Role of glutamate on T-cell mediated immunity

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Abstract

The pivotal role that glutamate plays in the functioning of the central nervous system is well established. Several glutamate receptors and glutamate transporters have been extensively described in the central nervous system where they, respectively mediate glutamate effects and regulates extracellular glutamate levels. Recent studies have shown that glutamate not only has a role as neurotransmitter, but also as an important immunomodulator. In this regard, several glutamate receptors have recently been described on the T-cell surface, whereas glutamate transporters have reportedly been expressed in antigen presenting cells such as dendritic cells and macrophages. On the other hand, an increasing number of reports have described a protective autoimmune mechanism in which autoantigen specific T cells in the central nervous system protect neurons against glutamate neurotoxicity. This review integrates and summarises different findings in this emerging area. A role of glutamate as a key immunomodulator in the initiation and development of T-cell-mediated immunity in peripheral tissues as well as in the central nervous system is suggested.
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1. Introduction

The amino acid glutamate is recognized as the primary excitatory neurotransmitter in the mammalian central nervous system (CNS). Neuronal synaptic activity generates the transient release of this amino acid in the synaptic cleft, thereby contributing to neurotransmission (Nakanishi, 1992). The glutamate may interact with multiple receptor types, divided into two main groups; namely ionotropic glutamate receptors (iGluRs), which form ion channels and mediate fast excitatory glutamate responses, and metabotropic glutamate receptors (mGluRs), which are heptaspanning-membrane-receptors and belong to the superfamily of G protein-coupled receptors (Nakanishi, 1992). So far, eight members of the mGluR family have been identified and classified into three subgroups (I, II, and III) according to their sequence homology, agonist selectivity and signal transduction machinery (Pin and Acher, 2002; Pin and Duvoisin, 1995). Group I contains mGlu1R and mGlu5R subtypes, which are mainly coupled to phospholipase C, and quisqualic acid is their most potent agonist. Group II consists of mGlu2R and mGlu3R, which negatively couple to adenylate cyclase in transfected cells and for which L-2-(carboxycyclopropyl)-glycine is the most potent agonist.

Group III contains mGlu4R, mGlu6R, mGlu7R and mGlu8R, which again negatively couple to adenylate cyclase and L-2-amino-4-phosphonobutyric acid is their most potent agonist. On the other hand, three major groups of iGluRs are recognized (Hinoi et al., 2004), based on their sequence homology and selective activation by the agonists *N*-methyl-D-aspartate (NMDA), kainate (KA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA).

Due to its pivotal role as neurotransmitter, extracellular glutamate levels are tightly regulated in the CNS. Under physiological conditions, clearance of glutamate is achieved by uptake via the high-affinity Na^+-dependent glutamate/aspartate/cystine transporter (X_{AG} system) expressed mainly in astrocytes (Bender et al., 2000; Schwartz et al., 2003) and via excitatory amino acid transporters (EAATs or X_{AG}^- system) expressed on astrocytes, oligodendrocytes and neurons (Matute et al., 2006; Nedergaard et al., 2002; Schwartz et al., 2003). In contrast, after an insult the site of injury is depleted of astrocytes, and instead repopulated by microglia. Depending on the context of activation, microglial cells express different levels of the cystine/glutamate antiporter (X_c^- system) and of glutamate/aspartate transporters (X_{AG}^- system) (Piani and Fontana, 1994; Rimaniol et al., 2001; Schwartz et al., 2003). Thus, this balance of glutamate transporter systems can lead to either accumulation or clearance of extracellular glutamate (Piani et al., 1991, 1992; Piani and Fontana, 1994; Schwartz et al., 2003).

Glutamate-mediated signaling has not only been described in the CNS (Ciruela et al., 2001, 2005; Ferre et al., 2002; Nakanishi, 1992), but also in several peripheral tissues (Boldyrev et al., 2005; Hinoi et al., 2004; Nedergaard et al., 2002). In recent years, growing evidence points to an important role of iGluRs (Boldyrev et al., 2004; Ganor et al., 2003; Lombardi et al., 2001) and mGluRs (Boldyrev et al., 2004; Miglio et al., 2005; Pacheco et al., 2004; Poulopoulou et al., 2005a,b; Rezzani et al., 2003; Storto et al., 2000) on T cell-mediated immunity and T-cell development. In addition, it has recently been described that dendritic cells (DC), which have a key role in the initiation and regulation of T-cell mediated immunity (Banchereau and Steinman, 1998; Lanzavecchia and Sallusto, 2001), release glutamate thereby contributing to modulate the T-cell activation (Pacheco et al., 2006). Also, a significant number of clinical studies have consistently shown strong correlation between deregulation of plasma glutamate levels and immunodeficiency, such as AIDS (Droge et al., 1993; Eck et al., 1989; Ferrarese et al., 2001) or malignancies (Eck et al., 1990; Ollenschlager et al., 1989). In addition to DC, other cells that belong to the immune system are also able to release glutamate such as neutrophils (Collard et al., 2002) and macrophages (Rimaniol et al., 2001). Despite this knowledge, more studies are necessary to elucidate the relevance of macrophage-or neutrophil-derived glutamate in the regulation of T-cell mediated immunity.

Although resting T cells patrolling in the periphery are unable to enter into CNS, activated T cells can enter into CNS via a not well understood mechanism (Hickey et al., 1991; Ludowyk et al., 1992). In this regard, after an insult to the integrity of the CNS, self-antigen-specific T cells protect neurons against glutamate neurotoxicity (Kipnis et al., 2001, 2002a; Nevo et al., 2003; Schori et al., 2001a, 2002; Schwartz et al., 2003, Shaked et al., 2004, 2005). This protective autoimmunity occurs by inducing different microglial phenotypes via a mechanism regulated by autoreactive T-cell-derived IFN-γ (Shaked et al., 2005). Although the implication of glutamate transport regulation on the surface of glial cells during protective autoimmunity have been described (Korn et al., 2005; Shaked et al., 2005), the role of glutamate receptors expressed on the surface of activated T cells in the CNS has not been yet explored.

