

# Amino Acids and Immune Response: A Role for Cysteine, Glutamine, Phenylalanine, Tryptophan and Arginine in T-cell Function and Cancer?

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**Abstract** While proteins are critical for immunity, T-cells constitute a critical component of adaptive immunity by clearing cancerous cells among other abnormal cells. However, cancer cells exhibit a potential to escape T-cell control by employing mechanisms not completely delineated. Interesting work has investigated how certain amino acids affect the proliferation rate of T-cells as well as their effectiveness in clearing tumors. The role of amino acids cysteine, glutamine, phenylalanine, tryptophan and arginine in immunomodulation and particularly regarding T-cell proliferation and activation is discussed. The redox balance is reported to affect T-cell proliferation via modulation of cysteine availability. In addition antigen presenting cells (APCs), similar to myeloid cells determine the availability of amino acids in the extracellular microenvironment affecting T-cell proliferation and activation. A better mechanistic understanding of T-cell function modulation via amino acid signaling or metabolic properties may be helpful towards optimization of adaptive immunity with implications for cancer prognosis and treatment.

**Keywords** T-cell · Amino acids · Cysteine · Glutamine · Phenylalanine · Tryptophan · Arginine · Immune response · Signaling · Immunomodulation · Avidity

## Major Types and Roles of T-cells

T-cells (T-lymphocytes) are essential for human immunity as they circulate surveying the human body, scanning, detecting and resolving cellular abnormalities and infections. Two

major types of T-cells orchestrate the immune response by either directly attacking infected or cancerous cells (killer T-cells or cytotoxic T lymphocytes, CTLs or T<sub>C</sub>), or via an array of signaling mechanisms that collectively facilitate the adroitness of the immune response (helper T-cells, T<sub>H</sub>) [1, 2].

Another T-cell type, natural killer (NK) T-cells (NKT), functions at the interface of the adaptive and innate immune system [2]. While CTLs are patrolling for antigen fragments protruding on the surfaces of antigen presenting cells (APC) bound to self-major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigens presented by means of cluster of differentiation (CD)1d (cells lacking self-MHC molecules).

## Maturation

T-cell maturation undergoes two distinct selection processes in the thymus, positive and negative. Positive selection permits the survival of only those T-cells with T-cell receptors (TCR) fully capable of accurately recognizing self-MHC molecule (MHC restriction), thus allowing highly efficient, effective and specific targeting ability [3]. Negative selection eliminates T-cells that react too strongly to self-MHC plus self-peptide/self tolerance, a strategy that safeguards against autoimmune phenomena [3].

## The Concepts of Activation, Affinity and Functional Avidity of T-cells

### Activation

A T-cell is activated upon TCR binding to its cognate antigen on the APC and the subsequent signaling cascade leading to phospholipase C –  $\gamma$  (PLC- $\gamma$ ) recruitment and intracellular

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$\text{Ca}^{++}$  mobilization, thus evoking an immune response under physiological circumstances. A significant difference in the degree and responsiveness of T-cell activation exists between naïve and experienced or primed T-cells. In vitro priming of T-cells could lead to approximately 75-fold PLC- $\gamma$ 1 upregulation along with significant increase of TCR signaling as evidenced by significantly increased  $\text{Ca}^{++}$  flux [4]. In line with the latter finding, naïve T-cells were reported to exhibit a significantly reduced  $\text{Ca}^{++}$  release subsequently to TCR activation compared to primed T-cells [5–7]. Of note, administration of the hormonal form of vitamin D (1,25-(OH) $_2$ -D) was shown to induce PLC- $\gamma$ 1 upregulation (via mechanisms implicating the vitamin D receptor, VDR) and subsequent  $\text{Ca}^{++}$  release in T-cells, suggestive of a nutritional modulation potential, at least regarding the sensitivity of TCR activation [4]. Additionally, evidence for microRNA regulation of activation has been presented particularly for miRNA181a, shown to target multiple phosphatases which otherwise would inhibit TCR signaling [8–10].

### Affinity

Affinity is a measure of the binding strength between one TCR and one peptide major histocompatibility complex (pMHC) (i.e., strength of association between receptor and target). It is determined via the dissociation constant value ( $K_D$ ) and the “dwell time”,  $t_{1/2}$ . Low  $K_D$  signifies a tighter association, while optimal affinity conditions are determined to be in the following range:  $K_D=1\text{--}10\ \mu\text{M}$ ,  $t_{1/2}=5\text{--}20\ \text{s}$  [11]. Too long of a dwell time does not lead to adequate binding events while too short dwell time leads to incomplete TCR activation [8].

### Functional Avidity

The relative capacity of a T-cell to produce effector-type cytokines or lyse target cells in an antigen-specific manner is measured by its functional avidity. Essentially, functional avidity describes how sensitive or responsive a T-cell is in terms of effectively recognizing, binding and responding to its target APC at a given concentration of the ligand. The concentration required to induce a half-maximal response ( $\text{EC}_{50}$ ) is typically used to describe the functional avidity of T-cells [8].

Functional avidity is determined via *ex vivo* quantification of biological functions such as IFN- $\gamma$  production, cytotoxic activity (lysing ability) or proliferation.

It is generally accepted that higher functional avidity CD8 T-cell responses are more efficient and effective in clearing acute viral infections and eliminating cancer cells [8]. In the case of tumors, better T-cell responses are evoked by high functional avidity T-cells [11]. In addition, an attenuated functional avidity exhibited by T-cells in cancer can partially

explain why cancer cells persist and proliferate, thus expanding their population [8, 11].

Therefore, strategies that can increase the functional avidity of T-cells are of particular interest for enhancing the effectiveness of treatments for viral infectious diseases and cancer. Antigen-experienced T-cells (primed) demonstrate markedly enhanced functional avidity (sensitivity to cognate antigen) compared to naïve T-cells, a sensitivity induction that is achieved in a process termed “functional avidity maturation” [12–15].

