutritional status

in malnourished subjects (McMurray *et al.* 1981), this is not feasible for patients who cannot tolerate enteral feeding. Consequently, defining the roles of individual amino acids in immune responses can aid in developing effective strategies to improve health and prevent infectious diseases. However, only in the past 15 years have the underlying cellular and molecular mechanisms begun to unfold (e.g. Wu & Brosnan, 1992; Yaqoob & Calder, 1997; Newsholme *et al.* 2003; Field, 2005; Calder, 2006). The major objective of this article is to provide an insight into the specific roles of amino acids in immune function.

### An overview of the immune system and assessments of immune function

#### *The immune system*

Because leucocytes are important targets for the actions of amino acids, here we briefly review the pertinent literature related to the defence against infectious diseases. The immune system protects the host from various pathogens and

**Abbreviations:** BCAA, branched-chain amino acids; GSH, glutathione; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NK, natural killer; NO, nitric oxide; TNF, tumour necrosis factor; TPN, total parenteral nutrition; UCA, urocanic acid.

\* **Corresponding author:** Dr Guoyao Wu, fax +1 979 845 6057, email g-wu@tamu.edu

consists of the innate (natural, non-specific) and the acquired (adaptive, specific) systems (Calder, 1995). These two systems are highly inter-related through cytokines and signalling molecules (Table 1). The innate immune system consists of physical barriers (e.g. skin, the endothelial cell layer in the respiratory tract, and the gastrointestinal tract), mononuclear phagocytes (e.g. monocytes and macrophages), dendritic cells, polymorphonuclear granulocytes (e.g. neutrophils, eosinophils and basophils), mast cells, natural killer (NK) cells, platelets and humoral factors including collectins, complements, lysozymes, C-reactive proteins and interferons. Recently, neutrophil extracellular traps, comprising DNA and proteins as major structural components, were discovered as a mechanism against bacterial infection (Buchanan *et al.* 2006). The innate immune system can rapidly respond to invading microbes, but its major disadvantages include non-specificity and a lack of memory effect. When infection cannot be fully cleared by the innate immunity over a short period, the adaptive immune system is activated to destroy infectious agents.

The adaptive (acquired) immune system consists of T lymphocytes, B lymphocytes and humoral factors (Calder, 2006). The bone marrow is primarily responsible for haematopoiesis and lymphopoiesis, while the thymus is required for T-cell development. The spleen, lymph nodes and the mucosa-associated lymphoid tissues in the gastrointestinal, respiratory and reproductive tracts, and other organs are secondary lymphoid tissues. Because each lymphocyte carries surface receptors for a single antigen, the acquired immune response is highly specific. This immune system becomes effective over days after initial stimulation and possesses immunological memory. B lymphocytes are unique in their ability to produce and release specific antibodies in the humoral immunity. The antibodies can neutralise microorganisms (including viruses) or toxins by binding to them; activate complement proteins in plasma for the destruction of bacteria by phagocytes; immobilise bacteria; and opsonise various pathogens. When pathogens escape the humoral immunity, they are targeted by the cell-mediated immunity that involves the production of cytokines (e.g. interferon- $\gamma$  (IFN $\gamma$ )) and other cytotoxic proteins by T lymphocytes. Antibodies are highly effective against extracellular pathogens. In contrast, host cells infected with intracellular pathogens (e.g. viruses and certain bacteria)

are generally cleared by cytotoxic T lymphocytes after the fragments of pathogens and the major histocompatibility complex are presented on the host cell surface and recognised by the T cells.

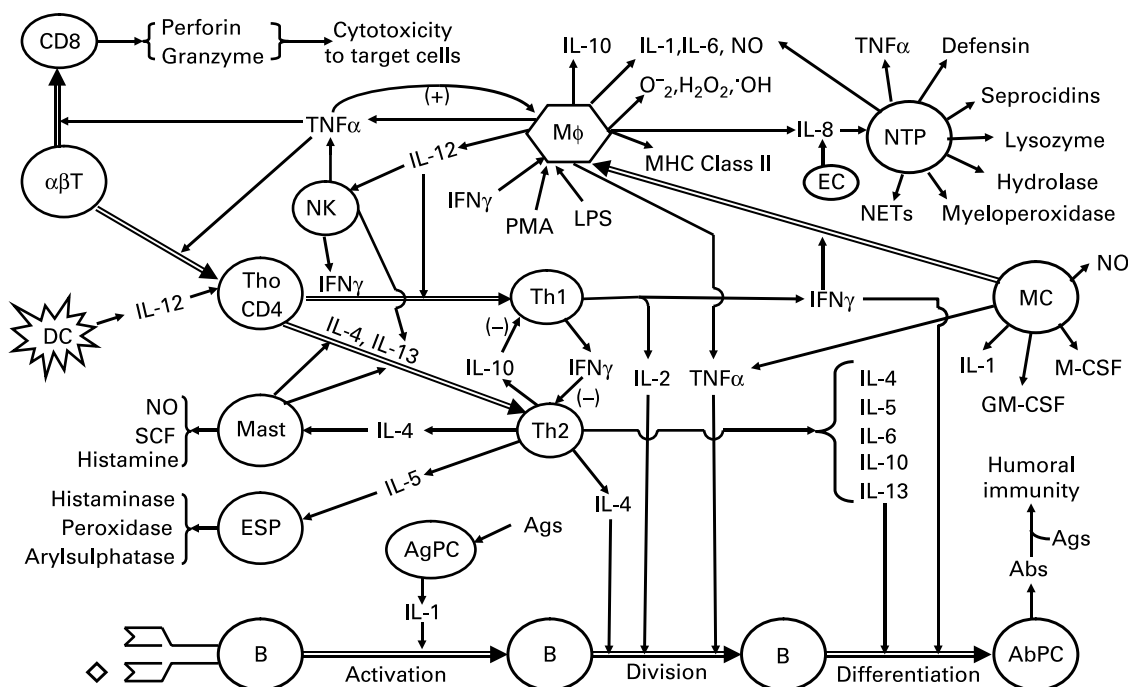
Innate and acquired immune systems are regulated by a highly interactive network of chemical communications (Fig. 1), which includes the synthesis of the antigen-presenting machinery, immunoglobulins and cytokines (Calder, 2006). Both immune systems are highly dependent upon an adequate availability of amino acids for the synthesis of these proteins and polypeptides, as well as other molecules with enormous biological importance (Kim *et al.* 2007). These substances include nitric oxide (NO), superoxide, hydrogen peroxide, histamine, glutathione and anthranilic acid (Table 2). Individual amino acids affect immune responses either directly or indirectly through their metabolites. While the immune system is vital to health, it can be dysfunctional under certain conditions, resulting in the development of autoimmune and hypersensitivity diseases, such as insulin-dependent diabetes mellitus, rheumatoid arthritis and asthma (Wu, 1995; Field, 2005).

#### Assessments of immune function

It is likely that nutrients influence several or all aspects of the immune system. Thus, there are multiple, complex methods for assessing immune function in individuals, depending on experimental conditions, the availability of analytical facilities and the investigator's interest (Field, 1996; Calder & Yaqoob, 1999). The classic functional measurements *in vivo* include: (1) the delayed-type hypersensitivity response measured by skin testing; (2) serum antibody titres or the humoral immunity in response to primary or secondary (booster) immunisation; (3) blood levels of different lymphocyte subsets as well as serum concentrations of cytokines and other immune mediators; (4) the weights of lymphoid organs; and (5) morbidity and recovery from infectious disease. The *in vitro* assays of immune function often consist of: (1) the metabolism of immunocytes; (2) lymphocyte blastogenesis (cell proliferation) in response to mitogens; (3) cell morphology and apoptosis; (4) the phagocytosis of particles by monocytes and macrophages; and (5) the production of antibodies, cytokines and low-molecular weight cytotoxic substances.

