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New role of glutamate as an immunoregulator via glutamate receptors and transporters

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1. ABSTRACT

Accumulating evidence suggests that the amino acid glutamate (Glu) may play a role in mediating immune function. The demonstration of Glu receptors (GluR) and Glu transporters (GluT) on a variety of immune cells suggests that Glu has a functional role in immunoregulation well beyond its role as a neurotransmitter. The extracellular Glu concentration plays a key role in the regulation of GSH synthesis in immune cells via 2 key GluTs (i.e., Xc^- and X^-_{AG} systems). Emerging evidence also suggests a role of Glu as signaling molecule in immune cells via ionotropic GluRs (iGluRs) and metabotropic GluRs (mGluRs). *In vitro*, extracellular Glu concentration has been shown to exert a dose-dependent regulation on lymphocyte activation/proliferation. Specifically, Given the exceedingly high intestinal Glu concentration, these finding are suggestive of a potential role for Glu in modulating immune function and promoting tolerance in the gut associated lymphoid tissues.

2. INTRODUCTION- ROLE OF GLUTAMATE (GLU) IN IMMUNE FUNCTION

The amino acid Glu plays a central role in amino acid metabolism in the body, most importantly in the disposal of the dietary protein (reviewed by (1)) and in neurotransmission and function (reviewed by (2)}. A speculation that Glu may play a regulatory role in immune system comes from early clinical studies reporting elevated plasma Glu concentrations in conditions associated with immune deficiency such as HIV infection and advanced cancer (3-7). Glu concentration in the plasma is normally tightly regulated between 10-50 μM (8, 9). Elevated plasma Glu has been reported in patients with gastrointestinal tumors (7, 10), bronchial carcinomas (11), lymphomas (11, 12), breast cancer (7, 13) , ovarian cancer (13), and various other types of tumors (14, 15). The concentrations appears to increase with advancing severity (stages) of the disease (7). It has been suggested that both Glu release from tumour and reduced Glu utilization by peripheral tissues of

Table 1. Altered plasma/serum Glu concentrations in neurologic disorders, malignancies and immunodeficiency

Conditions		Plasma/serum Glu concentration (µM)[1]	Plasma/serum Glu concentration of healthy controls reported (µM)	Reference
Malignancies	Breast cancer (all stages)	54–83	27±18	(7)
	Colorectal carcinoma (T2-T4)	38–86	28±14	(7)
Immunodeficiency	HIV+ (WR5,6)	59–70	44±19	(7)
Autoimmune disorder	Systemic Lupus Erythematosus	69±32 (serum)	166±64 (serum)	(16)
Trauma		115±21 (serum)	63.8.±6 (serum)	(110)
Neurologic disorders	Amyotrophic lateral sclerosis (ALS)	33±19	25±12	(111)
		49±3 ('Spinal type' ALS)	40±2	(112)
	Parkinson's disease	72±9	34±11	(113)
	HIV dementia	200±30	34± 6	(5)
	Alzheimer's disease	38±2	28±2	(114)

[1]Unless specified, all data are plasma concentrations

the tumour-bearing host and hypercatabolism-related muscle protein breakdown contribute to the increased plasma Glu pool size in cancer (7). Dysregulated plasma Glu has also been observed in other conditions. Patients infected with HIV have markedly elevated average plasma Glu level with highest Glu levels seen in HIV+ patients with opportunistic infections (WR 6) (3, 7). Elevated plasma Glu has also been reported in neurological disorders with an immune etiology or pathology (Table 1). Further, a depressed serum Glu was observed in certain autoimmune disorders such as SLE, which leads to lupus T-cell hyperreactivity (16). This suggests that the presence of altered Glu metabolism and its effect on immune function extends beyond the presence of a tumour. Together, these observations consistently show that increases in the plasma or serum Glu concentration are associated correlates with an immune deficiency or suppression in a variety of conditions, whereas decreases in plasma Glu concentration are associated with immune hyper-reactivity. Such an intriguing correlation observed in a variety of conditions led to the hypothesis that extracellular Glu could play an independent role in immune function and may thus exert certain immunoregulatory effects (17).

