Regulatory Roles of Amino Acids in Immune Response

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Abstract: Amino acids are not only the building blocks of proteins but are also key regulators of various pathological and physiological processes, including immune responses, in living cells. However, the mechanisms responsible for these effects of amino acids are largely unknown. The regulatory roles of amino acids in the immune system can be considered from two perspectives, namely, the enhancement of the immune response that protects individuals from infections and malignant neoplasms, and the reduction of over-responses such as inflammation and autoimmunity. In this review, we focus on the regulatory roles of amino acids in the immune response, showing how several amino acids (e.g., glutamine, arginine, tryptophan, cystine/cysteine, glutamate, and branched-chain amino acids) enhance immunity. Additionally, we describe how one amino acid, histidine, functions as an anti-inflammatory agent in colitis.

Keywords: Amino acids, metabolism, protein synthesis, immunoregulatory function(s).

IMMUNO-ENHANCING AMINO ACIDS

Our immune system protects us from attacks of a range of infectious pathogens, including viruses, bacteria, protozoa, and parasites. It is well known that a variety of amino acids play a pivotal role(s) in preserving immune responses. Indeed, a protein-malnourished status is known to cause disturbance of the immune system, whereas the provision of several amino acids, including arginine and glutamine, can ameliorate such immunological impairment. In recent years, it has been clearly demonstrated that several amino acids play a significant role in regulating a variety of immune responses, including the activation of lymphocytes, NK cells, and macrophages; proliferation of lymphocytes; regulation of intracellular redox states; gene expression; and production of cytokines. It appears to be particularly important to regulate immune responses both safely and efficiently by the administration of amino acids. In this section, the functions of several representative amino acids in regulating the immune system are described.

(1) Glutamine

Glutamine is a member of the glucogenic amino acid family and is nutritionally known as a non-essential amino acid. However, glutamine is also designated as a semi-essential amino acid because in markedly hypercatabolic individuals, or under conditions of metabolic stress, the capacity to synthesize glutamine is insufficient to meet the body's requirements. In human glutamine kinetics, a large proportion of dietary glutamine is usually catabolized in the gut, and approximately 90% of the glutamine pooled in a living body is produced in muscles [1]. Under conditions of increased glutamine demand, such as in sepsis or following injury, glutamine is rapidly recruited from the muscles. If this supply is insufficient to meet demands, low plasma glutamine levels result, leading to the disturbance of extracellular glutamine-dependent immune responses; namely, a reduction in human B cell differentiation to antibody-producing cells [2], suppressed T cell proliferation, decreased IL-2 production and expression of the IL-2 receptor, downregulation of MHC class II antigen expression on human macrophages, reduced antigen presentation potential, and inefficient phagocytosis [3]. Provision of exogenous glutamine can not only prevent the degradation of muscular proteins but also stimulates protein synthesis in the muscles and, additionally, can restore impaired immune responses in rats model [4].

What is the Role(s) of Glutamine in Immuno-Competent Cells?

Glutamine plays different roles in T cells, which can progressively proliferate after activation, and in non-proliferative phagocytic cells such as macrophages. In rat T cells, glutamine is usually used as a resource in nucleic acid synthesis and as an energy source. For example, the nitrogen contained in purine rings originates from the amide residue of the glutamine molecule, and pyrimidine bases are produced *in vivo* by an interaction between glutamine-derived carbamoyl phosphate and aspartate. The energy required by a living individual is produced by both the glucose metabolic pathway (approximately 80%) and the glutamine metabolic pathway (approximately 20%) [5].

In macrophages, a fundamental role of the glutamine metabolic pathway is NADPH production [6]. NADPH is well recognized as a cofactor required in fat synthesis and is also involved in the *in vivo* synthesis and degradation of various biomaterials, such as NO production and the synthesis of proteins, DNAs, and RNAs. Generally, NADPH is generated by and supplied via another glucose oxidation pathway, the pentose phosphate pathway. However, when murine macrophages and neutrophils are engaged in phagocytosis and pinocytosis, NADPH derived from glutamine rather than the pentose phosphate pathway is used

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because the glucose in this latter pathway will be utilized in fat synthesis [7].

(2) Arginine

Arginine, which was initially isolated more than 100 years ago, is categorized as a basic amino acid and is found abundantly in meats and nuts. Certain amounts of arginine (3–5 g daily) are generally consumed in ordinary diets [8]. Arginine was originally classified as a non-essential amino acid [9]; however, growing rodents are unable to meet their daily requirements. Thus, arginine is recognized as a conditionally essential amino acid for infants [10].