### Amino Acid Availability Affects T-cell Proliferation and Activation

Higher avidity T-cells are preferentially triggered, thus leading to a much more rapid and effective target (tumor)-cell elimination. Ideally, an organism would exhibit a high proliferative rate for T-cells as well as a high functional avidity of T-cells both at the individual T-cell level (clone level) as well as at the entire population level. However, cancer-induced mechanisms result in lower T-cell proliferation rate (in part by changing amino acid availability), as well as reduction of functional avidity.

All mammalian cells require nutrients, particularly amino acids, acquired by the extracellular environment to survive, function properly, grow and proliferate [16, 17]. Hence, amino acid availability through constant supply is critical. Regarding the T-cells, amino acid availability can exert an impact on the regulation of both proliferation and activation, thus affecting the overall effectiveness of a T-cell population towards clearing cancerous or infected cells. There are limited studies investigating the direct role of amino acids, in terms of mechanisms and/or availability, in immunomodulation. In this brief review the relationship between amino acid availability and T-cell activation/functional avidity potential is discussed along with how these observations could be used as a potential treatment strategy in the case of cancer.

#### The Cysteine-Cystine-Glutathione Axis

Cellular cysteine concentration is defended within a narrow range in most cells via regulation of amino acid transport (SLC and ABC families), glutathione synthesis and cysteine catabolism [18–20]. Regulation of cellular cysteine and glutathione levels is essential due to the many critical roles these thiols play in the cell. Cysteine is necessary for synthesis of proteins, glutathione and coenzyme A; while cysteine catabolism generates the essential metabolites hypotaurine/taurine and inorganic sulfur plus pyruvate. Glutathione is a major mediator of the cellular thiol-disulfide redox state and an essential component of antioxidant and detoxification

pathways. Inside the cell, both cysteine and glutathione are maintained in a highly reduced state; while cysteine levels are maintained quite low, glutathione levels are much higher (~50- to 100-times those of cysteine) [20].

In lymphocytes, cysteine has been identified as rate limiting for cell growth and proliferation with a series of studies demonstrating a role for cysteine in regulating the proliferation of T-cells [21–26]. Redox conditions determine the equilibrium between the levels of cysteine (free thiol form) and cystine (disulfide bonded form). Under oxidative conditions cysteine equivalents are primarily found in the form of cystine, whereas in a reducing environment the primary form of the amino acid is cysteine. In mammalian cells the extracellular environment is typically oxidative while the intracellular reducing [25]. Consequently, cysteine equivalents outside the cell are in the form of cystine, while in the cell in the form of the free thiol (i.e. cysteine).

In general, cysteine levels in the plasma are relatively low, while lymphocytes do not express SLC7A11 (xCT), which is the transporter subunit of the cystine-glutamate exchanger  $Xc^-$ , neither cystathionase (CTH), which is involved in cysteine synthesis from methionine. Hence, although cysteine is considered semi-indispensable amino acid since it can be synthesized via transsulfuration when intake of its indispensable amino acid precursor methionine is adequate, for lymphocytes in particular cysteine is practically essential. Given this idiosyncratic characteristic, in naïve T-lymphocytes, it has been proposed that T-cell activation is dependent on cysteine concentration increase induced by APCs (Fig. 1) [25, 27]. Work conducted by Levring et al. [23], demonstrated that although naïve T-cells express very low levels of the cysteine transporter, if any at all, these transporters become very strongly up-regulated during T-cell activation hence providing T-cells with the appropriate cysteine amounts. The extracellular amount of cysteine uptaken by T-cells is still provided by APCs through an act of exchange between cystine and cysteine (cystine-in for cysteine-out) (Fig. 1) [23]. Therefore, APCs play an important role in regulating the actual extracellular local availability of cysteine and subsequently the proliferative rate of T-cells. Furthermore, as T-cells must strike a balance between clearing invaders but also be tolerant towards self-antigens, it is critical that the degree of activation of T-cells can serve this delicate balance. Signaling via APCs provides for a fine-tuning in the T-cell activation response, with cysteine availability serving such a goal. Depending on the availability of cysteine, T-cells can regulate protein synthesis, proliferation and secretion of immunoregulatory cytokines [23, 25].

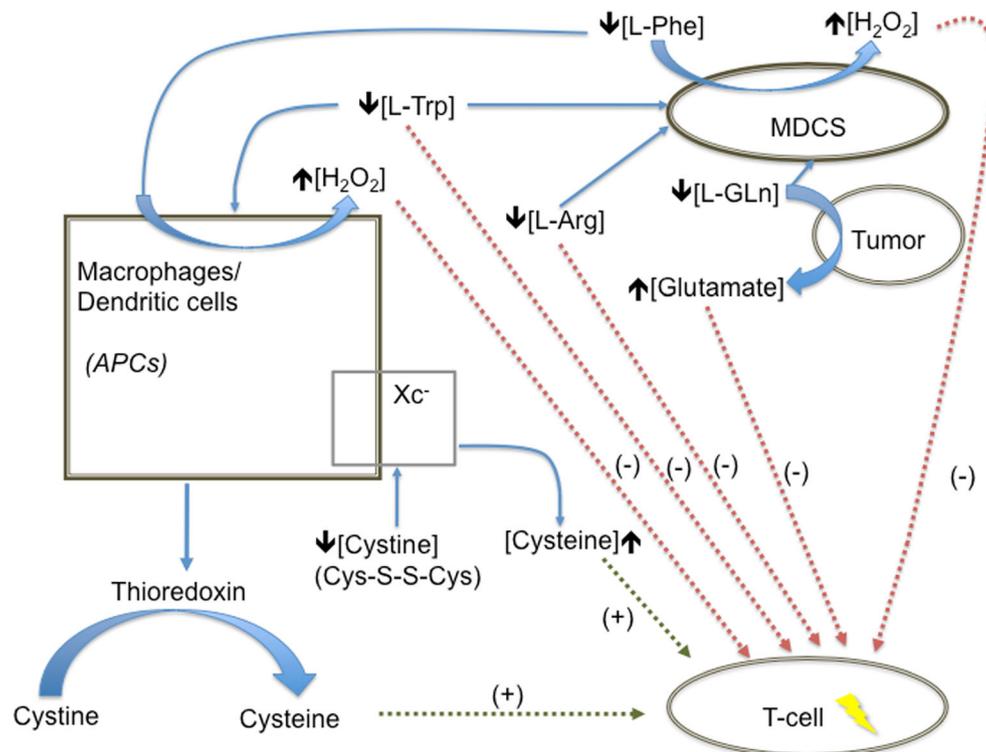
Cysteine is however fairly quickly oxidized to cystine in the extracellular space leading to decreased levels of available cysteine for T-cells. Thioredoxin secretion constitutes a mechanism for reducing cystine back to cysteine, thus maintaining/defending cysteine levels in the extracellular space during the immune response (Fig. 1) [28].