**Table 1.** Innate and adaptive immunity

	Innate/non-specific	Adaptive/acquired
Anatomical components	Skin, respiratory tract, gastrointestinal tract	Bone marrow, thymus, mucosal-associated lymphoid tissue, spleen, lymph node
Cells	Neutrophils, monocytes, macrophages, dendritic cells, mast cells, eosinophils, basophils, natural killer cells	B lymphocytes, T lymphocytes
Protein Receptors	Interferons, complements, collectin and lysozymes Pattern recognition receptors, encoded in a germline	Immunoglobulins Antigen-specific receptors, rearranged during development (somatic recombination)
Distribution of receptors	Non-clonal	Clonal: clones of cells have distinct specificities and express different receptors
Specificity	Non-specific nature of activity	Discriminate and specific molecular entities
Rapidity	No induction time	Take days to activate
Immunological memory	Functioning is not increased as a result of previous exposure	Increased functioning as a result of previous exposure
Stage	Early stage of defence	Later stage of defence
Self-discrimination	Distinguish self from non- self components, but indiscriminate tissue damage can occur	Distinguish self from non- self components, but is imperfect (autoimmunity)



**Fig. 1.** Interactions among immunocytes through production of regulatory molecules. Abbreviations:  $\alpha\beta$  T,  $\alpha\beta$  T cell; Abs, antibodies; Ags, antigens; AbPC, antibody-producing cells; AgPC, antigen-presenting cells; B, B lymphocytes; CD8, cytotoxic T cells carrying CD8 maker; DC, dendritic cell; EC, endothelial cells; ESP, eosinophil; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN $\gamma$ , interferon  $\gamma$ ; IL, interleukin; LPS, lipopolysaccharide; M $\phi$ , macrophage; M-CSF, macrophage colony-stimulating factor; Mast, mast cells; MC, monocyte; NETs, neutrophil extracellular traps; NK, natural killer cells; NO, nitric oxide; NTP, neutrophil; PMA, phorbol myristate acetate; SCF, stem cell factor; Th0 CD4, T cells carrying CD4 marker; Th1, T helper cell 1; Th2, T helper cell 2; TNF $\alpha$ , tumour necrosis factor  $\alpha$ .

## Roles of amino acids in immune function

### Alanine

Alanine is a major substrate for the hepatic synthesis of glucose, a significant energy substrate for leucocytes (Newsholme & Newsholme, 1989), thereby influencing immune function. There is evidence that supplementation with 2 mM-alanine to the culture medium prevented apoptosis, enhanced cell growth and augmented antibody production in B-lymphocyte hybridoma (Duval *et al.* 1991; Franek & Sramkova, 1996). This supplemental concentration of alanine is approximately 2–4 times that in the plasma of animals and represents 8% of that in ovine allantoic fluid on day 60 of gestation (Kwon *et al.* 2003). The underlying mechanism is not known, but may involve an alanine-mediated inhibition of protein degradation in immunocytes, as reported for hepatocytes through cellular signalling pathways (Meijer & Dubbelhuis, 2004). At present, little information is available regarding an effect of dietary supplementation with alanine on the immune response in any animal species. However, in patients with total parenteral nutrition (TPN), inclusion of alanine could be highly beneficial for supporting gluconeogenesis and leucocyte metabolism (Kudsk, 2006).

### Arginine, citrulline and ornithine

Arginine is synthesised from citrulline as an immediate precursor in virtually all cell types (Wu & Morris, 1998). The small intestine of most mammals, except for cats and ferrets, is capable of synthesising citrulline from glutamine, glutamate

and proline (Wu, 1998). Plasma concentrations of both arginine and citrulline decrease markedly in subjects with protein malnutrition, fasting, trauma, burn injury, inflammation, sepsis and liver transplantation (Bansal & Ochoa, 2003). Under these conditions, arginine must be provided from the diet to support nitrogen balance and the health of animals and humans (Flynn *et al.* 2002).

Due to membrane depolarisation coupled to transport of the positively charged amino acid, arginine is a potent secretagogue for insulin, growth hormone, prolactin and insulin-like growth factor-I (Newsholme *et al.* 2005). These hormones can mediate an NO-independent effect of arginine on immune function. In particular, insulin and growth hormone regulate the metabolism of glucose and amino acids in major tissues, including skeletal muscle, adipose tissue, liver and heart (Meijer & Dubbelhuis, 2004), thereby influencing the availability of these nutrients for leucocytes. Growth hormone can also increase the production of T-lymphocytes in the thymus, the number of haematopoietic progenitor cells in the bone marrow, the response of T cells to cytokines and the antigen-presenting capability of dendritic cells (Calder & Yaqoob, 2004). Interestingly, prolactin enhances the release of cytokines by Th1 lymphocytes and expression of the antigen-presenting major histocompatibility complex class II molecules (Dorshkind & Horseman, 2000). In addition, insulin-like growth factor-I promotes the maturation of lymphocytes in the bone marrow, ameliorates ageing-related thymic involution and increases lymphocyte number and activity (Dorshkind & Horseman, 2000).

**Table 2.** Roles of amino acids in immune responses

Amino acid	Products	Major functions
Amino acids	Proteins	Humoral and cellular immune factors and enzymes
Alanine	Directly	Inhibition of apoptosis; stimulation of lymphocyte proliferation; and enhancement of Ab production probably through cellular signalling mechanism
Arginine	NO	Signalling molecule; killing of pathogens; regulation of cytokine production; and mediator of autoimmune diseases
BCAA	Directly	Regulation of protein synthesis and activation of cytokine and Ab production through cellular mTOR signalling
	Glutamine	A major fuel for cells of the immune system; regulation of T-lymphocyte proliferation, protein synthesis, as well as cytokine and Ab production; activation of macrophage function; inhibition of apoptosis
Cysteine	Taurine	Antioxidant; regulation of cellular redox state
Glutamate	GABA	Neurotransmitter; inhibition of T-cell response and inflammation
Glutamine	Glu and Asp	Neurotransmitters; components of the malate shuttle; cell metabolism
Glycine	Directly	Calcium influx through a glycine-gated channel in the cell membrane
	Serine	One-carbon unit metabolism; ceramide and phosphatidylserine formation
	Haem	Haemoproteins (e.g. haemoglobin, myoglobin, catalase, and cytochrome <i>c</i> ); production of carbon monoxide (CO, a signalling molecule)
Histidine	Histamine	Allergic reaction; vasodilator; and central acetylcholine secretion
	Urocanic acid	Modulation of the immune response in skin
Leucine	HMB	Regulation of immune responses
Lysine	Directly	Regulation of NO synthesis; antiviral activity
Methionine	Homocysteine	Oxidant; inhibitor of NO synthesis
	Betaine	Methylation of homocysteine to methionine; one-carbon unit metabolism
	Choline	Synthesis of betaine, acetylcholine and phosphatidylcholine
	Cysteine	Glutathione synthesis and production of H <sub>2</sub> S (signalling molecule)
	DCSAM	Methylation of proteins and DNA; polyamine synthesis; gene expression
Phenylalanine	Directly	Regulation of tetrahydrobiopterin (a cofactor for NO synthesis) synthesis
	Tyrosine	Synthesis of neurotransmitters that regulate neuronal function and cell metabolism
Proline	H <sub>2</sub> O <sub>2</sub>	Killing pathogens; intestinal integrity; a signalling molecule; immunity
	P5C	Cellular redox state; DNA synthesis; lymphocyte proliferation; ornithine and polyamine formation; gene expression
Serine	Glycine	Antioxidant; one-carbon unit metabolism; neurotransmitter
	Directly	Inhibition of apoptosis; stimulation of lymphocyte proliferation; and enhancement of Ab production probably through cellular signalling mechanism
Taurine	TauCl	Anti-inflammation
Threonine	Directly	Synthesis of the mucin protein that is required for maintaining intestinal immune function; inhibition of apoptosis; stimulation of lymphocyte proliferation; and enhancement of Ab production
Tryptophan	Serotonin	Neurotransmitter; inhibition of the production of inflammatory cytokines and superoxide
	NAS	Inhibitor of tetrahydrobiopterin synthesis; antioxidant; inhibition of the production of inflammatory cytokines and superoxide
	Melatonin	Antioxidant; inhibition of the production of inflammatory cytokines and superoxide
Tyrosine	ANS	Inhibiting production of proinflammatory T-helper-1 cytokines; preventing autoimmune neuroinflammation; enhancing immunity
	Dopamine	Neurotransmitter; regulation of immune response
	EPN and NEPN	Neurotransmitters; cell metabolism
Arg and Met	Melanin	Antioxidant; inhibition of the production of inflammatory cytokines and superoxide
	Polyamines	Gene expression; DNA and protein synthesis; ion channel function; apoptosis; signal transduction; antioxidants; cell function; lymphocyte proliferation and differentiation
Arg, Met and Gly	Creatine	Antioxidant; antiviral; antitumour
Arg, Pro and Gln	Ornithine	Glutamate, glutamine and polyamine synthesis; mitochondrial integrity
Cys, Glu and Gly	Glutathione	Free radical scavenger; antioxidant; cell metabolism (e.g. formation of leukotrienes, mercapturate, glutathionylspermidine, glutathione–NO adduct and glutathionylproteins; signal transduction; gene expression; apoptosis; cellular redox state; immune response)
Gln, Asp and Gly	Nucleic acids	Coding for genetic information; gene expression; cell cycle and function; protein and uric acid synthesis; lymphocyte proliferation
Gln, Glu and Pro	Uric acid	An antioxidant
	Citrulline	Antioxidant; arginine synthesis
Gln and Trp	NAD(P)	Coenzymes for oxidoreductases; substrate of poly(ADP-ribose) polymerase
Lys, Met and Ser	Carnitine	Transport of long-chain fatty acids into mitochondria for oxidation; storage of energy as acetylcarnitine