In the 1980s, it was reported that in experimental animals housed under stressful conditions, arginine supplementation restored the reduced number of T cells. Subsequently, several commercial arginine products have been widely marketed to enhance immunity, and arginine is used along with n-3 fatty acids, nucleotides, and certain micronutrients to create the so-called immune-enhancing diets (IED) [11]. It has also been reported that in patients subjected to surgical operations with a high risk, such as colon excision or pancreatectomy, IED supplementation successfully reduces the incidence of various postoperative infections. However, it is necessary to administer such IED before or immediately after the surgical operations in order to prevent the patients succumbing to infection. Furthermore, the therapeutic efficacy of IED administered to patients with sepsis remains unproven [12].

Arginine Depletion by Arginase 1

In mammals, arginase 1, which is known as an enzyme capable of catabolizing arginine to ornithine and urea, plays a role in regulating the immune functions of activated T cells through depleting arginine in the local microenvironment [13]. Arginase 1 has been demonstrated to regulate the bioavailability of arginine to control nitric oxide production by nitric oxide synthase (NOS), particularly by inducible NOS, the only substrate for which is arginine, and also modulates T cell functions in a similar manner. Cancers have been well documented to impair host-mediated immune functions, and arginase 1 has been found to be elevated in the splenocytes of tumor-bearing mice. In addition, it has been reported that the mononuclear cells of patients with pancreatic cancers secrete arginase 1. Arginase 1 is produced in murine myeloid cells (myeloid dendritic cells, monocytes, macrophages, and eosinophils) after stimulation with Th2 cytokines (IL-4 and IL-13) [14], IL-6, IL-10, TGF-α [15], prostaglandins (PGE) [16], and so on. Such myeloid cells that can deplete arginine from their local microenviron-ment to downregulate T cell functions are designated as myeloid suppressor cells (MSC) [17]. MSC have been demonstrated to be present in patients with traumas, several infectious diseases, and cancers. In an experimental murine model of trauma, rapid infiltration of MSC into the splenic marginal zones has been observed. Furthermore, the following arginine-dependent T cell functions were found to be impaired after T cells were co-cultured with MSC: T cell proliferation, expression of the genes encoding the T cell receptor complex and ξ chain, and differentiation to memory T cells.

Impairment of T Cell-Mediated Functions by Arginine Depletion

There are two plausible explanations for the mechanisms underlying the suppression of T cell proliferation and impairment of T cell functions observed after the local depletion of arginine: (1) reduced expression of the CD3 ξ chain and the subsequent decreased expression of the T cell receptor complex; and (2) reduced expression and enzymatic activity of the cyclin D3/cdk4 complex, which is known to regulate cell cycle progression.

First, in the absence of arginine, the number of T cell receptors expressed on the surface of murine T cells was found to be reduced to approximately 25% of the normal level. This is caused by downregulating the arginine-depen-dent translation of the ξ chain molecule that is an essential component of the T cell receptor complex. In experimental murine models of cancers or trauma, MSC were demons-trated to suppress T cell proliferation, expression of the ξ chain, and IL-2 production [18]. Both in patients with cancers and in post-operative humans, a lack of ξ-chain molecules has been observed and, in addition to an elevated arginase 1 enzymic activity, dysfunction of T cells and a reduced number of T cells were also frequently observed.

Second, cyclin/cdk complexes usually phosphorylate retinoblastoma (Rb) proteins to release the E2F transcript-tional factor bound to them, and the released E2F is subse-quently translocated into the nucleus to induce expression of the genes required for cell-cycle progression to the S phase and DNA replication (e.g., cyclin E). However, arginine depletion may result in reduced cyclin D3 and cdk4 gene expression and subsequently may suppress cell-cycle progression to the S phase [19].

How does arginine depletion provoke such reduced expression of the CD3 zeta chain and the cyclin D3 molecules? The most probable explanation is the utilization of the common regulatory system of amino acid metabolism, which was originally investigated in yeasts [20]. In yeast cells cultured under conditions of amino acid depletion, the biosynthesis of various proteins was transiently inhibited and translation of a variety of enzymes involved in the amino acid synthesis was activated to promote amino acid production. Recently, a similar system was found in mammals [21].