In this article the role of glutamate as a key immunomodulator in the initiation and development of adaptive immune responses is presented, including data on the expression and function of glutamate receptors and glutamate transporters in T cells and other cells involved in the T-cell mediated immunity. Furthermore, this review highlights recent findings that point toward a role of glutamate as a link between the immune system and the nervous system.

2. Glutamate receptors expressed on immune system cells

In 1997, Kostanyan et al. (1997) performed pioneer studies which described the specific binding of radio-labelled glutamate to human blood lymphocytes, thus suggesting the presence of glutamate receptors and/or glutamate transporters in these cells. Three years later, Storto et al. (2000) reported the presence of functional group I and group II mGluRs in murine thymocytes, the T lymphocyte precursors. Addressing the group I mGluRs expression on thymocytes, Storto et al. (2000) showed that mGlu1R but not mGlu5R is expressed in immature $CD4^-$/$CD8^-$ thymocytes, whereas mGlu5R is present in more mature $CD4^+$/$CD8^+$ and $CD4^+$/$CD8^-$ cells. In addition, group II mGluRs (mGlu2/3R) are expressed in immature $CD4^-$/$CD8^-$ as well as mature $CD4^+$/$CD8^+$ and $CD4^+$/$CD8^-$ thymocytes (Storto et al., 2000). By immunohistochemical analysis Rezzani et al. (2003) demonstrated that immature thymocytes from cortical rat thymus, weakly express mGlu2/3R and mGlu4R, and that mature thymocytes from medullar rat thymus, express also mGlu5R.

In 2004, we described for the first time the presence of mGluRs on the surface of human T cells from peripheral blood (Pacheco et al., 2004). In such study, it was shown that mGlu5R is constitutively expressed on the T-cell surface and it mediates adenylate cyclase stimulation and, consequently, inhibition of anti-CD3 antibody-induced T-cell proliferation. Interestingly, expression of mGlu1R, which is coupled to MEK-ERK1/2-pathway, is only induced after T-cell activation. Stimulation of inducible mGlu1R in T cells undergoing activation counteracted the mGlu5R-mediated inhibitory effect in the T-cell proliferation (Pacheco et al., 2004).

More recently, we have demonstrated that the mGlu5R-mediated inhibitory effect on T-cell proliferation occurs via inhibition of IL-6 production (Pacheco et al., 2006), whereas the mGlu1R-triggered co-stimulatory effect is mediated by enhanced secretion of IL-2, IL-6, IL-10, TNF-α and IFN-γ(Pacheco et al., 2006). Three more studies showing expression of mGluRs in human resting T cells were subsequently reported. The first study, describes that stimulation of group I and group II mGluRs facilitate activation of potassium currents through Kv1.3 channels at low glutamate concentrations (1–10 μM), but decrease these potassium currents at high glutamate levels (>100 μM) (Poulopoulou et al., 2005a). The second study shows the expression of mRNAs for mGlu1R, mGlu2R, mGlu3R and mGlu8R in resting T lymphocytes from healthy donors; the functionality for these receptors was however not tested (Poulopoulou et al., 2005b). The third study, describes the increase of intracellular calcium and subsequent induction of c-*fos* and c-*Jun* expression promoted by group I mGluRs stimulation (Miglio et al., 2005). This finding contrasts with our previous report (Pacheco et al., 2004) where non-detectable changes on intracellular calcium levels were found after group I mGluRs stimulation in human T cells. Boldyrev et al. (2004) have reported that in contrast to data available for human T cells, rodent lymphocytes express group III mGluRs, but neither group I nor group II mGluRs are present in these cells. The expression and function of mGluRs in T cells and thymocytes are summarized in Table 1.

The first study describing the presence of iGluRs in human resting T lymphocytes was reported in 2001 by Lombardi et al. (2001). The authors demonstrated the functionality of NMDA, AMPA as well as KA receptors by mediating potentiation of PHA-or anti-CD3 antibody induced intracellular calcium rise (Lombardi et al., 2001). Two years later, Ganor et al. (2003), demonstrated the expression of the AMPA receptor iGlu3R in resting human T cells, which promotes integrin-mediated adhesion to laminin and fibronectin and SDF-1-induced chemotactic migration. In addition to studies performed in human T cells, Boldyrev et al. (2004) demonstrated that rodent lymphocytes express NMDA receptors, which mediate increases of intracellular calcium and reactive oxygen species (ROS). The expression and function of iGluRs in T cells are also summarized in Table 1.