ANS, anthranilic acid; BCAA, branched-chain amino acids (isoleucine, leucine and valine); DCSAM, decarboxylated *S*-adenosylmethionine; EPN, epinephrine; GABA,  $\gamma$ -aminobutyrate; HMB,  $\beta$ -hydroxy- $\beta$ -methylbutyrate; mTOR, the mammalian target of rapamycin; NAS, *N*-acetylserotonin; NEPN, norepinephrine; P5C, pyrroline-5-carboxylate, TauCl, taurine chloramine.

Early *in vitro* studies have identified that 0.2 mM-arginine (close to its post-absorptive level in plasma) is required for the maximal proliferation of rodent and human T lymphocytes in response to mitogens and for the killing of tumour cells by activated macrophages (Hibbs *et al.* 1987). There is also evidence that high concentrations of arginine (e.g. 2 mM) increase the cytotoxicity of monocytes and NK cells *in vitro* (Abumrad & Barbul, 2004). Note that an extracellular concentration of arginine >2 mM occurs in certain physiological fluids. For example, the arginine concentration is as high as 4–6 mM in porcine allantoic fluid during early pregnancy (Wu *et al.* 2006). Intensive research over the past decade has established that NO synthesis by inducible NO synthase (iNOS) in macrophages and neutrophils is an essential mechanism against viruses, bacteria, fungi, malignant cells, intracellular protozoa, and parasites in mammals, birds, terrestrial animals, low vertebrates (e.g. freshwater and marine fishes) and invertebrates (e.g. shrimp) (Bronte & Zanovello, 2005). Expression of iNOS is induced in leucocytes in response to IFN $\gamma$  and lipopolysaccharide (LPS), and NO is now recognised to play an important role in both innate and acquired immunity (Bogdan *et al.* 2000). Therefore, NO production by iNOS is of most relevance to the immune response. Because arginase and iNOS compete for arginine as a common substrate, modulating arginase expression and activity plays a critical role in NO generation by leucocytes (Kepka-Lenhart *et al.* 2000). Interestingly, some bacteria, such as *Helicobacter pylori*, develop a strategy of survival from NO killing through constitutive expression of arginase, which consumes arginine and thus reduces its availability for NO production by iNOS (Gobert *et al.* 2001). An exciting new development is that physiological levels of arginine (e.g. 150  $\mu$ M) modulates expression of the T-cell receptor  $\zeta$  chain (CD3 $\zeta$ ) that is required for T-cell receptor integrity (Rodriguez *et al.* 2003). Further, the addition to culture medium of citrulline at 0.1 mM (close to its plasma level) or 1 mM (10% of its concentration in ovine allantoic fluid during early pregnancy (Kwon *et al.* 2003)) promoted the synthesis of CD3 $\zeta$  by prolonging the half-life of its mRNA (Bansal *et al.* 2004). This result provides a basis for the use of citrulline to increase arginine availability under immunodeficient conditions associated with elevated arginase activity in blood.

A large body of evidence from animal studies indicates that adequate provision of arginine is required for lymphocyte development and that dietary arginine supplementation enhances immune function in various models of immunological challenges (Field *et al.* 2000; Calder & Yaqoob, 2004). In particular, a deficiency of arginine in mice (its plasma concentration <0.1 mM) resulting from overexpression of arginase in the small intestine impaired the development of progenitor-B to precursor-B lymphocytes in the bone marrow and decreased the number of B lymphocytes in secondary lymphoid organs (De Jonge *et al.* 2002). Importantly, these defects were reversed by subcutaneous administration with arginine (5 mmol/kg body weight twice daily). Also, inadequate intake of dietary arginine (e.g. 0.3% arginine in the diet) impairs NO synthesis by both constitutive NOS and iNOS in young rats (Wu *et al.* 1999) and reduces the immune response in growing chickens (Konashi *et al.* 2000). Further, dietary supplementation with 1 or 2% arginine (~1 or 2 times the arginine content of the regular diet) to healthy rodents and

tumour-bearing or septic rats increased thymic weight, the number of thymic lymphocytes, T-lymphocyte proliferation, the cytotoxicity of specific cells (T lymphocytes, macrophages and NK cells), interleukin (IL)-2 production, IL-2 receptor expression on T lymphocytes and the delayed-type hypersensitivity response (Calder & Yaqoob, 2004). In addition, supplementing 1 or 2% arginine to the diet for rats with trauma injury ameliorated thymic involution and body weight loss, while improving wound healing and survival rate (Field *et al.* 2002). In animals with burn injury, lethal bacterial peritonitis or intestinal damage, arginine supplementation (1% of the diet) reduced bacterial translocation, increased the bactericidal activity of host phagocytes and prolonged host survival (Abumrad & Barbul, 2004). Further, dietary supplementation with 0.83% arginine enhanced the immune status of pregnant sows and neonatal pigs, thereby reducing morbidity and mortality in response to infectious pathogens (Kim *et al.* 2007). Like arginine, ornithine  $\alpha$ -ketoglutarate supplementation (1 g/kg body weight per day) modulated immune function in various rat models of catabolic conditions, including burns, sepsis, tumour bearing and glucocorticoid-induced stress (Cynober, 2004).

Clinical studies have shown that enteral or parenteral provision of arginine (e.g. 8–20 g/d; corresponding to 1.5–3.6 times the arginine intake by an average adult) improves immune functions and clinical outcomes in patients with burn injury, cancer, HIV infection, major traumas and gastrointestinal surgical operations (Field *et al.* 2002; Suchner *et al.* 2002). The benefits are indicated by enhanced T-cell function, increased antibody production, accelerated wound healing mediated by immune cells or a reduction in infection, ventilator days, intensive care unit stay and hospital stay. The most important outcome of this research is the current availability of arginine-supplemented 'immunonutrition' (Kudsk, 2006). However, these formulas have supplemental L-arginine, *n*-3 fatty acids and nucleotides, making it unclear whether their benefit derives entirely from arginine. Nevertheless, recent studies with surgical patients have shown that these formulas may save hospital costs by decreasing the length of stay and reducing infection rates (Senkal *et al.* 1997; Braga *et al.* 1999). In contrast, benefits of arginine supplementation to critically ill patients with severe systemic inflammatory response syndrome, sepsis or multiple organ failure are less clear (Suchner *et al.* 2002). This is probably owing to the complex nature of arginine metabolism and NO production *in vivo* (Wu & Morris, 1998). Further, it is important to recognise that NO is an oxidant and inhibitor of enzymes that contain an iron-sulphur centre and that high levels of NO rapidly react with H<sub>2</sub>O<sub>2</sub> to form peroxynitrite (ONOO<sup>-</sup>) (Fang *et al.* 2002). NO and peroxynitrite oxidise biomolecules (e.g. proteins, amino acids, lipids and DNA), which leads to cell injury and death. Thus, large amounts of NO produced by iNOS can exert a deleterious effect on mammalian cells and mediate the pathogenesis of many diseases, including the autoimmune destruction of pancreatic  $\beta$ -cells in type I diabetes mellitus, arthritis, glomerulonephritis, inflammatory bowel disease and neurological disorders (Flynn *et al.* 2002). Under these conditions, arginine supplementation could 'fuel the fire' to worsen clinical outcomes (Wu *et al.* 2000).