Although no *in vivo* studies have been directed to study immune functional outcomes resulted from glutamate deficiency or excess exogenous supply, a multitude of *ex vivo* studies looking at mitogenic response of immune cells (primarily peripheral blood lymphocytes) are suggestive of an immunosuppressive role of Glu at supra-physiological levels (as compared to normal plasma concentrations) (Table 2). Initial *ex vivo* studies show that, within the range of 50 to 200µM, increase in Glu concentration reduced the blastogenic response of murine lymph node cells and splenocytes to mitogen stimulations in a dose-dependent manner (3, 18). Lombardi *et al.* (19) subsequently show that increase of Glu concentration from 10 to 100µM reduced mitogen-induced intracellular Ca^{2+} ($[Ca^{2+}]i$) rise in human peripheral lymphocytes incubated *ex vivo* by over 4-6 folds. Based on these findings, it could be hypothesized that elevated blood concentrations of glutamate as observed in various immunopathological conditions (Table 1) may suppress the function of immune cells *in situ*, but clinical studies are necessary to establish a cause and effect relationship. Other studies by Lombardi *et al* (20) and Sommer *et al.* (21) using human peripheral lymphocytes incubated *ex vivo* show that Glu consistently reduced ability of lymphocytes to respond (primarily blastogenic response) to the stimulation by various mitogens, within a wide range of supra-physiological concentrations (100µM to 10 mM). Of note, these high Glu concentrations may not necessarily be reached in blood *in vivo*, but can be reached in certain immune compartments such as intestinal epithelium and joint synovial fluid (22-25). These studies, however, may hint at the immunosuppressive role of glutamate, which has these comparably high concentrations in these particular microenvironments. In addition to blastogenic response, Lombardi *et al.* also studied the effect of Glu on another T cell effector function, cytokine production in response to mitogen stimulation (20). In their studies, Glu at 1mM reduced the blastogenic response, but potentiated secretion of IFN-γ and IL-10 after T cell stimulation (using anti-CD3 antibody) (20), This finding may hint at that Glu functions as a immune-regulator, rather than a merely immunosuppressor.

Consistent with an immuno-regulator role for Glu, incubation of lymphocytes in micromolar concentrations of Glu, which is at least one order of magnitude lower than normal plasma concentrations, has been reported to stimulate key biochemical and cellular events in lymphocyte activation. Lombardi *et al* reported that incubation with Glu between 0.001 and 1 µM potentiated both PHA- and anti-CD3 mAb-induced $[Ca^{2+}]i$ rise, an early event in cell activation (19). Ganor *et al* reported that Glu (in the absence of any additional stimulating molecules), at the picomolar to micromolar range (below 10µM), directly induced the adhesion of T cells to two principal extracellular matrix glycoproteins, laminin and fibronectin (26). Glu, at concentrations less than 10µM, positively modulates Kv1.3. channel gating, which would facilitate T lymphocyte responsiveness to stimuli (27). In this same study, maximal effects were achieved at 1µM; whereas Glu at 100µM or higher range, decreases potassium currents, inhibiting the ability of T cells to respond to stimuli (27). These findings with the micromolar concentrations of Glu are suggestive that the low Glu concentrations present in microenvironments such as cerebrospinal fluid (CSF) (28) may sensitize rather than inhibit immune cells to respond to stimuli. Together, these observations at the widely varied Glu concentrations suggest that Glu may play a major role in regulating the ability of immune cells to respond to stimuli. This manuscript will provide support to hypothesize a novel role of Glu as an immunoregulator at the cellular level.

Table 2. Effects of Glu on mitogen-stimulated responses by PBMCs

Ref	Subjects	Glu dose or concentration	Mitogen				
			Pokeweed Mitogen (PWM),	Concanavalin A (Con A)	Phytohaemagglutinin (PHA)	Anti-CD3 Ab	Anti-CD3 plus Anti-CD28
(20)	PBMCs from healthy donors	0.1.-10 mM			↓ proliferative response in a dose-dependent manner; IC50=2.1.4mM	↓ proliferative response in a dose-dependent manner; IC50=0.5.4mM	↓ proliferative response in a dose-dependent manner IC50=0.8.7mM
		0.5. – 5 mM				1mM Glu ↑ production of IFN-γ & IL-10; 5mM Glu ↓ production of IFN-γ, IL-10 & IL-5; NS difference TNF, IL-2 & IL-4 at tested concentrations of Glu	
(19)	PBMCs from healthy donors				Effect of Glu on anti-CD3 mAb or PHA-induced intracellular Ca^{2+} ($[Ca^{2+}]i$) rise was in a bell-shape concentration-dependent relationship. Low Glu concentrations between 0.001 and 1 μM potentiated $[Ca^{2+}]i$ rise, but such an effect was reversed with higher Glu concentrations larger than 3 μM. The maximum effect occurred at 1 μM Glu was +90±3% for anti-CD3 mAb, and +57±2% for PHA compared to control (no Glu) conditions. NMDA, (S)-AMPA, and KA all modulated $[Ca^{2+}]_i$ rise similar to Glu; the concentration-response curves of these agonists exhibited the same bell shapes seen with Glu with the maximum effects obtained at 1 μM; Glu potentiating effect on $[Ca^{2+}]_i$ rise could be abrogated by the use of NMDA or/and AMPA antagonists.		
	PBMCs with monocytes removed	0.1.-1 mM			↓ proliferation in concentration-dependent manner		
(21)	Peripheral lymphocytes from healthy donors	2, 4, 8 mM	↓ proliferation response with increasing Glu concentration	↓ proliferative response with increasing Glu concentration	↓ proliferation response with increasing Glu concentration		
(6)	Whole peripheral blood cells from patients with colorectal carcinoma before and after curative surgery; whole blood from healthy donors as reference	Patients before surgery had a plasma Glu concentration 99% higher than healthy donors; but it returned to a comparable level as healthy donors, within 1 week after surgery	Proliferative response depressed even 6 months post-surgery	Proliferative response was normalized 3-15 months after surgery	Proliferative response still depressed even 6 months post- surgery		
(18)	Splenocytes from C57BL/6 mice	50- 200 μM	Up to 50% ↓ in proliferative response with Glu concentration at 200 μM compared to 50 μM		Up to 30% ↓ in proliferative response with Glu concentration at to 200 μM compared to 50 μM		