Experiments, performed by Dröge et al. [29] revealed that the extracellular supply of cysteine influences strongly the intracellular level of glutathione (GSH) as well as the activity of the transcription factor NF $\kappa$ B, which subsequently regulates the expression of several immunologically relevant genes [30]. Furthermore, in vitro work conducted by the same group, including double-chamber experiments with macrophages and lymphocytes, demonstrated a regulatory mediator role for cysteine between these two cell types [31]. As widely observed, cysteine supply is significantly impaired directly or indirectly in several immunodeficiency associated pathologic conditions, including a broad variety of cancer types [31].

Contrastingly, the relatively high cystine transport activity of the antigen-presenting macrophages, provides these cells with a “cysteine pumping” mechanism/function which in turn allows the antigen binding T-cells in the close environment to shift to the antioxidant state [32]. Essentially, the difference between the membrane transport activities for cysteine of T-cells and macrophages emerges as the key element of a mechanism that facilitates both, the pro-oxidant state of T-cells and their regulated shift to the antioxidant state by generating competition for cysteine between the two cell types. In the event that T-cells are not provided with sufficient amounts of cysteine, the intracellular glutathione levels and rates of DNA synthesis activity decrease significantly. In such a condition, cells may well undergo a variety of oxidative damage related manifestations, which can potentially lead to functional impairments and eventually apoptosis [23, 27, 32]. While cysteine availability is regulated by macrophages and dendritic cells, glutamate has been shown to inhibit cystine uptake by macrophages indirectly by lowering cysteine levels available for T-cells [27].

In human studies, Murphy et al. conducted a nested case-cohort study within the General Population Nutrition Intervention Trial in Linxian, China assessing 498 oesophageal squamous cell carcinomas (OSCC) and 255 gastric cardia adenocarcinomas (GCA) [33]. Higher concentrations of serum cysteine were significantly associated with a lower risk of both OSCC and GCA [33], suggesting that cysteine should be further investigated for its potential as a chemopreventive agent at least for upper gastrointestinal cancers [33].

In general, lymphocytes require a reducing environment for optimal activation [34]. Such a reductive environment may be protective against oxidative stress during T-cell activation, however low levels of ROS are necessary for the initiation of the immune response [34–36]. For instance increased GSH synthesis accompanies antigenic response induction [37]. Furthermore, physiological levels of  $H_2S$  generated by cysteine metabolism were reported to mediate TCR-induced activation of primary murine T-cells ( $CD3^+$ ), OT-II  $CD4^+$ , and the human Jurkat T-cells. In addition, nanomolar  $H_2S$  concentration (50–500 nM) enhance T-cell activation as assessed by CD25, CD69 and interleukine (IL-2) levels [38].



**Fig. 1** Conceptual schematic diagram on the regulation of T-cell immune responses modulated by amino acid availability: Reduced extracellular levels of L-Arg, L-Gln, L-Trp due to uptake by macrophages, dendritic cells and MDCS directly extend an inhibitory effect to T-cell proliferation. Additionally, L-Gln is taken up by tumor cells and via glutaminolysis converted to glutamate which is excreted into the extracellular environment. Glutamate in turn has a direct inhibitory effect to T-cell proliferation and activation. L-Phe is taken-up by macrophages, dendritic cells and MDCS and via IL-411 is being converted to H<sub>2</sub>O<sub>2</sub> which is exported and inhibits T-cell proliferation. Cysteine is exchanged for cysteine by APCs

via Xc<sup>-</sup>, hence cysteine's extracellular levels increase which in turn has a positive signaling effect (induction) on T-cells proliferation and activation. As cysteine is quickly oxidized back to cystine in the extracellular environment, produced thioredoxin catalyzes the conversion of cystine back to cysteine thus defending the extracellular cysteine levels. *Blue plain-arrows* signify movement of compounds and transmembrane movement (where applicable). *Blue block-arrows* signify conversion of compounds and transmembrane movement (where applicable). *Green dashed arrows* signify positive signaling (induction). *Red dashed arrows* signify negative signaling (inhibition)

### L-taurine (Tau)

Taurine (Tau), the highest abundant free nitrogenous compound in cells, similarly to sulfate constitutes an end-product of cysteine metabolism. Taurine controls overall cell stability via membrane stabilization function and regulatory effects on calcium flux [32]. Additionally Tau has antioxidant properties as well as the capacity to regulate the release of pro-inflammatory cytokines in hamsters, rats, and humans [39–41].

Substantial impairment of immune function has been reported under Tau deprivation in cats, whereby taurine is practically an essential nutrient since cats are unable to synthesize the compound [40]. More specifically, the immunological effects of Tau deprivation in the feline model include a significant decline of the lymphocyte population, along with a marked increase in mononuclear cells with a diminishing capacity to produce an effective “respiratory burst” and perform bacterial phagocytosis [32]. Furthermore, a significant rise in  $\gamma$ -globulin concentrations was observed in Tau-deficient animals, while examination of the spleen and lymph

nodes revealed significant regression of follicular centers and depletion of mature and immature B-lymphocyte pools [32]. According to the researchers, taurine inclusion in the diet reversed all the aforementioned changes. Studies conducted using other species as noted by Gribble RF, have also corroborated the positive effects of taurine supplementation on immune system and its function [32].

Administration of Tau prevented the aging-related decline of T-cell population and enhanced proliferative responses of T-cells in both young and old mice [42].