### Asparagine

An early study suggested that asparagine availability could modulate lymphocyte blastogenesis as a possible means to treat childhood acute lymphoblastic leukaemia (Ohno & Hersh, 1970). Depletion of asparagine by exogenous asparaginase had been considered to be immunosuppressive until Kafkewitz & Bendich (1983) reported that this effect might also be attributable to glutamine depletion by a glutaminase activity present in asparaginase. However, subsequent research has provided the following lines of evidence that asparagine plays a significant role in immune function. First, the expression of asparagine synthetase was markedly enhanced in lymphocytes and macrophages in response to mitogens and other stimuli (Suzuki *et al.* 2002). Secondly, an increase in intracellular provision of asparagine increased the expression and activity of ornithine decarboxylase for polyamine synthesis in thymocytes (Brand, 1987) and of iNOS in activated macrophages (Suzuki *et al.* 2002). Thirdly, asparagine (2 mM) prevented apoptosis and increased cell growth in lymphocytes (Duval *et al.* 1991). Thus, asparagine is beneficial for mounting a successful immune response in normal subjects but can also contribute to abnormal lymphoblastic growth in patients with leukaemia. At present, little is known about an effect of dietary supplementation with asparagine on immune function in animals or humans.

### Aspartate and glutamate

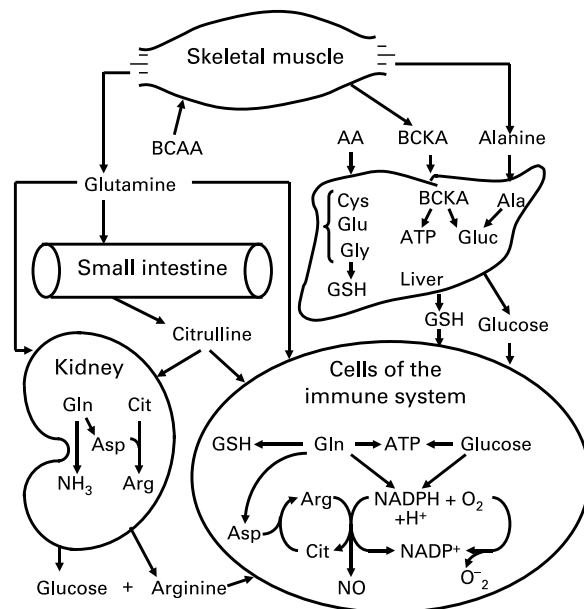
Aspartate and glutamate play versatile roles in the metabolism and function of leucocytes (Newsholme *et al.* 2003). As a substrate for the synthesis of purine and pyrimidine nucleotides, aspartate is crucial for the proliferation of lymphocytes (Newsholme & Calder, 1997). Moreover, aspartate is required for the recycling of the citrulline produced by iNOS into arginine in activated macrophages (Wu & Brosnan, 1992). This helps maintain an adequate intracellular concentration of arginine for sustaining a high rate of NO production in response to immunological challenges. As noted above, through asparagine synthesis, aspartate contributes to the modulation of immune function. In addition, glutamate regulates iNOS expression in certain tissues (e.g. brain), thereby indirectly modulating immunocompetence of animals (Wu & Meininger, 2002). Moreover, glutamate is a substrate for the synthesis of  $\gamma$ -aminobutyrate (GABA), which is present in both lymphocytes (Tian *et al.* 2004) and macrophages (Stuckey *et al.* 2005). Interestingly, T cells express GABA receptors, which mediate an inhibitory effect of GABA on their proliferation (Tian *et al.* 2004). Further, as an immediate precursor for glutathione synthesis, glutamate plays an important role in the removal of oxidants and regulation of the immune response (Wu *et al.* 2004a). Importantly, dietary aspartate and glutamate, along with glutamine, are the major fuels for enterocytes (Wu, 1998). Together, these amino acids help maintain intestinal barrier integrity and prevent the translocation of intestinal microorganisms to the systemic circulation (Van der Hulst *et al.* 1993). Besides their role in leucocyte metabolism as energy substrates, both aspartate and glutamate are excitatory neurotransmitters in central and peripheral nervous systems, acting on ionotropic and metabotropic receptors, which play a role in modulating the immune systems (Newsholme *et al.*

2003). Finally, glutamate and aspartate mediate the transfer of reducing equivalents across the mitochondrial membrane and thus regulate glycolysis and the cellular redox state in cells via the malate/aspartate shuttle (Newsholme *et al.* 1999).

GABA administration (0.6 mg/d) inhibited the development of the proinflammatory T-cell response and retarded the adoptive transfer of type I diabetes in NOD/SCID mice (Tian *et al.* 2004). Interestingly, Lin *et al.* (1999) reported that supplementation with 4 and 8% glutamate to a glutamine- and glutamate-free diet enhanced the delayed-type hypersensitivity and lymphoproliferation responses in rats recovering from methotrexate treatment. Notably, the beneficial effect of dietary glutamate was dose dependent and more pronounced after a longer period of supplementation (Lin *et al.* 1999). These results suggest that dietary glutamate is necessary for maintaining an optimal immune status under conditions of immunosuppression.

### Branched-chain amino acids (BCAA)

Lymphocytes express BCAA transaminase and branched-chain 2-oxoacid dehydrogenase for BCAA degradation (Schafer & Schauder, 1988). The transport and utilisation of BCAA by lymphocytes are dramatically increased in response to mitogens, with their uptake being highest during the S phase of the cell cycle (Koch *et al.* 1990). Importantly, BCAA provide the  $\alpha$ -amino group for the endogenous synthesis of glutamine primarily in skeletal muscle (Fig. 2), which has been considered as part of the immune system (Newsholme and Calder, 1997).



**Fig. 2.** Inter-organ metabolism of branched-chain amino acids, glutamine and arginine, and their role in immune function. Skeletal muscle takes up BCAA from the arterial blood, synthesises both alanine and glutamine from BCAA and  $\alpha$ -ketoglutarate, and releases these two amino acids into the circulation. The small intestine utilises glutamine to synthesise citrulline, which is converted into arginine in kidneys, cells of the immune system and other cell types. The liver is the primary organ for the synthesis of glutathione from glutamate, glycine and cysteine, and of glucose from alanine for use by extrahepatic cells (including leukocytes) and tissues. Abbreviations: Arg, arginine; Asp, aspartate; Cit, citrulline; BCAA, branched-chain amino acids; BCKA, branched-chain  $\alpha$ -ketoacids; Gluc, glucose; GSH, glutathione.

Also, leucine is an activator of the mTOR signalling pathway that regulates protein synthesis and degradation in cells (Meijer & Dubbelhuis, 2004). This may explain why reducing extracellular concentrations of each BCAA below 0.2 mM (close to the plasma level), as occurs in patients with protein malnutrition, impairs lymphocyte proliferation (Skaper *et al.* 1976).

There is a paucity of data on an effect of BCAA on the production of cytokines and antibodies by lymphocytes *in vitro* (Calder, 2006). Because the carbon skeletons of BCAA are not synthesised in leucocytes, a lack of leucine, isoleucine or valine in the culture medium results in the complete absence of protein synthesis or proliferation of lymphocytes in response to mitogens (Waithe *et al.* 1975). However, altering medium concentrations of BCAA between 0.2 and 1 mM (~1 and 5 times plasma levels) had no effect on lymphocyte proliferation (Skaper *et al.* 1976). This finding suggests that normal levels of BCAA in plasma do not limit T-cell responses to mitogens.