	Whole peripheral blood cells from patients with colorectal carcinoma, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC); whole blood from healthy donors as reference	All three groups of cancer patients had elevated plasma Glu. if compared			All three groups of cancer patients had depressed proliferative response. There was significant (correlation coefficient - 0.8.0, P<0.000001) correlation between Glu concentration and PWM-induced response that was independent of presence of tumor		
(3)	Murine lymph node cells and T cell-enriched nylon wool non-adherent spleen cells	50- 200 µM concentration comparable with that found in HIV+ patients		200 µM compared to 50 µM. extracellular Glu in media resulted in a 30-40% ↓. Pharmacological probes for the excitatory GluRs also inhibited the response, however inhibition was abrogated by adding cysteine			
(17)	Whole peripheral blood cells from patients with SCLC and NSCLC; whole blood from healthy donors as reference	All SCLC and NSCLC patients showed ↑ Glu concentration.	All SCLC and NSCLC patients had ↓ mitogenic response compared with healthy references. All cancer patients with Glu concentrations of > 120 µM had ↓ mitogenic response and ↓ mean survival than patients with Glu levels of < 120 µM				

3. REGULATORY ROLE OF GLUTAMATERGIC SYSTEM EXPRESSED ON IMMUNE CELLS

The conception that central nervous system and immune system can mutually influence each other in a sophisticated way was proposed at least two decades ago (29). Glu has been reported as one of the key mediators bridging the crosstalk between these two systems (19, 20). Glu is widely recognized as the primary excitatory neurotransmitter in the mammalian central nervous system (CNS), playing a crucial role in mental, sensory, motor, and affective and cognitive functions (30-32). In addition to its well-established neurotransmitter role in the CNS, there is growing evidence that Glu may also function as an extracellular signal mediator in peripheral tissues via glutamatergic mechanisms(33, 34). The demonstration of Glu receptors (GluR) and Glu transporters (GluT) on a variety of immune cells (19, 26, 27, 35-41) suggests that Glu has a functional role well beyond its well-recognized role as a neurotransmitter or precursor for protein synthesis.

3.1. GluTs on immune cells – a mechanistic focus on regulation of Glutathione (GSH) synthesis

Due to its essential role as a CNS neurotransmitter, extracellular Glu concentrations in CNS are tightly regulated, a function attributed to GluTs. Several GluTs have been identified, the first comprises a family of Na(+)-dependent excitatory amino acid transporters (EAATs). These transporters play a role in the removal of Glu from the extracellular space after neurotransmission is complete, via transport into neurons and glia cells (28, 42). This system is designated as X^{-}_{AG} system, through which inward transport of one L-Glu or D-/ L-aspartate molecule is coupled to the inward movement of three Na+ and one H+, and the outward movement of one K+ ion (43). The second transport system, X_c^{-} is a Na+-independent exchanger of anionic amino acids, with a high specificity for anionic form of L-Glu and L-cystine (44, 45). This transport system constitutes the major path for the provision cystine to the cell to be used for glutathione and/or protein synthesis (46). The third Glu transport system (X_{AG} system) has been identified in astrocytes and rat alveolar cells (47, 48). This system is also Na+-dependent and demonstrates affinity for cystine, Glu and aspartate (47, 48). Substrate competition

Table 3. Expression and function of GluT in immune cells

Species	Immune cell type	Glu transport system/receptor	Major function	References
Mouse	Macrophages	X_c^-	Regulating intracellular GSH store	(56, 58)
			Mediating macrophage-induced Glu-dependent neurotoxicity	(50, 59, 60)
Human	Monocyte-derived macrophages	XAG− and Xc− but not X_{AG} system	Regulating intracellular GSH store	(49, 61)
	Dendritic cells	Xc−	releasing Glu and modulate T cell activation	(53)
	Neutrophils	Not identified	Regulating brain endothelial permeability by releasing Glu	(54)

for the transporter between Glu and cystine is observed in the X_{AG} and X_c^- systems, but not in EAATs (49).