Arginine depletion leads to an intracellular accumulation of deaminoacyl tRNA, resulting in the activation of GCN2 kinase, the ligand for which is deaminoacyl tRNA, and subsequent phosphorylation of the eukaryotic translation initiation factor, eIF2. eIF2 is known to produce a ternary complex by interacting with the initiator methionine-tRNA and, after translocating the resultant complex to one of the ribosomal subunits, mRNA translation is initiated. There are two types of eIF2; one is GTP-bound eIF2, which can translocate the initiator methionine-tRNA to the ribosomes, and the other is GDP-bound eIF2, which is unable to translocate initiator methionine-tRNA. Once GDP-bound eIF2 has become phosphorylated by GCN2 activated by arginine depletion, it is difficult to convert it to the GTP-bound form, leading to a reduced content of the GTP-bound eIF2 [22]. Therefore, there is reduced translocation of the initiator methionine-tRNA to the ribosome subunits,

probably resulting in a decreased translational efficiency in expression of the CD3 ξ chain and cyclin D3. In T cells derived from GCN2-knockout mice, such untoward effects of arginine depletion as observed in normal mice were not observed, suggesting that GCN2 is essential [19].

(3) Tryptophan

Tryptophan is a glucogenic and ketogenic essential amino acid. Tryptophan-catabolizing indoleamine-2,3-dioxygenase (IDO) is regarded as a key enzyme in the host immune system. IDO can oxidatively degrade tryptophan, which is an essential amino acid involved in the kynurenine pathway, and has been recognized as an enzyme capable of modulating host-mediated immune responses. IDO enzymetically catalyzes the reaction of tryptophan to *N*-formyl-L-kynurenine that is the initial and late-limiting reaction in the kynurenine pathway [23]. It has been reported that IDO is induced in murine and human myeloid cells, epithelial cells, fibroblasts, vascular endothelial cells, and some types of cancer cells after treatment with IFN-γ. IDO once produced completely degrades tryptophan in the immediate vicinity, thereby limiting the growth of infectious pathogens in the tissues. On the basis of these observations, IDO was initially considered to be intimately involved in the host defense mechanism [24]. Among a variety of immunocom-petent cells, antigen-presenting cells such as myeloid dendritic cells and macrophages are able to modulate T cell functions *via* IDO induction. IDO-producing myeloid dendritic cells are included in the $CD11c^{+}CD8\alpha^{+}$ dendritic cell subset in mice and in the $CD123^{+}$ dendritic cell subset in humans. Myeloid dendritic cells are activated to produce IDO after either IFN-γ stimulation or an interaction between CTLA-4 expressed on $CD4^{+}CD25^{+}$ T cells (T_{reg}) and B7-1 or B7-2 expressed on myeloid dendritic cells.

Immunological Modulation via Tryptophan Catabolism and by its Metabolites

A large proportion of tryptophan (90%) is degraded in the kynurenine pathway [23] and the resultant intermediate of tryptophan catabolism, kynurenine, is further metabolized to 3-hydroxyanthranilic acid, kynurenic acid, and quinolinate by other enzymes. These metabolites can induce cell-cycle arrest and apoptosis in murine and human T cells. Accordingly, IDO is suggested to modulate T cell functions both through tryptophan depletion and *via* its cytotoxic metabolites [24]. At present, however, the underlying mechanism(s) of the tryptophan-related, metabolite-mediated downregulation in the host defense system is unclear. The mechanism underlying the downregulation of T cell functions by tryptophan depletion is thought to be the same as the GCN2 activation observed after arginine depletion. T cells derived from GCN2-knockout mice have been shown to proliferate even in the absence of tryptophan or even in a co-culture with tryptophan-catabolizing enzyme IDO-expressing cells [25]. The mechanism for triggering the initiation of this system after depletion of several particular amino acids, such as arginine and tryptophan, is still unclear.

In 1989, a syndrome whose main features were elevated eosinophil count and severe myalgia was reported mainly in the USA where at lease 37 deaths were reported. The syndrome was called eosinophilia myalgia syndrome (EMS) and was quickly associated with the consumption of dietary supplements containing L-tryptophan produced by one particular manufacturer during a period where a new producing microorganism was introduced and activated carbon used in the purification step was reduced [26-28]. A number of suspected contaminants were identifies and there have been studies on the effects of these contaminants on the immune system, but animal studies have so far failed to reproduce EMS seen in humans. However, as a result of this incident, a ban was placed on the importation of L-tryptophan in the USA and in Europe, the specifications for L-tryprophan was revised to include limits on contaminants [29].