A number of animal studies indicate that an inadequate intake of BCAA results in immune impairment. In particular, Jose & Good (1973) reported that dietary restriction of leucine and valine (25 and 50% of the control dietary intake) caused approximately 80–90% decreases in lymphocyte-mediated tumour cell lysis. Interestingly, leucine appears to exert a greater effect on immune function than isoleucine and valine (Konashi *et al.* 2000), which may be explained in part by their differential actions on the mTOR signalling (Meijer & Dubbelhuis, 2004). Also, mice fed a BCAA-deficient diet for 3 weeks exhibited enhancement of susceptibility to *Salmonella typhimurium*, impairment in antibody production, reductions in serum concentrations of transferrin and complement C3, and increased numbers of bacteria in liver and spleen (Petro & Bhattacharjee, 1981). In tetrachloride-induced cirrhotic rats fed a 14% casein diet, supplementing 10% BCAA increased the number of liver-associated lymphocytes (especially CD8-positive cells) and NK cells, as well as lectin-dependent cellular cytotoxicity, compared with the control rats consuming a 24% casein diet (Tsukishiro *et al.* 2000). Consistent with the animal studies, supplementing 35% BCAA (or 0.7 g/kg body weight per day) to TPN solution increased blood lymphocyte counts in patients recovering from surgery and decreased mortality in septic patients (Freund *et al.* 1978; Cerra *et al.* 1984). Further, dietary supplementation with a 6 g BCAA mixture (60% leucine, 20% isoleucine and 20% valine) to athletes increased IFN $\gamma$  production, decreased IL-4 release, prevented an exercise-associated decrease in tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) and IL-1 synthesis by mononuclear cells and stimulated lymphocyte proliferation (Bassit *et al.* 2002). Because BCAA share the same transporter on the cell membrane, an imbalance in their dietary composition can result in immunity impairment, especially when animals are fed a low-protein diet (Aschkenasy, 1979).

$\beta$ -Hydroxy- $\beta$ -methyl-butyrate (HMB), a leucine metabolite, may play a role in immune function. *In vitro* studies have shown that HMB (0.1–2 mM) increased the proliferation, phagocytosis and expression of Fc receptors in a chicken macrophage cell line (Peterson *et al.* 1999). Dietary supplementation with 0.1% HMB improved immune function and reduced mortality in several animal models of infection, including chickens, fish and pigs (Nissen & Abumrad, 1997).

### Glutamine

Glutamine is an abundant amino acid in plasma, skeletal muscle, fetal fluids and milk (Wu & Knabe, 1994; Newsholme & Calder, 1997; Self *et al.* 2004). As a major energy substrate for cells of the immune system (Wu *et al.* 1991; Newsholme *et al.* 1999), glutamine plays an important role in their function and homeostasis. Interestingly, glutamine is extensively catabolised via the glutaminolysis pathway to yield primarily glutamate and, to a lesser extent, aspartate, alanine, lactate, pyruvate and CO $_2$  in cells of the immune system, including thymocytes, lymph node lymphocytes, blood lymphocytes, intraepithelial lymphocytes, neutrophils and macrophages (Field *et al.* 1994; Wu, 1996; Newsholme *et al.* 1999). The action of the NADP $^+$ -dependent malate dehydrogenase, which is present in lymphocytes, macrophages, monocytes and neutrophils, converts malate and NADP $^+$  to pyruvate and NADPH (Newsholme, 2001). Notably, NADPH is required by NOS and NADPH oxidase for the production of NO and superoxide anion, respectively (Fang *et al.* 2002). As a major source of glutamate, glutamine regulates the synthesis of glutathione, a tripeptide crucial for defending cells from oxidative stress (Wu *et al.* 2004b). As an essential precursor for the synthesis of purine and pyrimidine nucleotides, glutamine is required for proliferation of lymphocytes (Ardawi & Newsholme, 1983; Wu *et al.* 1992). Increasing extracellular concentrations of glutamine from 0.01 to 0.5 mM (a physiological level in plasma) dose-dependently increases lymphocyte proliferation (Wu *et al.* 1992). There is also evidence that glutamine is required for NO synthesis in macrophages and monocytes via arginine synthesis. Indeed, arginine derived from glutamine appears to be essential for macrophage activity (Murphy & Newsholme, 1998). Mitogens, changes in cell volume (an early event in the activation of lymphocytes and macrophages in response to immunological stimulation), inflammatory cytokines and an acid–base balance are major regulators of glutamine metabolism in leucocytes (Wu & Flynn, 1995a, b; Newsholme *et al.* 2003).

Several lines of evidence from *in vitro* studies show that glutamine affects various components of the immune response. First, glutamine is necessary for the proliferation of lymphocytes in response to stimulation by T-cell mitogens and activation of protein kinase C (Parry-Billing *et al.* 1990; Wu *et al.* 1992; Wu, 1996). Secondly, addition of 2 mM-glutamine to the culture medium prevented apoptosis, stimulated cell growth and promoted antibody production in lymphocytes (Franeek & Sramkova, 1996). Thirdly, maximal NO production by activated macrophages occurs in the presence of an extracellular concentration of 1 mM-glutamine (Wu & Meininger, 2002). Fourthly, at or near physiological levels in plasma, glutamine (0.5–2 mM) modulates the production of cytokines by monocytes and macrophages (Spittler *et al.* 1997; Yaqoob & Calder, 1998). Indeed, a sufficient supply of extracellular glutamine (e.g. 2 mM) is required for the maximal production of IL-1 and TNF $\alpha$  by murine macrophages, and of IL-6 and IL-8 by human monocytes (Field *et al.* 2002). Fifthly, the maximum phagocytosis of murine macrophage depends on adequate provision of extracellular glutamine (0.6 mM) (Parry-Billing *et al.* 1990; Newsholme *et al.* 2003). Similarly, glutamine (0.5–2 mM) influences the expression of various genes related to (1) intercellular interactions; (2) the

production of cytokines by T lymphocytes; (3) phagocytosis of immunoglobulin G or complement-opsonised particles; (4) antigen presentation; and (5) opsonisation of human monocytes (Spittler *et al.* 1997; Yaqoob & Calder, 1997; Wells *et al.* 1999; Newsholme *et al.* 2003). Sixthly, glutamine (0.1–30 mM) in the culture medium enhances the bactericidal function of neutrophils isolated from burn patients in a dose-dependent manner (Ogle *et al.* 1994). Note that an extracellular concentration of glutamine >20 mM occurs in certain physiological fluids. For example, glutamine concentration is as high as 25 mM in ovine allantoic fluid during early pregnancy (Kwon *et al.* 2003). Finally, glutamine (2 mM) affects the lytic potential of cultured lymphokine-activated killer cells (Juretic *et al.* 1994) and is required for the activation of NK cells that are capable of spontaneous cytolytic activity against a variety of tumour cells (Liang *et al.* 1989). In addition, the activated NK cells participate in other functions through the production of cytokines (Fig. 1).

Animal studies show that enteral or parenteral provision of glutamine enhances the immunity of the host. For example, dietary supplementation with 4% glutamine maintained intramuscular glutamine concentrations and normalised lymphocyte function in early-weaned pigs infected with endotoxin (Yoo *et al.* 1997). Also, supplementation with 3.5% glutamine to a casein-based diet resulted in an increased ability of macrophages to produce TNF $\alpha$ , IL-1 $\beta$  and IL-6 (Wells *et al.* 1999), and lymphocyte responsiveness to mitogens (Kew *et al.* 1999), providing the first evidence that glutamine supplementation can enhance both macrophage and lymphocyte activities *in vivo*. Note that this supplemental glutamine corresponds to 1.8 times the glutamine content of the control diet. Further, dietary provision of 2% glutamine was essential for the maintenance of gut-associated lymphoid tissues and for the synthesis of secretory immunoglobulin A by the small intestine, thereby preventing TNF $\alpha$ -induced bacterial translocation from the lumen of the gut into the circulation (Alverdy, 1990). Moreover, dietary supplementation with 2 or 4% glutamine increased the survival of mice to bacterial challenges (Adjei *et al.* 1994), improved tumour-directed NK cell cytotoxic activity in rats (Shewchuk *et al.* 1997) and reduced the growth of implanted tumours in rats (Shewchuk *et al.* 1997). Likewise, parenteral provision of glutamine (2 g/100 ml) attenuated the adverse effects of TPN on the gut and respiratory tract immunity in rats (Li *et al.* 1997), and increased the survival of rats to endotoxin and bacterial challenges (Ardawi, 1991; Inoue *et al.* 1993).