Although less extensively than in T cells, glutamate receptors have also been studied in other cells of the immune system. The mGlu7R is expressed in human CD19^{+} B cells from healthy donors, but this receptor is down-regulated by hypermethylation of the *GRM7* gene promoter in CD19^{+} B cells from patients with chronic lymphocytic leukemia, (Rush et al., 2004). Also, mGluRs have been found in rat thymic DC (Rezzani et al., 2003). Whereas cortical DC express mGlu2/3R and mGlu4R weakly, medullar DC show moderate mGlu2/3R and mGlu4R and strong mGlu5R expression (Rezzani et al., 2003). However, we have recently demonstrated that neither mGlu1R nor mGlu5R are expressed on human monocyte-derived DC surface (Pacheco

Table 1
Expression and functionality of glutamate receptors in T cells and T cell precursors

Specie	Cells	mGluRs/effect	iGluR/effect
Human	Resting T cells	mGlu5R, ↑cAMP (a)	KA, modulate Kv1.3 channels? (b)
		mGlu1/5R, modulate Kv1.3 channels (b)	NMDA, potentiation of ↑Ca2+(c)
		mGlu2/3R, modulate Kv1.3 channels (b)	AMPA, potentiation of ↑Ca2+(c)
		mGlu1/5R, ↑Ca2+(d)	KA, potentiation of ↑Ca2+(c)
		mGlu1R, mGlu2R, mGlu3R, mGlu8R (e)	AMPA (iGlu3R), ↑adhesion, ↑chemotaxis (f)
	Activated T cells	mGlu5R, ↑cAMP (a)	ND
		mGlu1R, ↑ERK1/2 (a)	
Mouse	CD4−/CD8− thymocytes	mGlu1R, ↑ IP3 (g)	ND
		mGlu2/3, ↓cAMP (g)	
	CD4+/CD8+ thymocytes	mGlu5R, ↑ IP3 (g)	ND
		mGlu2/3, ↓cAMP (g)	
	CD4+/CD8− thymocytes	mGlu5R, ↑ IP3 (g)	ND
		mGlu2/3, ↓cAMP (g)	
Rat	Immature cortical thymocytes	mGlu2/3R, mGlu4R (h)	ND
	Mature medullar thymocytes	mGlu2/3R, mGlu4R, mGlu5R (h)	ND
Mouse/ Rat/ Rabbit	Blood lymphocytes	mGlu4/6/7/8R, potentiate ↑ROS (i)	NMDA (NR1), ↑ROS (I)

Data from: a, Pacheco et al. (2004); b, Poulopoulou et al. (2005a); c, Lombardi et al. (2001); d, Miglio et al. (2005); e, Poulopoulou et al. (2005b); f, Ganor et al. (2003); g, Storto et al. (2000); h, Rezzani et al. (2003); i, Boldyrev et al. (2004). ND, not determined.

et al., 2006). This discrepancy in the expression of mGlu5R in DC may be due to a different pattern of expression of glutamate receptors in DCs from different species and in DCs from different locations.

3. Glutamate transporters expressed on immune system cells

Glutamate transporters are key regulators of extracellular glutamate levels acting by either releasing or uptaking glutamate. So far, three main glutamate transport systems have been described. First, a Na^{+}-dependent high-affinity glutamate transporters family, EAATs, expressed in mammalian tissues was cloned in 1992 (Kanai and Hediger, 1992; Pines et al., 1992; Storck et al., 1992). This transporters

family is essentially expressed in the CNS and it consists of five members: GLAST (or EAAT-1), GLT-1 (or EAAT-2), EAAC1 (or EAAT-3), EAAT-4 and EAAT-5, which protect against excitotoxicity by clearing extracellular glutamate (Nedergaard et al., 2002; Schwartz et al., 2003). EAATs, which can be inhibited by the competitive inhibitors DL-threo-β-hydroxyaspartic acid (THA) or L-*trans*-pyrrolidine-2,4-dicarboxylic acid (*trans*-PDC) (Rimaniol et al., 2001), transport L-glutamate and D-or L-aspartate and couple the electrochemical gradient of three co-transported sodium ions and one counter-transported potassium ion with that of the amino acids (X_{AG}^- system) (Zerangue et al., 1995). A Na^+-independent anionic amino acid transport system highly specific for cystine and glutamate (Bannai and Kitamura, 1980, 1981) has been cloned more recently (Sato et al., 1999). This system designated as cystine/glutamate antiporter (or X_c^- system) transports the anionic form of cystine in exchange with glutamate (Bannai, 1986). Because cystine concentration is very low in the cytosol due to a rapid reduction to cysteine, and glutamate concentration is much higher in cells than in extracellular fluids, the physiological flow via the X_c^- system is the uptake of cystine and the release of glutamate (Bannai, 1986). The cystine/glutamate antiporter is composed of CD98 (heavy chain), commonly found in various transporters, and the xCT (light chain), which confers substrate specificity (Sato et al., 1999). The X_c^- system-mediated transport may be inhibited by L-homocysteic acid (HCA), α-aminoadipic acid (AAA) or quisqualic acid (Bender et al., 2000; Pacheco et al., 2006; Rimaniol et al., 2001). A third glutamate transport system has also been described in rat alveolar type II cells and in astrocytes (Bender et al., 2000; Knickelbein et al., 1997). This system mediates the Na^+-dependent transport of cystine, glutamate and aspartate (X_{AG} system) and it may be blocked by THA or AAA (Rimaniol et al., 2001).

Initially, it was described that murine brain macrophages release glutamate, thus promoting neurotoxicity in neurons expressing ionotropic NMDA receptors (Piani et al., 1991, 1992). The glutamate derived from brain macrophages was released via the X_c^- system (Piani and Fontana, 1994). The X_c^- system-mediated glutamate release has been confirmed in human monocyte-derived macrophages and, additionally, the X_{AG}^- system has been described in these cells (Rimaniol et al., 2001). Both the X_c^- system as well as the X_{AG}^- system are implicated in the regulation of glutathione (GSH) biosynthesis in human macrophages: the X_c^- system uptakes cystine, which in the cytosol is reduced to cysteine and subsequently used for GSH synthesis, and the X_{AG}^- system uptakes the glutamate released via the X_c^- system, thus providing intracellular glutamate for direct insertion into GSH and also fuelling the cystine uptake by the X_c^- system. In addition, it has been described that lipopolysaccharide (LPS) (Sato et al., 1995) as well as oxygen (Sato et al., 2001) promote up-regulation of xCT expression, thus increasing the X_c^- system activity. On the other hand, extremely poor

transport for cystine has been reported in both human resting and activated T cells (Droge et al., 1991; Gmunder et al., 1991), which suggests that neither the X_c^- system nor the X_{AG}^- system are expressed in these cells. In agreement with these results, we have recently demonstrated that very low levels of mRNA for xCT light chain are found in human T cells (Pacheco et al., 2006).