Although less is known about their function, GluTs have also been detected in a variety of other cell types including immune cells (49-55). Reported expression and functions of these transported are summarized in Table 3. The cystine/Glu antiporter (X_c^- system) was first reported in mouse peritoneal (56-58) and brain macrophages (50, 59, 60). Presence of both X_c^- and X^-_{AG} systems but not X_{AG} system, has been reported on human monocyte-derived macrophages (49, 61). Interestingly, cystine/Glu antiporter (X_c^- system) activity in mouse macrophages can be upregulated upon stimulation by LPS or reactive oxygen species (ROS)-generating agents (56, 58). In addition to macrophages, Glu transport systems have also been reported in other cell types in the innate immune system, including human dendritic cells, and neutrophils (53, 54). The X_c^- system on dendritic cells has been suggested to modulate T cell activation by releasing Glu during the T-cell-dendritic-cell interaction (53); whereas Glu release via' GluTs on neutrophils has been suggested to play a role in mediating endothelial permeability after activation (54).

Glutathione (γ-glutamyl-cysteinyl-glycine; GSH), synthesized from Glu, cysteine and glycine, is the most prevalent cellular thiol and plays a key role in maintaining cellular thiol redox state and protecting cells from oxidative stress (62). This is particularly important for immune cells. An adequate intracellular GSH store has been demonstrated to be essential to support the activation and proliferation of immune cells (63, 64). The cellular redox status primarily determined by GSH/glutathione disulfide(GSSG) ratio has been reported to play an essential role for cytokine production and Th1/Th2 polarization (64).

Cysteine has been suggested to be the limiting amino acid for synthesis of GSH due to the proportionally small size of its intracellular pool, as compared to that of GSH (65). Cysteine readily autoxidizes to cystine in oxygenated extracellular fluid, which leads to a much higher plasma concentration of cystine (50–150 μmol/L) compared to that of cysteine (10–20 μmol/L) (66). Once extracellular cystine enters the cell, it is rapidly reduced to cysteine (67). Therefore, the supply (uptake of) cystine, the major intracellular source of cysteine, has been concluded to be the rate limiting step in the biosynthesis of GHS for many non-hepatic cells (68).

The extracellular Glu abundance, however, has been implicated as a regulator of GSH synthesis through its effect on cystine uptake. Accumulating evidence shows that extracellular Glu competitively inhibits cystine transport into cells via the cystine/Glu exchanger (X_c^- system), such that high Glu concentrations can decrease intracellular cystine availability (56, 57, 69) and inhibit GSH synthesis (70, 71). Macrophages are an important source of cysteine for lymphocytes as increased cysteine release by macrophages is associated with enhanced intracellular GSH concentration and DNA synthesis of their neighboring lymphocytes (51). Given the limited availability of cysteine in the extracellular fluids, releasing cysteine to provide an important GSH precursor or exogenous thiol source for lymphocyte has been suggested as a possible mechanism by which macrophages and dendritic cells might regulate T-cell-mediated immune responses (72, 73). Consistent with the results of the *in vitro* studies on Glu and T cell function, it has been shown that elevated extracellular Glu concentrations reduce the capacity of macrophages to release cysteine (72).

However, although competing with cystine uptake, Rimanial *et al.* found that extracellular Glu increased GSH synthesis in a dose-dependent manner in macrophages that co-express both EAATs and Xc^- antiporter. This suggests a cooperative mechanism between different Glu transport systems (ie, EAATs and Xc^- system). The inward Glu transport via the high-efficient EAATs directly provides intracellular Glu as precursor for GSH synthesis and also assists cystine inward transport via cystine/Glu exchanger (X_c^- system) by boosting intracellular Glu pool. Thus, Glu transport into the cells via EAATs forms a '*trans*-stimulation' mechanism to stimulate cystine inward transport via Xc, the rate limiting step for intracellular GSH synthesis (49).