Findings from a majority of human clinical trials indicate that glutamine supplementation in the form of free glutamine or alanyl-glutamine dipeptide (8–30 g of oral glutamine per day or 0.3 g of alanyl-glutamine/kg body weight per day in TPN) is beneficial for the immune system in patients with burn injury and gastrointestinal surgical operations, as well as in critically ill patients (Van der Hulst *et al.* 1993; Melis *et al.* 2004). These effects are indicated by increases in lymphocyte number and function, as well as reductions in infectious complications, hospital stay, morbidity and mortality. Notably, a randomised, double-blind study with 28 patients undergoing major abdominal surgery demonstrated that pre-operative glutamine supplementation via TPN improved nitrogen balance, increased lymphocyte function, augmented leukotriene production by neutrophils, reduced the incidence of infection and shortened hospital stay

(Morlion *et al.* 1998). Similar results have also been reported for patients receiving bone marrow transplants (McBurney *et al.* 1994). Consistent with the favourable clinical outcomes, patients receiving glutamine in TPN (5 g/100 ml) exhibited higher total counts of blood lymphocytes (including CD4 and CD8 T lymphocytes) when compared with patients receiving glutamine-free TPN (Ziegler *et al.* 1998). In addition, oral administration of glutamine (27 mg/kg body weight) increased concentrations of plasma growth hormone in humans (Welbourne, 1995), which in turn beneficially modulate the immune system (Newsholme *et al.* 2005). Thus, a reduced availability of glutamine may impair immune function, thereby increasing the susceptibility of humans to infectious diseases. Because these published clinical studies involved a relatively small number of subjects, the efficacy of glutamine should be verified in a larger, multicentre study.

### Glycine

Glycine participates in the synthesis of many physiologically important molecules, including purine nucleotides, glutathione and haem (Kim *et al.* 2007). In addition, glycine itself is a potent antioxidant, scavenging free radicals (Fang *et al.* 2002). Thus, glycine is essential for the proliferation and anti-oxidative defence of leucocytes. There is also molecular and pharmacological evidence for a glycine-gated chloride channel in leucocytes (Froh *et al.* 2002). The activation of this channel suppresses the agonist-induced opening of L-type voltage-dependent calcium channels and, thus, attenuates intracellular Ca<sup>2+</sup> concentrations. As a result, glycine plays a role in regulating the production of cytokines by leucocytes and immune function (Zhong *et al.* 2003). This notion is supported by *in vitro* studies showing that an increase in extracellular glycine concentration (0.1–1 mM) within the physiological range activated a glycine-gated chloride channel and hyperpolarised the plasma membrane in a variety of cells types, including macrophages, monocytes, lymphocytes and neutrophils (Froh *et al.* 2002). In macrophages stimulated by LPS, glycine (0.1–1 mM) reduced the influx of Ca<sup>2+</sup> and an increase in its intracellular concentration, thereby blunting the production of superoxide, IL-1 and TNF $\alpha$  (Wheeler & Thurman, 1999). Glycine did not affect IL-2 production in T-cells in response to stimulation by immobilised anti-CD3 antibody, but inhibited cell proliferation in a dose-dependent manner (0.1–1 mM) by attenuating an increase in intracellular Ca<sup>2+</sup> levels (Stachlewitz *et al.* 2000). Further, addition of 2 mM-glycine to the culture medium prevented apoptosis and enhanced antibody production in B lymphocytes (Duval *et al.* 1991).

There is also *in vivo* evidence that glycine reduces inflammatory reactions and morbidity in pathogen-infected animals. A deficiency of dietary glycine impaired immune responses in chickens treated with LPS, which was alleviated by its dietary supplementation (Konashi *et al.* 2000). Additionally, dietary supplementation with 5% glycine to rats infected with a lethal dose of LPS reduced plasma levels of TNF $\alpha$  and improved survival rate (Ikejima *et al.* 1996). Similarly, supplementation with 1% glycine to a liquid milk diet reduced inflammation and attenuated a rise in body temperature of calves infected with a low dose of endotoxin (Simon, 1999). Interestingly, glycine protected animals against peptidoglycan polysaccharide-induced arthritis; chemically and



stress-induced gastrointestinal mucosal injury; the ischaemia/reperfusion injury of a variety of organs; and shock caused by haemorrhage, endotoxin and sepsis (Zhong *et al.* 2003). In particular, dietary supplementation with 5% glycine prevented experimental colitis in rat models induced by an intracolonic injection of 2,4,6-trinitrobenzene sulphonic acid or oral administration of dextran sulphate sodium (Tsune *et al.* 2003). In both models of gut inflammation, dietary supplementation with 5% glycine abolished increases in colonic expression of IL-1 $\beta$  and TNF $\alpha$ , cytokine-induced neutrophil chemoattractant and macrophage inflammatory protein, thereby ameliorating diarrhoea and body weight loss (Tsune *et al.* 2003). Collectively, these findings indicate that glycine is a novel anti-inflammatory, immunomodulatory and cytoprotective nutrient. Clinical trials are warranted to determine the efficacy of glycine supplementation on improving immune function in humans.

### Histidine

Plasma contains a high level of a histidine-rich glycoprotein, which has a multidomain structure, interacts with many ligands and regulates a number of biological processes, including cell adhesion and migration, complement activation, immune complex clearance and phagocytosis of apoptotic cells (Jones *et al.* 2005). Notably, an immunologically significant pathway for histidine utilisation is initiated by histidine decarboxylase to produce histamine, a major mediator of inflammatory reactions (Tanaka & Ichikawa, 2006). It was previously thought that only mast cells and basophils could release histamine from storage during degranulation in response to various stimuli. However, it is now clear that many tissues and cell types express this enzyme to synthesise histamine. These cells include haematopoietic progenitors, macrophages, platelets, dendritic cells and T lymphocytes (Dy & Schneider, 2004). Histamine regulates various physiological and immunological functions by activating various histamine receptors on target cells. The overwhelming evidence that many cell types (e.g. medullary and peripheral haematopoietic cells, eosinophils, basophils, mast cells, T lymphocytes and dendritic cells) express a histamine 4 receptor (H<sub>4</sub>R) suggests a possible role for these leucocytes in inflammation, haematopoiesis and immunity (Tanaka & Ichikawa, 2006). In addition, histamine mediates platelet aggregation and promotes Th2 cell activity by both reducing IL-12 and enhancing IL-10 production (Dy & Schneider, 2004).

In addition to histamine synthesis, histidine can be deaminated by the catalytic enzyme histidine-ammonia lyase to form urocanic acid (UCA). This substance is a unique photoreceptor, and its conversion from *cis*-UCA to *trans*-UCA controls the initiation of the immune suppressive action of solar ultraviolet-B (De Fabo & Noonan, 1983). Further, *cis*-UCA decreases the following capacities of murine splenocytes: (1) serving as antigen-presenting cells; (2) proliferating in response to mitogens; (3) suppressing the production of IL-2 and IFN $\gamma$ ; or (4) increasing the production of IL-10 (Holan *et al.* 1998). These effects resulted in a reduced T-lymphocyte activity and an increased risk of UV carcinogenesis (De Fabo *et al.* 1997).

A limited number of *in vitro* studies show that supplementation of 2 mM-histidine to the culture medium prevented

apoptosis, increased cell growth and promoted antibody production in lymphocytes (Duval *et al.* 1991). Surprisingly, only a few studies have examined a role for dietary histidine in immune function of animals. Nonetheless, a deficiency of dietary histidine can decrease plasma concentrations of proteins, including histidine-rich glycoprotein (Jones *et al.* 2005), which in turn impairs the immune response. Further, an inadequate intake of dietary histidine reduced the immune response in chickens, which could be reversed by its dietary supplementation (Konashi *et al.* 2000). Moreover, feeding either a high (64 g/kg diet) or low (0.4 g/kg diet) level of dietary histidine to rats resulted in an increase of skin *trans*-UCA levels above those in the control group and attenuated the UV-induced immunosuppression (De Fabo *et al.* 1997). These findings suggest that dietary histidine supplementation may provide a potentially novel means to boost immune function, particularly in the skin.

### Lysine

Compelling evidence shows that a dietary deficiency of lysine limits the synthesis of proteins (including cytokines) and the proliferation of lymphocytes, and impairs immune responses in chickens, resulting in increases in morbidity and mortality in response to infection (Kidd *et al.* 1997; Konashi *et al.* 2000). There are also findings that an inadequate intake of dietary lysine reduced antibody responses and cell-mediated immunity in chickens (Chen *et al.* 2003). By sharing the same transport systems with arginine, the availability of dietary or extracellular lysine can modulate the entry of arginine into leucocytes and thus NO synthesis by iNOS (Wu & Meininger, 2002). Indeed, increasing extracellular lysine concentrations (0.3–2 mM) reduced the intracellular arginine concentration and NO synthesis in activated macrophages in a dose-dependent manner (Closs *et al.* 2000). The knowledge about an antagonism between lysine and arginine has been exploited to treat effectively dermal infections caused by the herpes simplex virus (Griffith *et al.* 1978). Topical application of lysine (a therapeutic dosage of 0.8–1 g daily during an overt infection) inhibited the replication of the virus, shortened the course and duration of the disease, and improved clinical outcomes (Griffith *et al.* 1978). The underlying mechanism involves a decrease in the transport of arginine into the virus and an inhibition of arginase activity by lysine, resulting in a depletion of polyamines for the growth of the virus (Griffith *et al.* 1981). The successful use of lysine to treat infections by the herpes simplex virus illustrates the power of basic research on amino acid metabolism to solve a significant problem in medicine.