(4) Cystine/Cysteine

Cysteine is a glucogenic non-essential amino acid and a constituent of the important intracellular radical scavenger, glutathione. Cystine is believed to be a rate-limiting precursor of glutathione. Cysteine easily autoxidizes to cystine in extracellular fluid, whereas cystine is rapidly reduced to cysteine once it enter cells. Cysteine regulates the redox potential of a variety of immunocompetent cells. T cells have no cystine-glutamate transporter and, therefore, cannot take up extracellular cystine to metabolize it to cysteine for use in glutathione synthesis [30]. This results in a low intracellular content of glutathione and usually in an oxidative state in T cells. However, in order for T cells to be activated and proliferate, it is essential to increase their intracellular glutathione content and to shift their intracellular circumstance to a reduced state since the activation of the NF-κB transcription factor is, in part, affected by the glutathione content in murine T cells [31].

Supplementation of T Cells with Cysteine from Antigen-Presenting Cells

It is well known that antigen-presenting cells such as macrophages and myeloid dendritic cells are capable of supplying cysteine to T cells. In murine macrophages and myeloid dendritic cells, an Na^{+}-independent anionic amino acid transport system that is highly specific for cystine and glutamate (X-c system), is expressed and mediates the influx of cystine and the simultaneous efflux of glutamate. After metabolizing cystine to cysteine, the cysteine is released extracellularly [32] and then incorporated into T cells through the ASC transport system. Simultaneously, human macrophages and myeloid dendritic cells extracellularly secrete thioredoxin to inhibit the released cysteine from reverting to cystine [33].

(5) Glutamate

Glutamate is a non-essential amino acid, and in a living individual both endogenous (*in situ* synthesized) and exogenous (dietary: 10 g or more daily) glutamate are utilized. Glutamate is mainly utilized as a nutrient source and as a transmitter. As a nutrient source, it functions as an energy source, a component of protein synthesis, a nitrogen resource, and a substrate in glutathione synthesis; as a transmitter, it functions as the umami taste substance (sense of taste), as an excitatory nerve transmitter, and as a chemosensory transmitter in the gastrointestinal tract (visceral information) [34, 35]. In addition, it has been demonstrated

that glutamate can modulate the immune response. In particular, it may contribute to T cell activation.

T Cell Regulation via the Glutamate Receptors

A variety of glutamate receptors are generally categorized into the following two subgroups: the ionotropic group, which functions as an ion-channel via a direct interaction between glutamate and its receptor, and the metabotropic group, which induces an increase or decrease in the related second messenger content after the binding of glutamate to its receptor [36]. Pacheco and his colleagues reported in 2004 that several glutamate receptors were expressed on the cell membrane of human peripheral T lymphocytes [37], and these glutamate receptors were all categorized as metabotropic (mGluRs).

Among the glutamate receptors expressed on T cells, mGluR1 and mGluR5 both function as regulatory receptors in the T cell activation mediated by antigen-presenting cells. Here, we initially describe the role(s) of mGluR5, which is constitutively expressed on resting mature T cells. In the resting state, T cells constitutively express mGluR5 that is functionally coupled to adenylate cyclase. The signals transduced through this glutamate receptor elevate the intracellular cAMP level and activate PKA. In human T cells, cAMP, as well as Protein kinase A (PKA), can suppress the activation of extracellular signal-regulated kinase (ERK) [38] and c-Jun N-terminal kinase (JNK) [39], activate C-terminal Src kinase (CSK) [40], and block the activation of NF-κB [41], resulting in the suppression of T cell proliferation and the inhibition of T cell activation accompanied by increased cytokine production [37]. Next, the expression of mGluR1 is known to be inducible after T cells are activated antigen-specifically. This mGluR1 receptor is functionally linked to the MEK-ERK1/2 pathway [33]. The signals transduced through mGluR1 abolish the mGluR5-mediated inhibition of T cell proliferation and preserve the activated T cell functions via promoting the production of cytokines, including Th_1 cytokines (IL-2 and IFN-γ) and pro-inflammatory cytokines [42].

Modulation of Microenvironmental Glutamate Content by Antigen-Presenting Cells

Antigen-presenting cells such as macrophages and myeloid dendritic cells have been well documented to regulate extracellular glutamate levels via the glutamate transporters. Among the glutamate transporters, the Na^+-independent cystine-glutamate antiporter X-c system and the Na^+-dependent acidic amino acid transporter X^-_{AG} system (glutamate transporter) are both closely associated with T cell activation. The X-c system promotes the influx of cystine and the simultaneous efflux of glutamate. In contrast, the X^-_{AG} system transport glutamate and couple the electrochemical gradient of three co-transported sodium ion into the cells.