3.2. GluR expression on immune cells

GluRs constitute an indispensable component for Glu-mediated-signaling input in neuronal cells. More recently, GluRs have been found to be broadly expressed on 'peripheral' non-neuronal tissues and cells including T cells (19, 26, 27, 35-41, 74), suggesting a role for Glu-signaling in these cell types. GluRs can be divided into two categories: the ionotropic GluRs (iGluRs) and metabotropic GluRs (mGluRs) (75). The iGluRs, which are permeable to cations, are gated ion channels that mediate classical Glu excitatory action (76), whereas mGluRs are transmembrane receptors that activate intracellular signaling mechanisms via associated G proteins (77, 78). Based on sequence homology and agonist preference, iGluRs have been further divided into N-methyl-D-aspartate (NMDA), DL-a-amino-3-hydroxy-5-methylisoxasole-4-propionate (AMPA), and kainate (KA) receptors, all of which are associated with ion channels permeable to particular cations (79). The mGluRs have been divided into three groups based on their amino acid sequence, pharmacological profile, and transduction mechanisms. Group I mGluRs (mGluR1 and -5) are coupled to phospholipase C (PLC), and their activation stimulates the release of Ca2+ from intracellular stores (80); whereas group II (mGluR2 and -3) and III (mGluR4, -6, -7 and -8) are negatively coupled to adenylate cyclase and their activation results in inhibition of voltage-sensitive Ca2+ channels, and activation of K+ channels (81, 82).

The early work by Kostanyan *et al.* (83), demonstrating the specific binding of Glu to human blood lymphocytes, initiated a new line of research that has begun to build a case for the existence of glutamatergic system in T lymphocytes. This hypothesis is supported by a series of elegant studies that have measured the expression of GluRs on a variety of immune cells and strengthened the evidence that Glu is an important immunotransmitter (84) (summarized in Table 4).

3.2.1. mGluRs expression in immune cells

3.2.1.1. Thymocytes

Storto *et al.* (39) identified differential expression of group I and group II mGluR in isolated murine thymocytes (detailed in Table 4), suggesting a role of these receptors in thymic maturation and T cell selection. The differential distribution of mGluRs were further confirmed in the rat thymus via *in situ* immunohistochemical analysis by Rezzani *et al.* (40) (Table 4).

3.2.1.2. T lymphocytes

Pacheco *et al* first identified Group I mGluR on both resting and activated human peripheral blood T lymphocytes (35, 85). Their studies suggest that the mGluR5 is expressed constitutively on surface of T cells; whereas mGluR1 is expressed in response to activation via the T-cell receptor (35). Unlike the classical group I mGluR in CNS, which is coupled to phospholipase C, mGluR5 on T cells activates adenylate cyclase and leads to the inhibition of CD3-mediated proliferation (35). This observation suggests a mechanism by which high concentrations of Glu inhibit T-cell activation/proliferation. However, such an inhibitory effects of Glu via mGluR5 can be reversed by stimulation of mGluR1 expressed in T cells undergoing activation. Activation of mGluR1 leads to activation of ERK1/2 pathway. The dependency on the activation state of the cell and the predominance of the GluR subtype expressed on the immune cells, suggests a fine regulatory mechanism through which Glu might regulate lymphocyte function (35, 53).

Further, mRNA expression for mGluR1b, mGluR2, mGluR3, and mGluR8 subtypes were detected in peripheral blood T lymphocytes of healthy individuals by Poulopoulou *et al.* (37). Pharmacological activation of group I and II mGluRs mimics the up and down regulation of T lymphocyte Kv1.3. channels and is consistent with the up and down regulation of lymphocyte function produced *in vitro* with low and high Glu concentrations, respectively (27). Consistent with this, Miglio *et al.* demonstrated that pharmacological activation of group I mGluR raised intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and up-regulated *c-jun* and *c-fos* mRNA expression in human T cells (38). Thus, T lymphocytes appear to express functional mGluR subtypes with differential affinity for extracellular Glu which is important in the regulation of their function (27).

3.2.1.3. Dendritic cells

mGluR have also been reported in rat thymus derived dendritic cells (40). However, neither mGluR5 nor for mGluR1 was detected on human monocyte-derived dendritic cells (53). The role of these receptors on dendritic cells has not been established.

3.2.2. iGluR expression in immune cells

3.2.2.1. Peripheral blood mononuclear cell (PBMC)

Presence of functional iGluR on human PMBCs was reported by Lombardi *et al* (19). Agonists of iGluRs (i.e., NMDA, (S)-AMPA, and KA) potentiated $[Ca^{2+}]_i$ rise in mitogen-stimulated lymphocytes in a similar bell-shape dose-response relationship as seen with Glu treatment (Table 2). The potential role of Glu via iGluR on increasing $[Ca^{2+}]_i$ could be blocked by incubation with NMDA or/and AMPA antagonists (19).