### Phenylalanine and tyrosine

Emerging evidence shows that an important function of phenylalanine is to upregulate expression and activity of GTP cyclohydrolase I, which is the first and rate-controlling enzyme for the synthesis of tetrahydrobiopterin, an essential cofactor for NOS (Shi *et al.* 2004). Thus, phenylalanine can regulate NO synthesis by leucocytes. Consequently, an adequate intake of dietary phenylalanine is required to maintain a sufficient provision of tetrahydrobiopterin for the production of NO by iNOS in activated macrophages and other leucocytes (Wu & Meininger, 2002).

Tyrosine, a product of phenylalanine degradation, is the immediate precursor for the synthesis of catecholamine hormones (epinephrine and norepinephrine), thyroid hormones (triiodothyronine and thyroxine), as well as dopamine and melanin (Kim *et al.* 2007). Norepinephrine is a key messenger released from the sympathetic nervous system to act on the immune system (Kin & Sanders, 2006). Interestingly, both Th1 cells and B cells express  $\beta$ 2-adrenergic receptors. The binding of epinephrine and norepinephrine to the receptors triggers the generation of cAMP from ATP and the subsequent activation of protein kinase A, which stimulates the differentiation and proliferation of Th1 cells and B cells (Kin & Sanders, 2006). In addition, thyroid hormones regulate many important physiological processes, including gene expression, and the metabolism and differentiation of leucocytes (Dorshkind & Horseman, 2000). Moreover, dopamine and melanin reduce the synthesis of proinflammatory cytokines (including TNF $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10) by monocytes and macrophages, induce the production of anti-inflammatory mediators by leucocytes, and regulate lymphocyte proliferation, platelet aggregation and the phagocytic activity of neutrophils (Basu & Dasgupta, 2000; Mohagheghpour *et al.* 2000). These biochemical bases explain the finding that a deficiency of dietary phenylalanine plus tyrosine impaired the immune response in chickens, which could be reversed by their supplementation to the diet (Konashi *et al.* 2000).

#### Proline

Proline is catabolised via proline oxidase in a variety of organs, including the small intestine, liver, kidney, lymphoid organs and placenta, to generate pyrroline-5-carboxylate (P5C) and H<sub>2</sub>O<sub>2</sub> (Wu, 1997; Wu *et al.* 2005). Interestingly, P5C can be reduced to proline by the widespread NADPH-dependent P5C reductase. This proline–P5C cycle functions to regulate the cellular redox state and the proliferation of cells, including lymphocytes (Phang, 1985). This may provide a cellular mechanism responsible for a role for proline in protecting lymphocytes from apoptosis, stimulating cell growth and promoting antibody production (Duval *et al.* 1991). Moreover, proline constitutes one-third of amino acids in collagen, and, thus, is crucial for wound healing and injury recovery mediated by cells of the immune system (Abumrad & Barbul, 2004).

An intriguing new discovery is that proline oxidase may play an important role in immunity (Ha *et al.* 2005). Notably, a lack of proline catabolism due to a deficiency of intestinal proline oxidase impairs immune function in the gut (Ha *et al.* 2005). H<sub>2</sub>O<sub>2</sub>, a major product of proline oxidation, is a signalling molecule (Shi *et al.* 2004) and a cytotoxic agent for pathogenic bacteria (Kim *et al.* 2007). Therefore, a high activity of proline oxidase in the porcine placenta (Wu *et al.* 2005) and the small intestine of piglets (Wu, 1997) may play a crucial role in protecting these organs from infections during the critical periods of fetal and neonatal development. Further, proline oxidase is present in milk and may aid in protecting the neonatal intestine from bacterial and viral challenges (Sun *et al.* 2002). This may explain, in part, why neonates fed a diet of non-mother's milk have a high risk of intestinal dysfunction in comparison with those nursed by their mothers (Wu *et al.* 1996; Field, 2005).

#### Serine

There are multiple pathways for serine utilisation, which include one-carbon unit metabolism; the hepatic and renal synthesis of glucose, particularly in ruminants; and the synthesis of glycine, ceramide and phosphatidylserine as structural components and signalling molecules of cells, including T and B lymphocytes (Jones *et al.* 1999; Kim *et al.* 2007). Indeed, phosphatidylserine has been implicated in the regulation of IL-2 production and T-lymphocyte activation in response to an immunological challenge (Pelassy *et al.* 1991). Because glucose is a major fuel for lymphocytes and macrophages (Newsholme *et al.* 1999), an adequate availability of serine is necessary for the function of these cells, particularly during late pregnancy in ruminants (Wu *et al.* 2006). Interestingly, addition to the culture medium of 2 mM-serine (~7 times its plasma levels and 10% of its concentration in ovine allantoic fluid during late gestation (Kwon *et al.* 2003)) prevented apoptosis, stimulated cell growth and increased antibody production in lymphocytes (Duval *et al.* 1991; Franek & Sramkova, 1996). Further, there is evidence that inadequate intake of dietary serine reduced the immune response in chickens, which could be reversed by its dietary supplementation (Konashi *et al.* 2000).

#### Sulphur-containing amino acids

A sufficient intake of dietary methionine and cysteine is important for the synthesis of proteins of the immune system (Grimble, 2006). Through the generation of decarboxylated S-adenosylmethionine, methionine is a donor of the methyl group that participates in the methylation of DNA and proteins, the synthesis of spermidine and spermine, and regulation of gene expression (Wu *et al.* 2006). Because polyamines are important for the proliferation and differentiation of lymphocytes (Flynn *et al.* 2002), methionine may play a role beyond a protein constituent. In addition, methionine is a substrate for the synthesis of choline and thus phosphatidylcholine and acetylcholine that are essential for nerve function and leucocyte metabolism (Kim *et al.* 2007).

Cysteine is the precursor of glutathione (GSH) and H<sub>2</sub>S (a signalling molecule) in animal cells, and its metabolism is markedly altered in response to infection (Malmezat *et al.* 2000). Glutathione synthesis is influenced by dietary intakes of sulphur amino acids (Wu *et al.* 2004b). Thus, there is a positive correlation between the transsulphuration pathway activity and glutathione concentrations in the liver, spleen and muscle (Malmezat *et al.* 2000). Glutathione scavenges free radicals and other reactive oxygen species (e.g. hydroxyl radical, lipid peroxy radical, peroxy nitrite and H<sub>2</sub>O<sub>2</sub>) and conjugates with various electrophils and xenobiotics for their detoxification (Fang *et al.* 2002). There is evidence that the intracellular concentrations of GSH play an important role in regulating cellular signalling pathways (including the nuclear transcription factor  $\kappa$ B pathway) in response to immunological challenges (Fratelli *et al.* 2005). In addition, GSH concentrations in antigen-processing cells modulate immune responses, including T-helper cell function and antibody production (Peterson *et al.* 1998). Thus, a deficiency of extracellular cysteine or intracellular GSH decreases the number of CD4 cells, reduces the production of IFN $\gamma$ , impairs the

proliferation of lymphocytes in response to mitogens and diminishes cytotoxic T-cell activity (Obled *et al.* 2004). Further, GSH depletion is associated with many diseases, such as malaria, tuberculosis, cancer, AIDS and rheumatoid arthritis, and the requirement for sulphur amino acids increases during trauma, sepsis and injury (Obled *et al.* 2004; Grimble, 2006).