The expression of glutamate transporters in DC, the most potent antigen presenting cells (APC), has also recently been reported. In 1993, glutamate-like immunoreactivities were described in skin-residing human DC- and macrophages-like cells (Nordlind et al., 1993). Furthermore, immunoreactivity is increased in inflamed versus normal skin (Nordlind et al., 1993), suggesting that glutamate could play an important role in these cells during inflammatory processes. In addition, in 2002 Angelini et al. (2002) described that DC uptake cystine from extracellular fluids by a mechanism potentiated by maturative stimuli such as LPS, therefore suggesting that the X_c^- system or the X_{AG}^- system could be operating in these cells. We have recently reported the presence of the X_c^- system in human DC, which plays a key role releasing glutamate during the T-cell-DC interaction, thus modulating T-cell activation (Pacheco et al., 2006).

Furthermore, Collard et al. (2002) have demonstrated that human neutrophils, during inflammatory processes, release glutamate. The neutrophil-derived glutamate might act on group I and group III mGluRs expressed in endothelial cells, thus leading to an increase in endothelial permeability (Collard et al., 2002). Although the most probable target for neutrophil-derived glutamate has been identified in endothelium, the mechanism by which glutamate is released from these cells remains unknown. Expression and functionality of glutamate transporters in cells of the immune system are summarized in Table 2.

4. Modulation of T-cell function by glutamate in peripheral tissues

Because human T cells express several glutamate receptors and also differential expression of these receptors

Table 2

Expression and function of glutamate transport systems in human cells of immune system

Cell	Transport system			Function
	X_{AG}	X_{AG}^-	X_c^-	
Macrophages	−	+	+	Release glutamate (neurotoxicity)/ Modulate GSH synthesis (a,b)
T cells	−	ND	−	Require extracellular cysteine to GSH synthesis (c,d,e)
Dendritic cells	ND	ND	+	Release glutamate: Modulate T cell activation (f)
Neutrophils	ND	ND	ND	Release glutamate: ↑brain endothelial permeability (g)

Data from: a, Rimaniol et al. (2001); b, Piani and Fontana (1994); c, Droge et al. (1991); d, Gmunder et al. (1991); e, Angelini et al. (2002); f, Pacheco et al. (2006); g, Collard et al. (2002). ND, not determined.

Table 3
Elevation of plasma glutamate levels in neurologic disorders, malignancies and immunodeficiency

Classification	Pathology	[Glutamate] (μM) [a]	Glutamate increase (ln-fold) [b]	Reference
Neurologic disorders	Amyotrophic lateral sclerosis	164±117	4.8±3.4	Iwasaki et al. (1992a)
	Epilepsy	53±25	2.7±1.3	Janjua et al. (1992)
	Headache (cerebral infarction)	321±150	1.4±0.6	Castillo et al. (1995)
	HIV-associate dementia	200±30	5.9±0.9	Ferrarese et al. (2001)
	Parkinson's disease	72±9	2.1±0.3	Iwasaki et al. (1992b)
Malignancies [c]	Breast cancer	54–83	2.0–3.0	Ollenschlager et al. (1989)
	Colorectal carcinoma	47–86	1.7–3.1	Ollenschlager et al. (1989)
Immunodeficiency [c]	AIDS	54–70	2.0–2.6	Ollenschlager et al. (1989)

[a] Plasma glutamate levels.
[b] Increase of plasma glutamate levels are expressed as the ratio of average of glutamate concentration in plasma from patients versus average of glutamate concentration in plasma from healthy donors.
[c] The Range of plasma glutamate levels or range of elevation of plasma glutamate levels are respectively indicated.

is triggered by T-cell activation (Table 1), it is inferred that glutamate is an important regulator of T-cell function. Moreover, differential expression of glutamate receptors has also been described in rodent thymocytes during different maturational stages (Table 1), which suggest that the amino acid could play a role during T-cell development.

Patrolling resting T cells encounter plasma glutamate, whose concentration fluctuates in the range 10–60 μM (Divino Filho et al., 1998; Graham et al., 2000; Tsai and Huang, 1999) in physiological conditions. After thymic maturation, resting T cells express group I mGluRs (mGlu5R), group II mGluRs (mGlu2/3R), the group III mGluR member mGlu8R and ionotropic receptors NMDA, AMPA and KA (Table 1). By stimulating the adenylate cyclase-coupled mGlu5R (Pacheco et al., 2004), plasma glutamate may keep elevated intracellular cAMP levels with subsequent PKA activation. It should be noted that mGlu5R in cells of the nervous system are usually coupled to phospholipase C and not to adenylate cyclase. This constitutes a distinctive feature of signalling via mGlu5R in T lymphocytes (Pacheco et al., 2004). In T cells, PKA as much as cAMP evoke inhibition of ERK (Ramstad et al., 2000) and JNK activation (Harada et al., 1999), activate C-terminal Src kinase (CSK) (Vang et al., 2001) and block NF−κB activation (Hershfield, 2005; Jimenez et al., 2001). All of these intracellular biochemical events induce a marked impairment on T-cell activation with inhibition of T-cell proliferation and of cytokine production (Aandahl et al., 2002). In this regard, a considerable number of clinical studies have shown elevation of plasma glutamate levels under pathophysiological conditions such as in neurologic disorders, malignancies and immunodeficiencies (Table 3). Thus, mGlu5R stimulation by plasma glutamate would promote a high threshold for T-cell activation (Fig. 1), which could be exacerbated in some pathologies (Table 3). Regarding the function of the AMPA receptor iGlu3R in T cells, it seems that upon stimulation it mediates impairment of IL-10 production (Pacheco et al., 2006), and allows the chemotactic migration and integrin-mediated adhesion of patrolling resting T cells (Ganor et al., 2003) (Fig. 1). In addition, the amplitude of calcium influx during the initiation of T-cell activation may be regulated by NMDA and KA receptors via their cation channel activity (Lombardi et al., 2001), and by group II mGluRs, which modulate potassium currents through Kv1.3 channels (Poulopoulou et al., 2005a). Further experimental work is necessary to provide an integrated view of the role of these glutamate receptors in the T-cell physiology.