3.2.2.2. Lymphocytes

Glu was demonstrated, *in vitro*, to trigger the chemotactic migration of normal resting T cells and adhesion of these cells to two principal extracellular matrix glycoproteins, laminin and fibronectin (26). These effects were mimicked by AMPA receptor agonists and blocked by AMPA receptor antagonists, suggesting the functional effects of Glu on these T cells are mediated via specific AMPA iGluRs (26). Consistent with these observations, Ganor *et al.* (26) demonstrated the expression of AMPA receptor iGluR3 on human peripheral T cells. Boldyrev *et al.* (36) demonstrated that rodent lymphocytes also express mRNA for iGluR NR1 and group III mGluR Pharmacological activation of iGluR NR1 increased [Ca2+]i, ROS levels and activation of caspase-3; whereas specific activation of the group III mGluR increased ROS production without altering[Ca2+]i (36, 86).

3.3. The regulatory role of Glu in T cell activation/proliferation occurs via GluRs

Despite the rapidly growing novel findings in the glutamatergic systems in various types of immune cells, an integrated view of the role of GluR in the regulation of the innate and acquired immune response has not been established. The most intriguing questions is what is the physiological importance of Glu -mediated signaling in regulating immune function and how could changing Glu concentrations extracellular/intracellular be used therapeutically.

Glu's role as a neurotransmitter involves the transmission of a signal from a neuron to a target cell across synapse, an essential structure for the signal-passing (presynaptic) neuron to communicate with its target (postsynaptic) cell. Pacheco *et al.*'s findings (35, 53) represent a prime example of how the 'synapse' concept in neurobiology was applied to the communication between immunocompetent antigen presenting cell (i.e., dendritic cells and macrophages) and T-cell. This is termed an 'immunosynapse' in which Glu serves as an immunotransmitter. In such a model, Pacheco *et al* (35)

Table 4. Expression and function of GluR in immune cells

Species	Immune cell type	GluR			Expression feature	Major function	Ref
		mGluR /iGluR	Sub-class/group	Receptor member			
Human	PMBCs	iGluR	NMDA, (S)-AMPA, and KA receptors	Not identified		NMDA, (S)-AMPA, and KA all potentiated $[Ca^{2+}]_i$ rise; Glu's potentiating effect on $[Ca^{2+}]_i$ rise could be further abrogated by NMDA or/and AMPA antagonists	(19)
	T cells	mGluR	Group I	GluR5	constitutively expressed in both resting and activated T cells	Activating adenylate cyclase and inhibiting CD3-mediated proliferation	(35, 53)
				GluR1	only expressed in response to T-cell receptor activation	Activating ERK1/2 pathway	
				GluR1b	mRNA expressed on resting T cells	Not identified	(37)
				Not identified		pharmacological activation upregulating T lymphocyte Kv1.3. channel gating that has been observed with low and high extracellular Glu levels respectively	(27)
				Not identified		Raising intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) via PLC activation and up-regulated c-jun and c-fos mRNA expression	(38)
			Group II	GluR2, GluR3	Resting T cells	Not identified	(37)
				Not identified		pharmacological activation down regulating T lymphocyte Kv1.3. channel gating	(27)
			Group III	GluR8	Resting T cells	Not identified	(37)
		iGluR	AMPA receptor	iGluR3	Resting T cells	Mediating Glu's role in triggering the chemotactic migration and adhesion of resting T cells to laminin and fibronectin	(26)
	CD19+ CD20+ B-type chronic lymphocytic leukemia (B-CLL) cell line WaC3CD5	mGluR	Group III	Not identified	Resting B cells	Not identified	(115)
mouse	thymocytes	mGluR	Group I	mGluR1	Expressed in immature CD4–/CD8– thymocytes	Stimulating polyphosphoinositide (PPI) hydrolysis	(39)
				mGluR5	Expressed in more mature double positive CD4+/CD8+ and mature CD4+/CD8– thymocytes		
			Group II	mGlu2/3	Expressed in both immature and mature thymocytes	inhibiting cAMP formation	
Rat	thymocytes	mGluR	Group I	mGluR5	Strong expression in mature thymocytes from medullar thymus	Differential expression of mGluRs may suggest a role of these receptors in thymic maturation and T cell selection ?	(40)
			Group II	mGluR 2/3	Moderate expression in mature thymocytes from medullar thymus; weak expression in immature thymocytes from cortical thymus		
			Group III	mGluR4	Moderate expression in mature thymocytes from medullar thymus; weak expression in immature thymocytes from cortical thymus		