Further evidence supports an immunomodulatory role for Tau, shown to improve inflammation status in trinitrobenzene sulfonic acid-induced colitis. Taurine interacts with hypochlorous acid, produced during the marked increase of oxidants of stimulated macrophages, to produce taurine chloramine (TauCl), an immunomodulating compound that may be responsible for signaling properties previously attributed to taurine. In vitro studies have demonstrated that an increase in Tau concentration from physiological to supraphysiological concentrations carries no effect on proinflammatory cytokine production by peripheral blood mononuclear cells (PBMC).

Nonetheless, TauCl impairs both NF $\kappa$ B activation and the capacity for proinflammatory cytokine production, thus generating an overall antiinflammatory effect [41]. In *in vitro* experiments, TauCl inhibited NO, PGE<sub>2</sub>, TNF- $\alpha$ , and IL-6 production from stimulated macrophages and the ability of APCs to process and present ovalbumin [42]. In studies with human PBMCs, taurine (0 to 500 mmol/L) was not found to influence TNF- $\alpha$  or IL-1 $\beta$  production, while TauCl concentration of 250 mmol/L or higher was shown to decrease the production of both cytokines [43]. An oral dose of 0.1 mg/kg L-methionine is shown to raise plasma Tau to 120 mmol/L; suggesting that the effect may not be physiologically meaningful [44]. Work with murine dendritic cells revealed that TauCl altered the balance of Th1 to Th2 cytokines, suggesting that it may play a role in maintaining the balance between the inflammatory response and the acquired immune response [44].

#### L-glutamine (Gln)

Glutamine, the most abundant amino acid in the human body, is the most important nutrient for cellular proliferation, second only to glucose [45].

Extracellular glutamine appears to regulate T-lymphocyte proliferation, the rate of IL-2 production and IL-2 receptor expression [46]. Cancer cells present an increased glutamine turnover partly because of higher glutaminase expression which converts glutamine into glutamate [45], therefore cancer patients exhibit low plasma glutamine and high glutamate levels with glutamate secreted by tumor cells inhibiting T-cell activity.

As glutamine is highly utilized by immunologic tissues and cells, increased glutamine consumption in inflammatory states leads to an overall negative glutamine balance in blood, muscle and immunologic tissue as well as a limited function of immune cells [46]. Estimates prefigure that when plasma glutamine levels fall in a “glutamine-deficient” state, e.g., 0.4 mmol compared with “normal” levels of plasma glutamine, e.g., 0.6 mmol, then the immune system is severely compromised [47]. In this context, administration of exogenous glutamine in conditions of low glutamine levels, such as those observed during inflammation, should enhance immunologic responses and improve outcome. In addition, as glutaminolysis contributes to myeloid-derived suppressor cells (MDSC) maturation through the supply of energy and metabolic intermediates [48], maintaining optimal glutamine levels is critical in preventing the MDSC-mediated immunosuppression.

#### L-arginine (Arg)

Arginine (Arg), a non-essential amino acid, plays a key role in various biological processes including the immune response

[22, 49]. In cases of hepatic transplantations impaired T-cell function induced by immunosuppression is paralleled by markedly reduced Arg plasma levels, a relationship also observed with *in vivo* models. Impaired T-cell function can be reversed upon enteral or parenteral Arg supplementation, suggesting an immunomodulation capacity of Arg. [49, 50].

Low Arg levels induce loss of the CD3 $\zeta$  chain, inhibition of T-cell proliferation and diminished cytokine production, highly resembling conditions met in cancer patients, while these phenomena are completely reversed by excess addition of Arg in culture [51, 52].

Besides dietary intake, Arg levels are controlled through various ways including *de novo* synthesis from citrulline, protein catabolism, as well as Arg degrading enzymes such as arginase (ARG) I and II, and inducible nitric oxide synthase (iNOS) [53]. Arginase converts Arg to ornithine and urea, while iNOS produces NO, which besides vasodilation, plays an important role in the control and eradication of parasites, bacteria, viruses and cancer cells [54]. Interestingly, humoral, anti-inflammatory cytokines (IL-4, IL-10, IL-13 and TGF- $\beta$ ) induce ARG I expression, while pro-inflammatory cytokines (IL-1, TNF- $\alpha$ , IFN- $\gamma$ , and IL-2) induce iNOS [54].

Murine peritoneal macrophages stimulated with Th2 cytokines (IL-4 and IL-13) produce ARG I and express the cationic amino acid transporter CAT2B, leading to depletion of Arg from the environment and in turn T-cell dysfunction. However, addition of the ARG I inhibitor Nor-NOHA or excess Arg can reverse this effect [52].

Suppression of tumor-specific T-cell functions by MDSCs is a dominant mechanism of tumor escape [21], while both ARG I and iNOS are expressed by MDSCs, further indicating that Arg bioavailability is crucial for T-cell proliferation and proper function. As reported by Popovic et al., T-cell maximal proliferation is achieved with Arg levels of approximately 100  $\mu$ mol/L, with further Arg increase having no additional effect on T-cell proliferation rates [54]. On the other hand increased Arg levels in an oxidative environment (due to excessive superoxide (O<sub>2</sub><sup>-</sup>) generation or compromised O<sub>2</sub><sup>-</sup> quenching ability) can lead to peroxynitrite (ONOO<sup>-</sup>) formation. The subsequent nitration/nitrosylation conferred upon the TCR  $\alpha$  and  $\beta$  subunits of the CD8 co-receptor causes CD3 $\zeta$  chain dissociation from the TCR molecule and ultimate TCR signaling disruption [55]. Reactive Nitrogen Species (RNS) in general induce nitration/nitrosylation of either MHC class I molecules or peptide ligands on tumor cells and TCR chains on T-cells, hence leading to impaired recognition of tumor cells, rendering Arg availability to T-cells a critical parameter for their proper function, especially in the case of tumors [55]. However, as too high or too low levels can lead to TCR signaling disruption and apoptosis and/or T-cell proliferation failure, it appears critical to optimize Arg levels for proper T-cell function.