Not only plasma glutamate may activate glutamate receptors expressed in patrolling resting T cells, but also homocysteine (HC) and HC metabolic derivatives such as homocysteine sulfinic acid (HCSA), homocysteic acid (HCA), cyteine sulfinic acid (CSA) and cysteic acid (CA) may stimulate either mGluRs or iGluRs (Lazarewicz et al., 2003; Shi et al., 2003). Normal plasma HC levels are in the range 5–15 μM, but they may increase up to >100 μM in
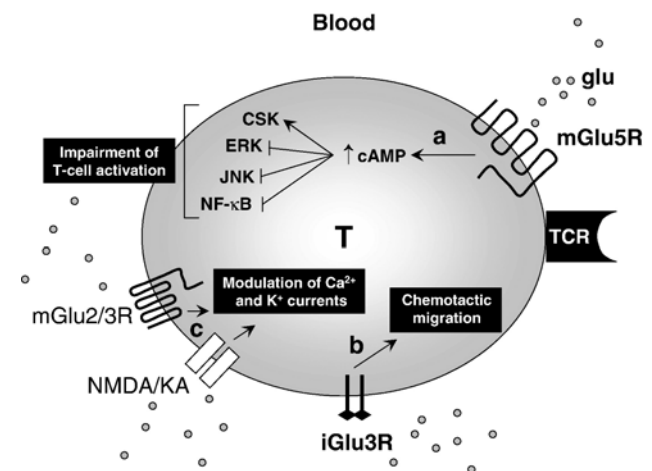

Fig. 1. Function of glutamate receptors in resting T cells. Glutamate receptors expressed on the surface of resting T cells are subject to action of plasma glutamate (glu, gray dots). Stimulation of mGlu5R increases intracellular cAMP levels, which in turn induces activation of C-terminal Src Kinase (CSK), inhibition of Ras/ERK pathway, inhibition of JNKs and inactivates NF-κB (a). All these mGlu5R-mediated effects lead to an impaired state for T-cell activation. On the other hand, stimulation of iGlu3R by plasma glutamate allows chemotactic migration in patrolling T cells, probably by inhibiting IL-10 production (b). Activation of NMDA and KA iGluRs as well as mGlu2/3R could have a role by regulating the amplitude of Ca²+ and K+ currents, respectively (c). Arrow heads represent activation, while flat heads represent inhibition.

pathophysiological conditions such as in neurodegenerative or cardiovascular diseases (Gortz et al., 2004).

Adaptive immune responses are initiated when the antigen is presented to specific T cells by an APC and, consequently, T cells become activated. DCs are the most potent APC specialized in the initiation of immune responses by directing the activation and differentiation of naïve T lymphocytes (Banchereau and Steinman, 1998; Lanzavecchia and Sallusto, 2001). Immature DC (iDC) reside in most tissues in order to uptake antigen; they are engaged when exposed to danger signals produced by microorganisms, inflammatory cytokines, nucleotides, and cell damage (Gallucci and Matzinger, 2001). Upon exposure to such factors, DC lose their phagocytotic capacity, migrate to draining lymph nodes, and undergo a maturation process, acquiring high levels of membrane major histocompatibility complex (MHC) and co-stimulatory molecules such as CD80 and CD86.

In the lymph nodes, mature DC (mDC) present the captured and processed antigen to specific T cells, thereby directing the initiation and development of immune responses. Depending on the context, DC can stimulate the polarized outgrowth of distinct T cell subsets, including T helper 1 (Th1) and T helper 2 (Th2) (Banchereau and Steinman, 1998; Lanzavecchia and Sallusto, 2001; Pacheco et al., 2005). Th1 or Th2 polarization orchestrates the immune effector mechanism most appropriate to combat the invading pathogen. Th1 cells promote cellular immunity protecting against intracellular infection and cancer, whereas Th2 cells promote humoral immunity, which is highly effective against extracellular pathogens, and play a role in tolerance mechanisms and allergic diseases (Del Prete, 1998).