Species	Immune cell type	GluR			Expression feature	Major function	Ref
		mGluR /iGluR	Sub-class/group	Receptor member			
	Thymus dendritic cells	mGluR	Group I	mGluR5	Strong expression in DCs from rat medullar thymus; no expression detected in dendritic cells from cortical thymus	Not identified	
			Group II	mGluR2/3	Moderate expression in DCs from rat medullar thymus; no expression detected in dendritic cells from cortical thymus		
			Group III	mGluR4	Moderate expression in DCs from rat medullar thymus; no expression detected in dendritic cells from cortical thymus		
human	monocyte-derived dendritic cells	mGluR	Group I	mGluR1, mGluR5	No expression detected	-	(53)
Rodent (mouse, rat and rabbit)	Peripheral lymphocytes	iGluR	NMDA receptor	NR1	Not identified	Increasing [Ca2+]i, ROS levels and activation of caspase-3	(36)
		mGluR	Group III	Not identified		increasing ROS level, not affecting [Ca2+]I	

demonstrated that the constitutively expressed mGlu5Rs on T cells are tonically activated at the micromolar Glu concentrations that are present in plasma. Such a tonic activation of mGlu5R maintains the increase in cAMP levels and subsequent PKA activation, which inhibits T cell proliferation and cytokine production (87, 88). Theoretically, through tonic activation of mGlu5R the threshold for T-cell activation would be increased (35). By contrast to the constitutive expression of mGlu5R the mGlu1R appears to be only expressed when T cells are activated (35, 53)., Glu released from antigen-presenting cells, such as DC, via the cystine/Glu antiporter (X_c^- system) could bind to the mGlu1R expressed on T cells undergoing activation and activate the ERK-pathway (35). This would counteract the mGlu5R-mediated inhibitory effect and facilitate T-cell proliferation and production of Th1 (IL-2 and IFN-γ) and pro-inflammatory (IL-6 and TNF-α) cytokines (53). In this model, mGlu5R/mGlu1R may act as regulatory trigger for T cell activation. The tonic activation of mGlu5R may exist to function as a brake mechanism to shield T cells from becoming activated inappropriately (35, 53). Nonetheless, mGluR1 induction and activation appears able to effectively counteract the inhibitory effects of Glu and selectively assist specific-antigen or TCR- triggered T cell activation (35, 53). In this model, Glu seems to also function as a co-stimulatory molecule T cell activation via the upregulated mGlu1R on activated T cells (35, 53).

A number of *in vitro* and *ex vivo* studies have demonstrated that extracellular Glu at low (no higher than micromolar) and high (higher than micromolar) concentrations modulates key biochemical/cellular events in T cells , suggesting There is also evidence that Glu can exert a dose-dependent dual regulatory role in T cell function and activation, (19, 37). An important role of T cells in the peripheral tissue is to adhere to the extra cellular matrix via the expression of integrins (89). Ganor *et al.* (26) demonstrated that picomolar to micromolar concentrations of Glu, promoted T cell adhesion to laminin; whereas adhesion was reduced at Glu concentrations in excess of 10 μM). Antigen activation of T cells is mediated through the TCR/CD3 receptor complex which results in Ca^{2+} release from the endoplasmic reticulum, and the activation of calcium-release activated Ca2+ channel (CRAC) resulting in a Ca2+ influx (90). Lombardi *et al.* (19) demonstrated that extracellular Glu modulates mitogen-induced [Ca2+]i rise in a 'bell-shape' dose-dependent manner with maximal [Ca2+]i rise occurring at 1 μM Glu. When Glu was provided at lower concentrations (0.0.01 and 1 μM) the[Ca^{2+}]i rise was potentiated by Glu, however when Glu concentrations were increased to higher than 3 μM, Glu inhibited [Ca^{2+}]i rise in a dose-dependent manner (19). The activity of K+ channels (i.e., voltage-gated Kv1.3. and Ca2+-activated IKCa1channel) modulates the membrane potential of T cells, thereby regulating the influx of Ca2+, which subsequently alters gene transcription and subsequent proliferation (91, 92). Given the important role of Kv channels in regulating Ca2+ influx, Poulopoulou *et al.* (93) work demonstrating Glu modulates Kv1.3. gating, further supports the role of Glu to regulate T cells function in a dose-dependent manner (93). More specifically extracellular Glu concentrations below or within normal plasma levels (i.e., 1 μM and 10 μM) appears to potentiate Kv1.3. currents ; whereas Kv1.3. currents are suppressed with elevated Glu concentrations above normal plasma levels (i.e., 100 μM and 1 mM) (93). These authors further postulated that at low Glu concentrations the effect is mediated via Group I mGluR whereas high concentrations of Glu blunts Kv1.3. responses via Group II mGluR (27). Although intriguing, the precise role of these biochemical/biophysical events needs to be examined in relation to key function of immune cells such as proliferation and cytokine production (20, 36).

Such a dose-dependent dual effect of small changes in extracellular Glu concentrations may constitute a means of how the microenvironment would regulate immune cell's function. For example, Glu concentration of cerebrospinal fluid (CSF) is much lower than that of plasma (no higher than 1 μM (28) vs. 10-50 μM (8, 9, 94, 95)), facilitating immune cells in this tissue to be more sensitive to activation (such as migration or integrin-mediated adhesion) than in plasma where T cell are more tonically suppressed with high Glu concentration (26). The increase in plasma Glu that occurs in pathological conditions (cited earlier in this review) would exert an inhibitory effect on lymphocyte activation or in local tissue such as joint synovial fluid where high Glu concentrations might inhibit T cell function during inflammation (22, 23).