### L-phenylalanine (Phe)

There is evident involvement of Phe metabolism in modulating suppression of T-cell immune responses [21]. Suppression of T-cell proliferation along with signaling impairments can occur as a result of the decreased Phe and the increased hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) resulting from the oxidative deamination of Phe by a Phe oxidase encoded by IL-4 induced gene 1 (IL4I1) [21]. IL4I1 can be expressed by APCs in humans and mice [56]. In addition, microarray data have delineated a significant increase of IL4I1 mRNA levels in tumor-induced murine MDSCs, suggesting a role for IL4I1 in the negative feedback regulation of T-cell activation [57]. It appears highly possible that IL4I1 constitutes an immunomodulatory factor via the inhibition of CD3 $\zeta$  chain expression and T-cell proliferation through the production of H<sub>2</sub>O<sub>2</sub> [58]. Overall, these observations strongly support a regulatory role for Phe and its metabolism regarding the proliferation and activation of T-cells and subsequent immune responses.

### L-tryptophan (Trp)

Macrophages are reported to adjust extracellular Trp levels and limit T-cell proliferation by degrading Trp, a process identified to play an important role in the generation of T-cell tolerance [22]. Macrophages cultured in the presence of macrophage colony-stimulating factor (MCSF) were shown to suppress T-cell proliferation by generating the Trp degrading enzyme indoleamine 2,3-dioxygenase (IDO) [22]. A negative effect on proliferation, function and survival has been observed in T-cells in the proximity of IDO-expressing cells [59]. Two genes have been identified encoding for the heme containing enzymes that catalyse Trp oxidative degradation, IDO and tryptophan 2,3-dioxygenase (TDO). Both enzymes catalyze the oxidative cleavage of the 2,3 double bond in the indole ring, which is the first and rate-limiting step in Trp catabolism. While TDO is mainly expressed in the liver, IDO is detected in several tissues [22]. A broad variety of cellular types like myeloid-lineage cells (monocyte derived macrophages), fibroblasts, endothelial cells, as well as certain tumor cell lines, express IDO upon exposure to INF- $\alpha$ , lipopolysaccharide (LPS) or the inflammatory cytokines IL-1 and TNF $\alpha$  [60].

Excess Trp supply or IDO chemical inhibition, can abolish the T-cell anti-proliferative effect exerted by MCSF matured macrophages [61]. Interestingly, the Trp degrading activity of these macrophages is not continuously engaged but instead carefully regulated via interactions with activated T-cells [60, 61]. Therefore, APC and T-cell intercommunication can emerge as a potentially important factor for the control of Trp catabolism. Tryptophan degradation can also be induced if INF- $\gamma$  is administered to macrophages with INF- $\gamma$  acting to generate IDO. Notably, macrophages can escape growth

limitation due to Trp degradation and deficiency, by upregulating the expression and increasing the translation for Trp t-RNA synthetase [28]. In addition, in vitro activated human and mouse T-cells undergo cell-cycle arrest when cultured in the absence of Trp, exhibiting a much higher sensitivity to apoptosis [61, 62]. Low serum Trp concentrations predict poor prognosis for melanoma patients, suggesting an immunosuppressive role of Trp deprivation since T-cell activation leads to increased demand for Trp [63].

Clinical data demonstrate an association between IDO upregulation and low infiltration of tumor by infiltrating lymphocytes, linked to increased malignancy of colorectal cancer [64]. Certain metabolites of Trp degradation by IDO induce lymphocyte apoptosis [65], while low Trp concentration in combination with high kynurenin (downstream metabolite) decreases the expression of the TCR  $\zeta$ -chain leading to T-cell anergy [66]. Several studies providing associations between cancer and Trp catabolism, have identified the kynurenin/tryptophan ratio as a good measure of Trp catabolism and hence effectiveness to cancer treatment [67, 68]. However, the kynurenin/tryptophan ratio can change in response to non-specific inflammation and is induced in all states of chronic immune activation since IDO gene expression is highly responsive/sensitive to IFN- $\gamma$  levels [69]. Thus, IDO appears critical in regulating Trp levels and subsequently Trp-dependent immunity. Interestingly, cells expressing IDO (macrophages, dendritic cells, etc.) are commonly present in cancer patients [70]. Particularly MDSCs as well as macrophages and other IDO-positive cells are determined to play a key role in blocking immunotherapy by interfering with amino acid metabolism, thus exerting an inhibitory effect on T-cell activation [24, 71].

### A Case for Optimal Amino Acid Signaling by Striking Optimal Amino Acid Balance

There is evidence from both in vitro [16] and in vivo [17, 72] studies to suggest that a mild and carefully regulated sulfur amino acid limitation can induce a pro-survival response via the Integrated Stress Response (ISR) and 4E-BP1 induction levels independent of the mTORC1 system [73]. Although it is not yet clear how critical the role of sulfur containing amino acids can be in bringing about these effects, the relationship between repressed global protein synthesis and growth/proliferation and resistance to disease and longevity is of great interest, and nutrient sensing/regulatory pathways such as the amino acid deprivation pathway as well as thiol-disulfide redox balances may well play a role in these processes. Such ISR-mediated cellular responses could be beneficial in cancer cases among other conditions (including ageing-related diseases) due to induction of selective upregulation of pro-

survival genes [74]. In these studies though, whether amino acids exerted a direct effect on T-cells activation was not investigated yet cannot be excluded. There is evidence to support that the availability of cysteine is necessary for proper T-cell activation as previously discussed here. It remains unclear however, if mild limitation of cysteine in a carefully tailored dietary scheme could evoke the positive signaling effects by the way of ISR and mTOR and still provide enough resources for optimal T-cell activation.

The use of amino acid limitation to control the proliferation of lymphocytes has been clinically validated and constitutes a common medical practice via the use of L-asparaginase in the treatment of patients with acute lymphoblastic leukemia. The notably high rate of cured lymphocytic leukemia in children treated with L-asparaginase combined with other chemotherapeutics provides a very good example of how effective limited/optimized levels of specific amino acids can be in regulating cellular proliferation in the case of tumors [28].

In another approach, it has been reported that intracellular leucine concentrations critically regulate mTORC1 signaling to promote Th1, Th2, and Th17 CD4<sup>+</sup> T-effector cell differentiation [75]. In addition tryptophan depletion has been reported to result in T-cell anergy and apoptosis through the GCN2 pathway [69]. As noted by the same group, depletion of tryptophan, by IDO or TDO inhibition, in the tumor would arguably result not only in the ISR activation in T-cells but also in cancer cells (most probably at different thresholds/doses).