Recently, we have demonstrated that undergoing maturation and during antigen presentation, DC release glutamate via the X_c^- system (Pacheco et al., 2006). In agreement with
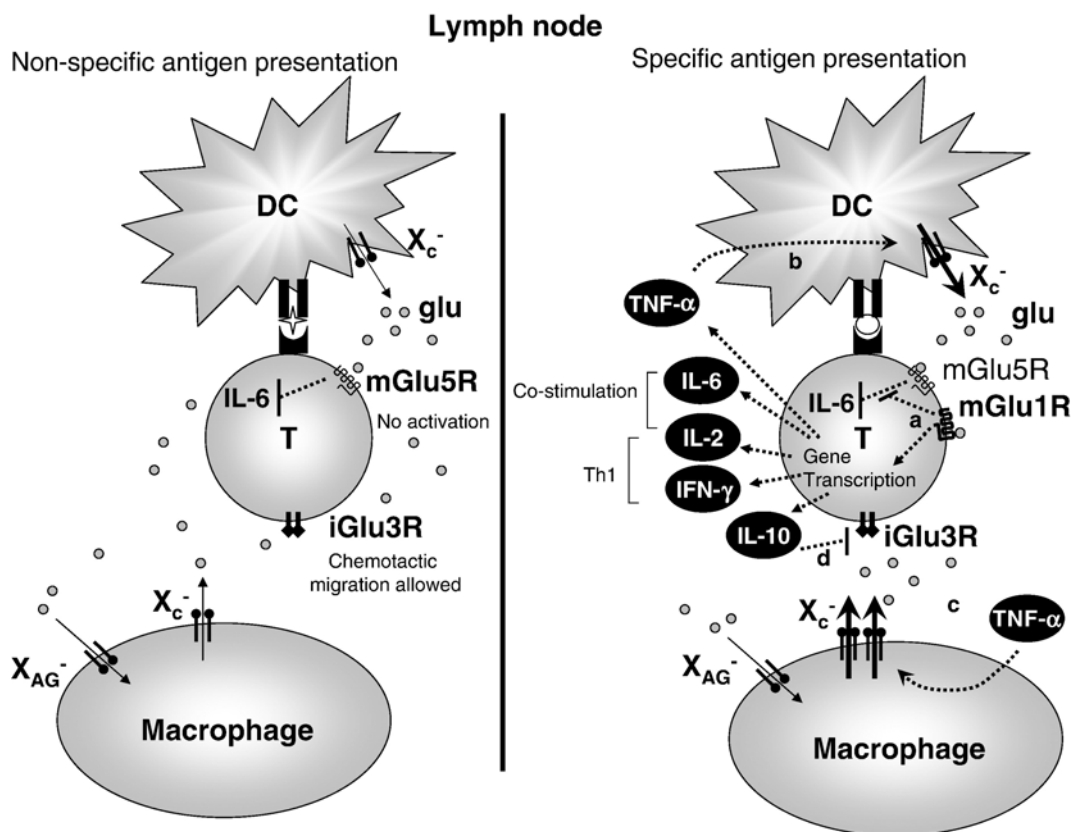

Fig. 2. Putative role of DC-released glutamate during T-cell-DC interaction in the lymph nodes. Left panel: When a non-cognate antigen is presented by DC to T cells in the lymph nodes, glutamate released by DC (gray dots) via the cystine/glutamate antiporter (X_c^- system) stimulates the constitutively expressed mGlu5R in resting T cells. The mGlu5R stimulation evokes an increase of intracellular cAMP, thus avoiding the erroneous T-cell activation (i.e. by inhibition of IL-6 production). On the other hand, stimulation of constitutively expressed iGlu3R by promoting an inhibition of IL-10 production, allows chemotactic migration of resting T cells. In addition to DC-released glutamate, the macrophage-derived glutamate contributes to regulate glutamate levels in the microenvironment of lymph nodes. Right panel: When cognate peptide is presented by DC to a T cell, the TCR-triggered pathway is strong enough to overcome the mGlu5R-triggered inhibitory pathway and therefore T-cell activation begins. During cell activation, mGlu1R expression is induced in T cells and subsequently this receptor is stimulated by extracellular glutamate inducing a bypass of mGlu5R-triggered pathway and promoting enhanced production of Th1 (IL-2 and IFN-γ) and pro-inflammatory cytokines (IL-6 and TNF-α) as well as IL-10 (a). The increase of IL-2 and IFN-γ levels further promotes a Th1 response, whereas IL-2 and IL-6 promote co-stimulation by parallel pathways. The mGlu1R-induced secretion of TNF-α establishes a positive feedback promoting enhanced glutamate secretion (bold line arrows) by DC (b) as well as by macrophages (c). Also, the mGlu1R-induced potentiation of IL-10 secretion counteracts the iGlu3R-mediated effect on chemotactic migration (d), thereby mediating T cell retention into lymph nodes during activation. Dotted lines with arrow heads represent stimulation/up-regulation, while the flat heads represent inhibition/down-regulation. Solid line arrows represent glutamate flows.

these findings, it has been reported that after freund's adjuvant injection, glutamate levels are increased in lymph nodes (Bonacho et al., 2001). From these results it can be surmised that DC-released glutamate acts early during T-cell-DC interaction via mGlu5R, which is positively coupled to the adenylate cyclase (Pacheco et al., 2004), thereby impairing IL-6 production and, consequently, T-cell proliferation (Pacheco et al., 2006). Therefore, this mGlu5R-mediated mechanism may contribute to impede T-cell activation when a non-specific antigen is presented by a DC. Thus, mGlu5R stimulation could lead to the unresponse of non-specific T cells in the lymph nodes (Fig. 2, left panel). In addition, stimulation of iGlu3R by DC-released glutamate could contribute to keep migratory activity in resting T cells (Ganor et al., 2003). On the other hand, when DC present the cognate-antigen to T cells, the latter are engaged and undergo an activation process. In these conditions the mGlu1R expression is induced on activated T cells, which so far, has been the only T-cell activation-inducible glutamate receptor described (Pacheco et al., 2004, 2006). Subsequently, DC-released glutamate acts on the induced mGlu1R, which are coupled to the ERK-pathway (Pacheco et al., 2004). In this way, glutamate causes the attenuation of the inhibitory mGlu5R-triggered effects as well as the enhancement of Th1 (IL-2 and IFN-γ) and pro-inflammatory (IL-6 and TNF-α) cytokine secretion inducing co-stimulation (Fig. 2, right panel) (Pacheco et al., 2006). The mGlu1R-mediated co-stimulatory effect in combination with the mGlu5R-mediated inhibitory effect could constitute a fine regulatory mechanism by which little differences in the intensity of TCR-triggered stimulus would allow different T-cell-fate decisions between antigen-specific and non-specific T cells (Fig. 2). Thus, it is expected that the deregulation of either glutamate release by DC or mGluRs-mediated signaling could promote erroneous cell-fate decisions with respect to T-cell maturation, activation and differentiation. Such mechanisms would have important implications for pathophysiological mechanisms of autoimmunity as well as for immunodeficiency diseases.