3.3.1. A potential link between high intestinal Glu content and oral tolerance development?

In contrast to CNS, intestinal mucosal tissue is exposed to an exceedingly high concentrations of Glu from the diet (24) as Glu is one of the most abundant amino acids in dietary proteins (96). Postprandial luminal Glu concentrations can easily be at the order of magnitude of mM, which may accordingly result in an exceptionally high intramucosal Glu concentration (24, 25). It's a vital task for the intestinal immune system to mount protective immune responses against harmful intestinal pathogens while preventing excessive responses to dietary antigens and the microbiome. Oral tolerance is an active physiological mechanism to sustain immune 'unresponsiveness' to dietary and commensal bacterial antigens and is crucial for preventing harmful hypersensitivity responses in the intestine (97). The high Glu concentration in the intestinal microenvironment could theoretically impose a tonic inhibitory effect on the intestinal lymphocytes (e.g., intraepithelial distributed lymphocytes in Peyer's Patches and the lamina propria) and prevent an inappropriate response to dietary antigens by conditioning T lymphocytes with anergic or immunosuppressive properties. Consistent with the hypothesized role of high Glu concentrations it is reported that the microenvironment of Peyer's Patches render naïve T cells hyporesponsive to several stimuli (98) and favors Th2/Treg differentiation (99, 100). Therefore, it would be of interest to investigate whether the high intestinal Glu concentration is important for maintaining this 'favorable' intestinal microenvironment which favors induction of tolerance against plethora of environmental antigens from microflora and diet.

It has been well recognized that modulation of the gut cytokine milieu profoundly affects the nature of T cell response to dietary antigens (101). Regulatory cytokines such as IL-10 and TGF-β play a key role in driving T cell differentiation into regulatory T cells (Tregs), primary executors for maintaining tolerance and homeostasis of the immune compartment (102-105). There are few studies that have examined the effect of high Glu concentrations on cytokine production by of immune cells. The study by Lombardi *et al.* (20) showed that incubation of PBMC in 1mM Glu increased the production of IFN-γ (+44.3.%) and IL-10 (+31.6%) after mitogen stimulation. Both of these cytokines are involved in the maintenance of oral tolerance (106, 107). Although one can not assume that intestinal lymphocytes would respond the same way upon antigen stimulation as the lymphocytes in peripheral blood (100, 108) this is intriguing and warrants future exploration.

4. CONCLUSIONS

Accumulating evidence suggests that Glu may play a regulatory role in immune system. The discovery of presence of glutamatergic system (GluR and GluT) on immune cells was the cornerstone that leads the way to mechanistic exploration of Glu's immunoregulatory function. Extracellular Glu appears key in the regulation of intracellular synthesis of GSH, a competence factor for immune cell function, via GluT primarily expressed in macrophages and dendritic cells. With the discovery of GluR on T cells, Glu has taken on a new role as an immunotransmitter (109), bridging the immunosynaptic crosstalk between APC (such as dendritic cells), which releases Glu via Xc^- system, and T cells, which use Glu as a molecule to signal activation via membrane GluRs (35, 53). Differential expression of Group I mGluR appear to finely regulate the activation status of T cells. Further, there is evidence to suggest that extracellular Glu concentrations mediate a dose-dependent regulation of lymphocyte activation/proliferation. This observation warrant future research efforts to explore the immunoregulatory role of Glu in tissues with varied Glu concentrations. More specifically, intestinal mucosal tissue is exposed to an exceedingly high concentrations of dietary Glu and the effects of this on immune function, based on what is known about peripheral blood cells, entails future research to investigate how it is involved in the maintenance of intestinal tolerance.

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Abbreviations: Glu: glutamate; GluR: glutamate receptor; iGluR: ionotropic GluR; mGluR: metabotropic GluR; GluT: glutamate transporter; CNS: central nervous system; EAAT: excitatory amino acid transporters; NMDA: N-methyl-D-aspartate; AMPA: DL-a-amino-3-hydroxy-5-methylisoxasole-4-propionate; KA: kainite; ROS: reactive oxygen species; GSH: Glutathione (γ-glutamyl-cysteinyl-glycine); Treg: regulatory T cell; ALS: Amyotrophic lateral sclerosis; Con A: concanavalin A; PWM: pokeweed mitogen; PBMC: Peripheral blood mononuclear cell; GSSG: glutathione disulfide

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