Research in the area of amino acid therapy in malignant disease has worked on the concept of manipulating the amino acid supply levels in a way that beneficial responses will be evoked for healthy cells while tumor cells will be suppressed. The strategy aims in striking the optimal balance point in terms of type and relative quantities of amino acids in order to shift the balance between the host and tumor in the host's favor. In this context and given the signaling effects of branched chain amino acids (BCAA) (leucine in particular) to mTORC1, some researchers have supported that selective BCAA limitation can damage the tumor more than the host [76]. On the other hand, the idea of BCAA supplementation on the basis that the overall benefit of the host will be greater than that of the tumor has also gained notable support. At least for the BCAAs, the few available data offer conflicting conclusions as to whether amino acid limitation would be more favorable or unfavorable in cancer patients although these experiments only considered a certain set of amino acids (BCAAs) and only the signaling pathway of mTORC1 as judged by phosphorylation of its direct targets (not taking into consideration the GCN2 – ISR pathway or the actual protein amounts of 4E-BP1). The possibility cannot be excluded that there may be an ideal level of amino acid supply, an optimum mixture with optimum relative ratios that would maximize the advantages to the host against the tumor.

From an immunology standpoint, tumors appear to employ mechanisms for inducing a severe deprivation of certain amino acids in the microenvironment of T-cells, leading to reduced T-cell proliferation and impaired proper/effective signaling. This strategy allows tumor cells to not be fully subjected to T-cell control. Thus it appears that a condition of a mild limitation of sulfur containing amino acids may on the one hand induce the pro-survival programming yet not impair T-cell proliferation/activation to a degree that would allow tumor cells to escape immunological control as the deprivation of amino acids would not be severe. Studies investigating the direct role of amino acids, in terms of mechanisms/availability, in immunomodulation are not very common. Furthermore, notably the existing research does not study all the amino acids. Identifying the optimal degree of such a limitation and striking the correct equilibrium point of balance is certainly challenging, nevertheless worth pursuing its investigation through further research for improving immunity in cancer and other conditions such as ageing. By and large, cancer is a disease of age progression. While several studies suggest that a natural defense mechanism seeks and destroys cancer cells [70], clinical studies also reveal that tumor-employed mechanisms bypass the immune response that attacks cancer cells, by markedly impairing the T-cell responses that would normally eradicate and remove tumor cells [22]. Hence, cancer management via harnessing amino acid immunomodulatory capacity, may prove useful and applicable in ageing and other related conditions as well. Enhancing immune responses can extend a great service in the context of disease and sickness in the elderly population for reduced mortality, more effective treatment and overall better health and quality of life.

**Conflict of interest** The author declares no conflict of interest.

## References

1. Steinke JW, Lawrence MG (2014) T-cell biology in immunotherapy. *Ann Allergy Asthma Immunol* 112:195–199
2. Smith KA (2014) Revisiting the first long-term culture of antigen-specific cytotoxic T cells. *Front Immunol* 5:194
3. Viret C, Janeway CA Jr (1999) MHC and T cell development. *Rev Immunogenet* 1:91–104
4. von Essen MR, Kongsbak M, Geisler C (2012) Mechanisms behind functional avidity maturation in T cells. *Clin Dev Immunol* 2012: 163453
5. Robinson AT, Miller N, Alexander DR (1993) CD3 antigen-mediated calcium signals and protein kinase C activation are higher in CD45RO<sup>+</sup> than in CD45RA<sup>+</sup> human T lymphocyte subsets. *Eur J Immunol* 23(1):61–68
6. von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Ødum N, Geisler C (2010) Vitamin D controls T cell antigen receptor signaling and activation of human T cells. *Nat Immunol* 11(4):344–349