Dietary supplementation with methionine or cysteine is beneficial for the immune system under various catabolic conditions. For example, increasing total methionine levels from 0.35 to 1.2 % in the diet for chickens infected with the Newcastle disease virus markedly enhanced the following key aspects of the immune responses: T-cell proliferation in response to mitogen stimulation (Tsiagbe *et al.* 1987a), plasma levels of immunoglobulin G (Tsiagbe *et al.* 1987a), leucocyte migration and antibody titre (Swain & Johri, 2000). Dietary cysteine provided similar effects to methionine with regard to the immune responses of chickens (Tsiagbe *et al.* 1987b). However, high supplemental levels of methionine or cysteine (> 1.45 % in the diet) were detrimental to the growth and immune responses of chickens (Tsiagbe *et al.* 1987a, b), probably due to the excess production of highly toxic substances (e.g. homocysteine and sulphuric acid) (Wu & Meininger, 2002).

Because cysteine is toxic at a high concentration, *N*-acetyl cysteine (NAC) and L-2-oxothiazolidine-4-carboxylate (OTC, an analogue of 5-oxoproline) are commonly used to deliver cysteine via intravenous infusion or drinking water to increase endogenous glutathione synthesis in cells (Wu *et al.* 2004b). Findings from clinical studies show that daily NAC provision to septic patients (a bolus dose of 150 mg/kg body weight, followed by a constant infusion of 50 mg/kg body weight over 4 h) increased blood GSH content, decreased plasma IL-8 levels and soluble TNF receptors, improved respiratory function, and reduced the number of days in the intensive care unit (Spapen *et al.* 1998). Further, oral administration of NAC (0.6 g/d every second day) was also effective in raising GSH concentrations in blood and CD4 T cells, increasing NK cell activity and lymphocyte proliferation in response to mitogens, and prolonging the survival time of HIV patients (Breitkreutz *et al.* 2000). Similarly, OTC supplementation to cirrhotic patients (0.2 g/kg body weight per day) increased GSH content in monocytes and reduced the production of inflammatory cytokines, including IL-1, IL-8 and TNF $\alpha$  (Obled *et al.* 2004).

Taurine is the most abundant free amino acid in lymphocytes and a potent antioxidant (Fang *et al.* 2002). Further, the reaction of taurine with hypochlorous acid, which is a microbicidal agent produced by activated monocytes and neutrophils, yields taurine chloramine (Wright *et al.* 1986). This long-lived oxidant reduces the production of proinflammatory cytokines (e.g. IL-1, IL-6 and TNF $\alpha$ ) and prostaglandin E<sub>2</sub> (Weiss *et al.* 1982; Choręży *et al.* 2002), and increases histamine release from neutrophils of carrageenin-induced rats (Wojtecka-Lukasik *et al.* 2004). Thus, dietary supplementation with taurine (1 % in drinking water) can reduce lung inflammation induced by bleomycin (Schuller-Levis *et al.* 2003).

### Threonine

Threonine is a major component of intestinal mucin and plasma  $\gamma$ -globulin in animals (Kim *et al.* 2007). Through protein synthesis and cellular signalling mechanisms, addition

of 2 mM-threonine to the culture medium prevented apoptosis, stimulated cell growth and promoted antibody production in lymphocytes (Duval *et al.* 1991). Animal feeding studies indicate that changes in components of the immune system are sensitive to dietary threonine intake (Li *et al.* 1999). Indeed, serum antibody titres increased with increasing dietary intake of threonine in chickens infected with the Newcastle disease virus (Bhargava *et al.* 1971). Also, dietary supplementation with threonine increased serum levels of IgG in sows (Cuaron *et al.* 1984). Further, increasing dietary threonine intake increased antibody production, serum IgG levels and jejunal mucosal concentrations of IgG and IgA, while decreasing jejunal mucosal concentrations of IL-6 in young pigs challenged with *Escherichia coli* (X. Wang *et al.* 2006). These findings provide support for a role of dietary threonine in modulating immune function in livestock and perhaps humans.

### Tryptophan

The products of tryptophan catabolism include serotonin, *N*-acetylserotonin, melatonin and anthranilic acid (Kim *et al.* 2007). Tryptophan catabolism is increased to generate anthranilic acid through the indoleamine 2,3-dioxygenase (IDO) pathway during inflammation or stimulation by LPS or certain cytokines (Platten *et al.* 2005). Serotonin, melatonin and *N*-acetylserotonin can enhance host immunity by inhibiting the production of superoxide, scavenging free radicals and attenuating the production of TNF $\alpha$  (Perianayagam *et al.* 2005). In addition, *N*-acetylserotonin is an inhibitor of sepiapterin reductase, an enzyme for the synthesis of tetrahydrobiopterin (Shi *et al.* 2004). By modulating inducible NO synthesis, this tryptophan metabolite can affect both innate and acquired immunity systems. Excitingly, anthranilic acid was recently found to inhibit the production of proinflammatory Th1 cytokines and prevent autoimmune neuroinflammation (Platten *et al.* 2005). Because there is a progressive decline in tryptophan concentrations in plasma of animals with inflammation, its catabolism plays a critical role in the functions of both macrophages and lymphocytes (Melchior *et al.* 2004).

Early work indicated that tryptophan starvation resulting from IFN $\gamma$  treatment was associated with the antiproliferative effect of this cytokine on intracellular parasites (Taylor & Feng, 1991) and tumours (Ozaki *et al.* 1988). Interestingly, progressively increasing concentrations of IFN $\gamma$  were required for its growth inhibition in the presence of elevated tryptophan concentrations (Pfefferkorn, 1984). Subsequently, Munn *et al.* (1998) demonstrated that a pharmacological inhibition of IDO suppressed T-cell activity and induced fetal allograft rejection in mice. More recently, *N*-(3,4-dimethoxycinnamoyl) anthranilic acid, an orally active synthetic derivative of the tryptophan metabolite anthranilic acid, was found to protect paralysed mice from experimental autoimmune encephalomyelitis (Platten *et al.* 2005). Available evidence suggests that tryptophan catabolism plays a role in immune responses by producing a local immunosuppressive environment that is able to control T-cell homeostasis and self-tolerance during inflammation (Platten *et al.* 2005).

A deficiency of dietary tryptophan impaired the immune response in chickens (Konashi *et al.* 2000). Conversely, oral administration of 300 mg of tryptophan to rats enhanced phagocytosis by macrophages and the innate immune response



(Esteban *et al.* 2004). Dietary supplementation with 0.22 % L-tryptophan also increased resistance to bacterial and parasitic infections in rats fed a 20 % zein diet (Watson & Petro, 1984). At present, a potential use of crystalline tryptophan for animal health management is not fully developed. However, there are reports that dietary supplementation with 0.36 or 0.5 % tryptophan (corresponding to eight or 11 times the tryptophan content (0.044 %, on a dry matter basis) of the commercial feed) reduced cannibalism in fish (Hseu *et al.* 2003) and cortisol-mediated immune suppression in the rainbow trout (Lepage *et al.* 2003).

### Conclusion and perspectives

Amino acids are required for the synthesis of a variety of specific proteins (including cytokines and antibodies) and regulate key metabolic pathways of the immune response to infectious pathogens. Arginine, glutamine and cysteine precursors are currently the best prototypes, with well-defined roles and expanded applications to human nutrition and livestock production. Adequate dietary provision of all amino acids is necessary for sustaining normal immunocompetence and protecting the host from a variety of diseases in all species. Because of a negative impact of amino acid imbalance and antagonism on nutrient intake and utilisation, an excess supply of amino acids in the diet can be deleterious to the immune system. Thus, care should be exercised in developing effective strategies of enteral or parenteral supplementation to achieve maximum health benefits. Such measures should be based on knowledge about the inter-organ metabolism of amino acids (Fig. 2), their roles in the immune response, nutritional and pathological states of individuals, and expected treatment outcomes. Although great advances have been made in the rapidly growing field of nutritional immunology, there is a paucity of information about the molecular mechanisms that regulate the actions of amino acids on the immune system. Discovery of this new knowledge will probably be facilitated through the integrative applications of modern high-throughput and high-efficient technologies, including genomics, transcriptomics, metabolomics, proteomics, bioinformatics, systems biology and epigenetics (Wu *et al.* 2004a; Mathers, 2006; J. Wang *et al.* 2006). Given the increasing commercial availability of food- and feed-grade amino acids for dietary supplementation, we enthusiastically predict that amino acids will widely become cost-effective nutraceuticals for improving health and preventing infectious disease in both humans and animals.

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