In 1993, it was reported that a glutamate transporter was expressed on the cell membrane of human myeloid dendritic cells [43]. In addition, Pacheco *et al*. demonstrated that during the interaction of myeloid dendritic cells with T cells, human myeloid dendritic cells modulate human T cell activation through extracellular secretion of glutamate via the X-c transporter.

Unlike myeloid dendritic cells, macrophages have both the X-c and X^-_{AG} systems. In activated macrophages, the activity of the X-c system was found to be elevated and glutamate was secreted from these cells into the surrounding lymph nodes, resulting in an increased extracellular glutamate level. In contrast, at a steady state, it is likely that macrophages have a role in maintaining extracellular glutamate in the lymph nodes at a low level [44].

(6) Branched-Chain Amino Acids

Branched-chain amino acids (BCAA) comprise the three structurally related essential amino acids leucine, isoleucine, and valine. These amino acids share common catabolic enzymes (transaminase and dehydrogenase) that catalyze the primary steps of degradation and also provide α-amino groups for the endogenous synthesis of glutamine, mainly in skeletal muscle. Furthermore, leucine is an activator of the Ser/Thr protein kinase mTOR (mammalian target of rapamycin) signaling pathway that upregulates protein synthesis and cell growth [45]. Rapamycin is clinically used as an immunosuppressant to inhibit antigen- or IL2-induced T cell proliferation. In addition to being a target of immunosuppressant drugs, recent studies have revealed that mTOR has a variety of immunoregulatory functions in various cell type, including effector T cells, regulatory T cells, monocytes/macrophages, and peripheral myeloid dendritic cells [46, 47]. It is plausible that BCAA, particularly leucine, have a variety of immunoregulatory functions involved in the mediation of mTOR activation. However, compared with glutamine and arginine, there is currently little literature describing the roles of BCAA in immune functions [48]. On the other hand, we recently discovered that the pharmacological oral supplementation of BCAA could be therapeutically useful in the prevention of dexamethasone-induced skeletal muscle atrophy in rat model, one of the adverse effects of glucocorticoids, which are used extensively as immunosuppressants or anti-inflammatory drugs (manuscripts in preparation).

In addition, it has been reported that isoleucine and valine have unique characteristics in innate immunity; namely, that isoleucine induces the antimicrobial peptide beta-defensin from Madin–Darby bovine kidney (MDBK) epithelial cells [49] and that valine improves the function of human monocyte-derived dendritic cells by increasing allostimulatory capacity and IL-12 production in culture [50].

HISTIDINE AS AN ANTI-INFLAMMATORY AMINO ACID

In this section, we describe a further aspect of the immunoregulatory role of amino acids—the anti-inflammatory functions of histidine in colitis.

Crohn's disease (CD) is a chronic inflammatory disorder of unknown etiology that primarily affects the gastrointestinal tract [51, 52]. Enteral nutrition therapy improves not only nutritional status but also clinical symptoms and morphologic findings of CD, and has been used as the first-line therapy for pediatric CD patients [53-55]. In Japan, enteral nutrition, particularly an elementary diet (ED), has now also become the first line of therapy for adult patients

with CD [56, 57]. The ED is a low-fat diet that includes amino acids. Some clinical trials have shown the ED to be as effective as steroids in achieving short-term remission [58]. Although the mechanisms of the actions of the ED remain uncertain, the following possibilities have been indicated: (1) a low-antigenicity diet reduces the mucosal immune reaction; (2) a low-fat diet is less pro-inflammatory; and (3) the ED alters the enteric microflora population. Recently, it has been reported that the ED reduces the mucosal production of pro-inflammatory cytokines such as TNF-α and IL-6 in CD [59]. These cytokines play an important role in the inflammatory process in CD. We hypothesize that the ED may function by suppressing intestinal inflammation directly. Certain amino acids have recently been reported to contribute to the modulation of gut inflammation in various animal colitis models [60, 61]. In this respect, we focused on the effect of the amino acids that are the main component of the ED.

Previously, it has been reported that the ED reduces colonic inflammation in a murine IL-$10^{-/-}$ transfer model of colitis as a Th-1 disease model that resembles CD [62, 63]. Furthermore, not only the ED, but also a mixture of the amino acids found in the ED reduces colonic inflammation in this colitis model [64]. These findings indicated that amino acids themselves have anti-inflammatory activity. Among these, oral supplementation with 5% histidine in a standard formula reduces histological damage, colon weight, and TNF-α mRNA expression [64]. The anti-inflam-matory effects of histidine have been confirmed in primary mouse peritoneal macrophages. Histidine inhibits the production of TNF-α and IL-6 by LPS-stimulated peritoneal macrophages in a concentration-dependent manner.