7. Ericsson PO, Orchansky PL, Carlow DA, Teh HS (1996) Differential activation of phospholipase C- $\gamma$ 1 and mitogen-activated protein kinase in naïve and antigen-primed CD4 T cells by the peptide/MHC ligand. *J Immunol* 156(6):2045–2053
8. Viganò S, Utschneider DT, Perreau M, Pantaleo G, Zehn D, Harari A (2012) Functional avidity: a measure to predict the efficacy of effector T cells? *Clin Dev Immunol* 153863
9. Ebert PJ, Jiang S, Xie J, Li QJ, Davis MM (2009) An endogenous positively selecting peptide enhances mature T cell responses and becomes an autoantigen in the absence of microRNA miR-181a. *Nat Immunol* 10(11):1162–1169
10. Garcia Z, Pradelli E, Celli S, Beuneu H, Simon A, Bouso P (2007) Competition for antigen determines the stability of T cell-dendritic cell interactions during clonal expansion. *Proc Natl Acad Sci U S A* 104(11):4553–4558
11. McMahan RH, Slansky JE (2007) mobilizing the low-avidity T cell repertoire to kill tumors. *Semin Cancer Biol* 17(4):317–329
12. Choi EML, Chen JL, Wooldridge et al (2003) High avidity antigen-specific CTL identified by CD8-independent tetramer staining. *J Immunol* 171(10):5116–5123
13. Xiao Z, Mescher MF, Jameson SC (2007) Detuning CD8 T cells: down-regulation of CD8 expression, tetramer binding, and response during CTL activation. *J Exp Med* 294(11):2667–2677
14. Slifka MK, Whitton JL (2001) Functional avidity maturation of CD8<sup>+</sup> T cells without selection of higher affinity TCR. *Nat Immunol* 2(8):711–717
15. Rechtsteiner G, Warger T, Hofmann M, Rammensee HG, Schild HJ, Radsak MP (2006) Precursor frequency can compensate for lower TCR expression in T cell competition during priming in vivo. *Eur J Immunol* 36(10):2613–2623
16. Sikalidis AK, Lee J-I, Stipanuk MH (2011) Cellular responses to indispensable amino acid deficiency are mediated through the integrated stress response pathway. *Amino Acids* 41(1):159–171
17. Sikalidis AK, Stipanuk MH (2010) Growing rats respond to a sulfur amino acid-deficient diet by phosphorylation of eIF2 $\alpha$  and induction of adaptive components of the integrated stress. *J Nutr* 140(6):1080–1085
18. Bella DL, Hirschberger LL, Hosokawa Y, Stipanuk MH (1999) The mechanisms involved in the regulation of key enzymes of cysteine metabolism in rat liver in vivo. *Am J Physiol* 276 (Endocrin. Metab. 39): E326–E335
19. Bella DL, Hahn C, Stipanuk MH (1999) The effects of non-sulfur amino acids and of sulfur amino acids on the regulation of hepatic enzymes of cysteine metabolism. *Am J Physiol* 277(Endocrin. Metab. 40): E144–E153
20. Cresenzi CL, Lee JL, Stipanuk MH (2003) Cysteine is the metabolic signal responsible for dietary regulation of hepatic cysteine dioxygenase and glutamate cysteine ligase in intact rats. *J Nutr* 133(9):2697–2702
21. Yang BH, Wang X, Ren X (2012) Amino acid metabolism related to immune tolerance by MDSCs. *Int Rev Immunol* 31(3):177–183
22. Rodríguez PC, Ochoa AC (2006) T cell dysfunction in cancer: role of myeloid cells and tumor cells regulating amino acid availability and oxidative stress. *Semin Cancer Biol* 16(1):66–72
23. Levring TB, Hansen AK, Nielsen BL, Kongsbak M, von Essen MR, Woetmann A, Odum N, Bonefeld CM, Geisler C (2012) Activated human CD4<sup>+</sup> T cells express transporters for both cysteine and cystine. *Sci Rep* 2:266
24. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S (2010) Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res* 70(1):68–77
25. Yan Z, Banerjee R (2010) Redox remodeling as an immunoregulatory strategy. *Biochemistry* 49(6):1059–1066
26. Sido B, Lasitschka F, Giese T, Gassler N, Funke B, Schröder-Braunstein J, Brunnemer U, Meuer SC, Autschbach F (2008) A prominent role for mucosal cystine/cysteine metabolism in intestinal immunoregulation. *Gastroenterology* 134(1):179–191
27. Yan Z, Garg SK, Banerjee R (2010) Regulatory T cells interfere with glutathione metabolism in dendritic cells and T cells. *J Biol Chem* 285(53):41525–41532
28. Edinger AL, Thompson CB (2002) Antigen-presenting cells control T cell proliferation by regulating amino acid availability. *Proc Natl Acad Sci U S A* 99(3):1107–1109
29. Dröge W, Eck HP, Gmünder H, Mihm S (1991) Dysregulation of plasma amino acid levels in HIV-infection and cancer and its relevance for the immune system. *Amino Acids* 1(2):193–198
30. Mihm S, Galter D, Droge W (1995) Modulation of transcription factor NF kappa B activity by intracellular glutathione levels and by variations of the extracellular cysteine supply. *FASEB J* 9(2):246–252
31. Dröge W, Eck HP, Gmünder H, Mihm S (1991) Modulation of lymphocyte functions and immune response by cysteine and cysteine derivatives. *Am J Med* 91(3C):140S–144S
32. Grimble RF (2006) The effects of sulfur amino acid intake on immune function in humans. *J Nutr* 136(6 Suppl):1660S–1665S
33. Murphy G, Fan JH, Mark SD, Dawsey SM, Selhub J, Wang J, Taylor PR, Qiao YL, Abnet CC (2011) Prospective study of serum cysteine levels and oesophageal and gastric cancers in China. *Gut* 60(5):618–623
34. Gostner JM, Becker K, Fuchs D, Sucher R (2013) Redox regulation of the immune response. *Redox Rep* 18(3):88–94
35. Yan Z, Garg SK, Kipnis J, Banerjee R (2009) Extracellular redox modulation by regulatory T cells. *Nat Chem Biol* 5(10):721–723
36. Matsue H, Edelbaum D, Shalhevet D, Mizumoto N, Yang C, Mummert ME et al (2003) Generation and function of reactive oxygen species in dendritic cells during antigen presentation. *J Immunol* 171(6):3010–3018
37. Los M, Schenk H, Hexel K, Baeuerle PA, Dröge W, Schulze-Osthoff K (1995) IL-2 gene expression and NF-kappa B activation through CD28 requires reactive oxygen production by 5-lipoxygenase. *EMBO J* 14(15):3731–3740
38. Miller TW, Wang EA, Gould S, Stein EV, Kaur S, Lim L, Amarnath S, Fowler DH, Roberts DD (2012) Hydrogen sulfide is an endogenous potentiator of T cell activation. *J Biol Chem* 287(6):4211–4221
39. Kontny E, Szczepanska K, Kowalczewski J, Kurowska M, Janicka I, Marcinkiewicz J, Maslinski W (2000) The mechanism of taurine chloramine inhibition of cytokine IL-6, IL-8 production by rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis Rheum* 43(10): 2169–2177
40. Grimble RF (1994) Sulphur amino acids and the metabolic response to cytokines. *Adv Exp Med Biol* 359:41–49
41. Huxtable RJ (1996) Taurine past, present and future. *Adv Exp Med Biol* 403:641–650
42. Grimble RF (1996) Theory and efficacy of antioxidant therapy. *Curr Opin Crit Care* 2:260–266
43. Chorazy M, Kontny E, Marcinkiewicz J, Maslinski W (2002) Taurine chloramine modulates cytokine production by human peripheral blood mononuclear cells. *Amino Acids* 23(4):407–413
44. Winder B, Lebilhuber F, Frick B, Laich A, Artner-Dworzak E, Fuchs D (2002) Moderate hyperchomocysteinaemia ad immune activation in Parkinson's disease. *J Neural Transm* 109(12):1445–1452
45. Gottfried E, Kreutz M, Mackensen A (2012) Tumor metabolism as modulator of immune response and tumor progression. *Semin Cancer Biol* 22:335–341
46. Newsholme P (2001) Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? *J Nutr* 131(9 Suppl):2512–2522
47. Wilmore DW, Shabert JK (1998) The role of glutamine in immunologic responses. *Nutrition* 14(4):618–626
48. Hammami I, Chen J, Bronte V, DeCrescenzo G, Jolicoeur M (2012) L-glutamine is a key parameter in the immunosuppression phenomenon. *Biochem Biophys Res Commun* 425(4):724–729
49. Raber P, Ochoa AC, Paulo C (2012) Metabolism of L-arginine by myeloid-derived suppressor cells in cancer: mechanisms of T cell