During T-cell-DC interaction IL-10 levels are not affected by glutamate depletion, but they are increased by mGlu1R-specific stimulation (Pacheco et al., 2006). As IL-10 has been implicated in the inhibition of chemotactic CD4$^+$ T cells migration (Jinquan et al., 1995; Tan et al., 1995; Zachariae et al., 1992), mGlu1R could also be involved in the retention of T cells undergoing activation in lymph nodes (Fig. 2, right panel).

Furthermore, because macrophages are also APC in secondary immune responses, the macrophage-derived glutamate may play a role during antigen presentation in secondary immune responses. However, macrophages not only express the X_c^- system, but also the X_{AG}^- system (Table 2) and therefore, the glutamate released by these cells is subsequently uptaked by the same cells (Rimaniol et al., 2001). Due to these reasons, macrophages may have a role in regulating glutamate concentrations in the lymph node microenvironment, such as it occurs with astrocytes and microglia in the CNS (Schwartz et al., 2003). In basal conditions in lymph nodes, a low extracellular glutamate concentration could be sustained by macrophages. In contrast, after a challenge with danger signals (Sato et al., 1995, 2001), an up-regulation of the X_c^- system is induced in macrophages and subsequently these cells begin to release glutamate (Piani et al., 1991, 1992; Piani and Fontana, 1994). Therefore, in addition to DC-released glutamate, the macrophage-released glutamate would contribute also to increase glutamate levels into lymph nodes during the first stages of the immune response (Fig. 2).

5. Modulation of T-cell function by glutamate in the central nervous system

High extracellular glutamate concentrations in the CNS promote excitotoxicity, which has been involved in various pathological conditions, including acute CNS trauma such as brain or axonal injury (Alessandri and Bullock, 1998), ischaemia (Lipton, 1999), and epilepsy (Fountain, 2000), as well as in chronic neurodegenerative disorders such as Parkinson's and Alzheimer's diseases, glaucoma and amyotrophic lateral sclerosis (Choi, 1988). On the other hand, it is known that T cells, when activated, are able to enter inside CNS (Hickey et al., 1991; Ludowyk et al., 1992). In fact, a number of studies have shown that high glutamate levels in the CNS, either directly or indirectly, elicits a systemic T-cell mediated immune response directed against immunodominant self-antigens that reside at the site of glutamate-induced damage (Kipnis et al., 2001,a; Nevo et al., 2003; Schori et al., 2001a, 2002; Shaked et al., 2004, 2005). In those studies, crush injury of the optic nerve or intravitreal injection of a toxic dosage of glutamate in mice or rats have been used as experimental model. After glutamate-induced damage, survival of retinal ganglion cells was much higher in wild type animals than in animals devoid of mature T cells (nude animals) (Schori et al., 2001a). Moreover, when nude animals were supplied with splenocytes derived from the wild type animals, survival of retinal ganglion cells after CNS insult was significantly improved (Schori et al., 2001a). The neuroprotective T cell-mediated immunity is attributed to autoreactive T cells specific to CNS-associated myelin antigens such as myelin basic protein (Hauben et al., 2000; Moalem et al., 1999a,b) and therefore, it is called protective autoimmunity (Kipnis et al., 2002a; Nevo et al., 2003; Schwartz et al., 2003; Shaked et al., 2004, 2005). Indeed, therapeutic vaccination with copolymer 1, a synthetic peptide that cross-reacts with myelin-derived antigens, improves neuron survival and prolongs life span in mice under conditions that simulate glaucoma (Schori et al., 2001b) or amyotrophic lateral sclerosis (Angelov et al., 2003).

Although these results point out to a relationship between high glutamate concentrations and T-cell-mediated protective autoimmunity, the role of glutamate receptors in this process has not been well defined. According to the direct implication of iGluRs expressed on the neuronal surface in the glutamate