Histidine is one of the most common naturally occurring amino acids and is classified as one of the conditionally essential amino acids. This is because adults, but not children, can independently produce an adequate amount of histidine. It has been reported that histidine has the potential to scavenge hydroxyl radicals and non-radical toxic oxygen species. Recently, it has been reported that histidine can inhibit the production of IL-8 by intestinal epithelial cell lines treated with TNF-α or H_2O_2 [65]. It has also been reported that several amino acids in addition to histidine act as radical scavengers during epithelial injury, and that the oral or rectal administration of these amino acids shows efficacy in dextran sodium sulfate (DSS) or trinitrobenzene sulfonate (TNBS) colitis models [51, 52]. In primary macrophages, D-histidine and carnosine exhibit reduced inhibition of TNF-α. On the other hand, there was no difference in the intracellular levels of histidine in L- and D-histidine-treated macrophages, and the intracellular level of carnosine was equal to that of histidine. As previously reported, not only L-histidine but also D-histidine and carnosine have anti-oxidative effects. These results clearly indicate that the antioxidant properties of histidine alone cannot explain its anti-inflammatory effect in macrophages.

The suppressive effects of histidine on the production of pro-inflammatory cytokines in these studies were, at least partially, dependent on the NF-κB signaling pathway [64]. However, further studies will be needed to clarify the mechanism of action of the histidine in macrophages. The anti-inflammatory effects of amino acids suggest a new mechanism of action of ED therapy and, more importantly, the potential of histidine as a novel therapeutic reagent for CD. Histidine also inhibits TNF-α secretion from LPS-stimulated CD14-positive human monocytes. Furthermore, as previously reported, other amino acids have been shown to contribute to the modulation of gut inflammation. It follows that, owing to their anti-inflammatory effects, amino acids may have potential as novel therapeutic agents for inflammatory bowel disease. In conclusion, these reports provide new insights into a therapeutic strategy for CD based on the observations that amino acids, particularly histidine, inhibit the production of pro-inflammatory cytokines from activated macrophages.

CONCLUSION AND FUTURE PROSPECT

Amino acids are involved in immune responses because they are required for the synthesis of a variety of specific proteins, including cytokines and antibodies. In addition to these roles as building-blocks, amino acids have a variety of immunoregulatory functions. In this review, we focused on the regulatory functions of amino acids; e.g., as an energy source, as components or precursors of various biomaterials, as neurotransmitters, and as inducers of signal transduction. Furthermore, local and systemic changes in the concentrations of free amino acids in the plasma and tissues, described as amino acid imbalances, act as a trigger for abnormal immune responses, followed by not only malnutrition but also certain pathological conditions such as infections, malignant neoplasm formation, autoimmunity, and inflammation. Because of the metabolic networks of amino acids in the body, plasma amino acid imbalances result in specific profiles of amino acid concentrations; furthermore, these profiles or an algorithm (AminoIndex) of multiple amino acid concentrations are useful diagnostic or predictive markers of specific pathological conditions [66]. AminoIndex is an algorithm for developing an index composed of multiple amino acid concentrations against any given target parameter [67], which has been registered as AminoIndex, and in the current version of the algorithm, the generation of Amino-Index is performed by statistical methods using a data mining approach, through conventional multivariate statistics such as logistic regression, linear regression, linear discriminant analysis and others. In order to prevent pathological abnormalities, it is suggested that supplementation of appropriate amino acids is a possible solution for maintaining the healthy life of an individual by regulating his/her immune responses. In the future, people will have a greater desire for such amino acid supplements for therapeutic and preventive applications even after diagnosis by profiles of plasma amino acid concentrations, such as AminoIndex. In order to realize amino acid-based healthcare, several outstanding problems need to be solved. One of these concerns the important need to deliver the required amino acid(s) to the necessary target site(s), at an appropriate dose, and in accordance with individual needs. Another would be possible unpredictable adverse reactions with excess intake of specific amino acids, followed by hyperphysiological increase of plasma or tissue concentrations of amino acids. In an attempt to address such problems, further investigations are required to clarify the functions of amino acids *in vivo* and the regulation of their metabolic networks.

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DISCLOSURE

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