- suppression and therapeutic perspectives. *Immunol Investig* 41(6–7): 614–634
50. Barbul A (1990) Arginine and immune function. *Nutrition* 6(6–7): 53–58
  51. Taheri F, Ochoa JB, Faghiri Z, Culotta K, Park HJ, Lan MS et al (2001) L-Arginine regulates the expression of the T-cell receptor zeta chain (CD3zeta) in Jurkat cells. *Clin Cancer Res* 7(3 Suppl):958s–965s
  52. Rodriguez PC, Zen AH, Culotta KS, Zabaleta J, Ochoa JB, Ochoa AC (2002) Regulation of T cell receptor CD3 zeta chain expression by L-arginine. *J Biol Chem* 277(24):21123–21129
  53. Albina JE, Caldwell MD, Henry WL Jr, Mills CD (1989) Regulation of macrophage functions by L-arginine. *J Exp Med* 169(3):1021–1029
  54. Popovic PJ, Zeh HJ 3rd, Ochoa JB (2007) Arginine and immunity. *J Nutr* 137(6 Suppl 2):1681S–1686S
  55. DeSanctis F, Sandri S, Ferrarini G, Pagliarello I, Sartoris S, Ugel S, Marigo I, Molon B, Bronte V (2014) The emerging immunological role of post-translational modifications by reactive nitrogen species in cancer microenvironment. *Front Immunol* 5:1–16
  56. Terabe M, Swann J, Ambrosino E et al (2005) A nonclassical non-Valpha14Jalpha18 CD1d-restricted (type II) NKT cells is sufficient for down-regulation of tumor immunosurveillance. *J Exp Med* 202(12):1627–1633
  57. Takahashi A, Hanson MG, Norell HR et al (2005) Preferential cell death of CD8<sup>+</sup> effector memory (CCR7<sup>+</sup>CD45RA<sup>+</sup>) T cells by hydrogen peroxide-induced oxidative stress. *J Immunol (Baltimore)* 174(10):6080–6087
  58. Schroder AJ, Pavlidis P, Arimura A, Capece D, Rothman PB (2002) Cutting edge: STAT6 serves as a positive and negative regulator of gene expression in IL-4-stimulated B lymphocytes. *J Immunol* 168(3):996–1000
  59. Godin-Ethier J, Hanafi LA, Piccirillo CA, Lapointe R (2011) Indoleamine 2,3-dioxygenase expression in human cancers: clinical and immunologic perspectives. *Clin Cancer Res* 17(22):6985–6991
  60. Mellor AL, Munn DH (2001) Tryptophan catabolism prevents maternal T cells from activating lethal anti-fetal immune responses. *J Reprod Immunol* 52(1–2):5–13
  61. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL (1999) Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* 189(9):1363–1372
  62. Lee GK, Park HJ, Macleod M, Chandler P, Munn DH, Mellor AL (2002) Tryptophan deprivation sensitizes activated T cells to apoptosis prior to cell division. *Immunology* 107(4):452–460
  63. Weinlich G, Murr C, Richardsen L, Winkler C, Fuchs D (2007) Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. *Dermatology* 214(1):8–14
  64. Brandacher G, Perathoner A, Ladurner R, Schneeberger S, Obrist P, Winkler C, Werner ER, Werner-Felmayer G, Weiss HG, Göbel G, Margreiter R, Königsrainer A, Fuchs D, Amberger A (2006) Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. *Clin Cancer Res* 12(4):1144–1151
  65. Ramanathan A, Wang C, Schreiber SL (2005) Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements. *Proc Natl Acad Sci U S A* 102(17):5992–5997
  66. Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR et al (2004) Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* 64(11):3892–3899
  67. Opitz CA, Litzenburger UM, Sahn F, Ott M, Tritschler I, Trump S, Schumacher T, Jestaedt L, Schrenk D, Weller M, Jugold M, Guillemin GJ, Miller CL, Lutz C, Radlwimmer B, Lehmann I, von Deimling A, Wick W, Platten M (2011) An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 478(7368):197–203
  68. Pilote L, Larrieu P, Stroobant V, Colau D, Dolusic E, Frédéric R, De Plaen E, Uytendhoeve C, Wouters J, Masereel B, Van den Eynde BJ (2012) Reversal of tumoral immune resistance by inhibition of tryptophan 2,3-dioxygenase. *Proc Natl Acad Sci U S A* 109(7):2497–2502
  69. Platten M, Wick W, Van den Eynde BJ (2012) Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. *Cancer Res* 72(21):5435–5440
  70. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoeediting: from immunosurveillance to tumor escape. *Nat Immunol* 3(11):991–998
  71. Mizoguchi H, O’Shea JJ, Longo DL, Loeffler CM, McVicar DW, Ochoa AC (1992) Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science* 258(5089):1795–1798
  72. Lee J-I, Dominy JE Jr, Sikalidis AK, Hirschberger LL, Wang W, Stipanuk MH (2008) HepG2/C3A cells respond to cysteine-deprivation by induction of the amino acid deprivation/integrated stress response pathway. *Physiol Genomics* 33(2):218–229
  73. Sikalidis AK, Mazor KM, Kang M, Liu H, Stipanuk MH (2013) Total 4EBP1 is elevated in liver of rats in response to low sulfur amino acid intake. *J Amino Acids* 2013:864757
  74. Sikalidis AK (2013) Cellular and animal indispensable amino acid limitation responses and health promotion. Can the two be linked? A critical review. *Int J Food Sci Nutr* 64(3):300–311
  75. Maciolek JA, Pasternak JA, Wilson HL (2014) Metabolism of activated T lymphocytes. *Curr Opin Immunol* 27:60–74
  76. Baracos VE, Mackenzie ML (2006) Investigations of branched-chain amino acids and their metabolites in animal models of cancer. *J Nutr* 136(1 Suppl):237S–242S