evoked-neuronal death, it has been reported that in absence of T-cell mediated response, activation of NMDA as well as AMPA/KA receptors exacerbate the glutamate-induced neuronal loss (Schori et al., 2002). When NMDA-induced neuronal death was compared between mice devoid of T cells and wild type animals, no differences were found (Schori et al., 2001a). This discards the possibility that NMDA receptors expressed in T cells play a role in the neuroprotection. On the other hand, the role of mGluRs expressed on the T-cell surface in the protective autoimmunity remains unknown. Because only activated T cells are enabled to enter into CNS (Hickey et al., 1991; Ludowyk et al., 1992), it is likely that the MAPK-coupled mGlu1R differentially expressed only in activated T cells, is involved in T-cell mediated protective autoimmunity. In agreement with this idea, we have recently described that mGlu1R stimulation in activated T cells from peripheral blood potentiates induction of Th1 phenotype with augmented secretion of IFN-γ (Pacheco et al., 2006). Both Th1 phenotype and IFN-γ production are strongly related to glutamate-induced autoimmunity (Shaked et al., 2004, 2005). In fact, in a mouse strain with spontaneous T cell-dependent ability to withstand the consequences of glutamate-induced neuronal damage, depletion of $CD4^+CD25^+$ regulatory T cells (Treg) was beneficial (Kipnis et al., 2002a, 2004). This inhibitory effect in the protective autoimmunity mediated by Treg was explained by Treg-induced down-regulation in the Th1 cell-mediated functions (Kipnis et al., 2002b, 2004). In this regard, it has been described that beneficial effects of Th1 cells in the protective autoimmunity induced by glutamate mediated-damage in the CNS, were attributed to the ability of these cells to induce a given microglial phenotype (Schwartz et al., 2003; Shaked et al., 2004, 2005). It has been suggested that depending on the way in which microglial cells are activated, different effector functions are induced resulting in two possible phenotypes (Schwartz et al., 2003; Shaked et al., 2004, 2005). The beneficial phenotype, with high levels of class II MHC (Schwartz et al., 2003; Shaked et al., 2004) and increased expression of the glutamate transporter GLT-1 (Jacobsson et al., 2006; Lopez-Redondo et al., 2000; O'Shea et al., 2006; Persson et al., 2005; Schwartz et al., 2003), would display an APC function and high capacity to uptake glutamate. The destructive phenotype would make cells release glutamate, nitric oxide and TNF-α. According to the beneficial role of autoreactive Th1 cells in the protective autoimmunity, it has recently been described that the Th1-derived cytokine IFN-γ mediates the induction of the beneficial phenotype on microglial cells (Shaked et al., 2005). Thus, in the CNS, glutamate may stimulate mGlu1R on the activated T-cell surface potentiating Th1 phenotype with high IFN-γ production. Subsequently, the Th1-cell secreted IFN-γ might induce the beneficial phenotype in microglial cells with high expression of GLT-1, thereby mediating the clearance of glutamate, thus achieving neuroprotection (Fig. 3).

Taken all the evidence, the mGlu1R expressed on the surface of T cells in the CNS becomes an excellent candidate
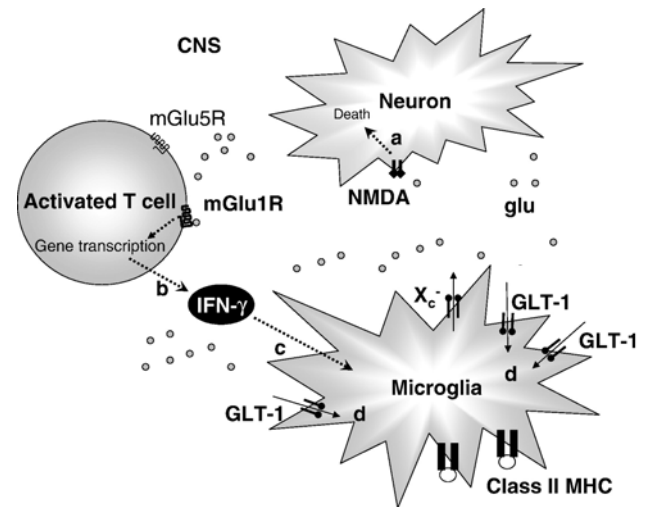

Fig. 3. Proposed role of mGlu1R expressed on the activated T-cell surface during protective autoimmunity in the CNS. When the CNS is injured, glutamate buffering cells, astrocytes, are lost and the damaged site is repopulated by activated microglial cells. Activated microglia release glutamate (gray dots) via the cystine/glutamate antiporter (X_c^- system), thereby increasing glutamate levels. In these pathological conditions, elevated glutamate concentrations promote excitotoxic neuronal death by excessive activation of NMDA receptors (a). Under such conditions, self-reactive Th1 cells specific against CNS-derived antigens are activated. In the damaged site, glutamate stimulates the mGlu1R expressed on the activated T-cell surface promoting strong production of IFN-γ (b). Subsequently, IFN-γ induces the beneficial phenotype in microglial cells up-regulating the expression of the glutamate transporter GLT-1 and of class II MHC (c). The high quantity of GLT-1 expressed in microglia mediates the glutamate clearance (d) thus preventing the damage spreading. Dotted lines represent effects, while solid lines represent glutamate flows.

to explain the mechanism by which self-reactive Th1 cells minimizes the glutamate-induced neuronal damage during protective autoimmunity (Fig. 3). Thus, glutamate seems to be a link between the immune system (modulating cytokine secretion by T cells) and the nervous system (where the amino acid is highly released under stressful conditions).

6. Concluding remarks

In the last years several glutamate receptors have been identified on the T-cell surface. On the other hand, glutamate transporters have been described in APC where, depending on the context, they may uptake or release glutamate. The release of glutamate by DC has recently been demonstrated and a regulatory role of this amino acid during the T-cell-DC interaction in lymph nodes is now evident. The DC-released glutamate, by stimulating differentially expressed glutamate receptors in T cells, may regulate the responsiveness of T cells to antigen presentation. Furthermore, a protective autoimmunity operates when glutamate-induced neuronal damage elicits a self-reactive Th1-mediated response in the CNS where T cell-derived IFN-γ plays a pivotal role. Thus the inducible mGlu1R, which potentiates IFN-γ secretion when stimulated in activated T cells, is proposed as a key part of the T-cell mediated mechanism involved in protective autoimmunity.

Consequently, the mGlu1R may be considered as a key mediator of glutamate-induced modulation of T-cell responsiveness as well as a receptor by which glutamate could establish a link between the immune system and the CNS.

Acknowledgment

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