Review

Metabolic mechanisms of cancer-induced inhibition of immune responses

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Abstract

During progression, tumors become refractory to the offensive weapons of the immune system. It has been known for a long time that the tumor microenvironment presents a profound modification in the metabolism of arachidonic acid and amino acids such as L-triptophan and L-arginine. However, only in the last decade we have started to appreciate how these changes might cause dysfunctions in cells of both adaptive and innate immune system. The knowledge of these complex and partially interconnected metabolic pathways is offering new targets for an integrated pharmacological approach aiming at freeing tumor-specific T lymphocytes from the latches of cancer influence.
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Contents

1. Introduction

The immune system possesses all the requisites to act as a powerful weapon against tumors. It can be very selective by

recognizing antigens that are solely or preferentially expressed on malignant cells. However, results from clinical trials have shown that the efficacy of different immunotherapeutic approaches, either active or passive, is not adequate for an immediate and widespread transfer to the clinic. In fact, even though cancer vaccination often succeeds in expanding circulating T lymphocytes recognizing the autologous tumor, only a limited number of clinically objective responses has been reported so far [1,2]. T lymphocytes activated by cancer vaccination acquire an antigen-experienced/memory phenotype and their functional analysis confirms that these T cells are virtually competent to attack and destroy neoplastic cells [3]. Thus, inefficacy of active immunotherapy likely depends either on the inability of sufficient lymphocyte numbers to reach the tumor site or the divertive maneuvers orchestrated by tumor

cells, a complex process often referred at as tumor immune escape.

Tumor escape mechanisms are quite diversified and have been extensively reviewed recently [4–6]. Among the most common causes, it is possible to enlist: loss of antigen, HLA molecules, or key proteins of the antigen processing machinery; local production of immunosuppressive molecules; recruitment and activation of suppressive lymphoid and myeloid cells; loss of costimulatory molecules [4–6]. Although there is no agreement about the prevailing mechanisms, studies conducted in the last years, however, have clearly indicated that tumor microenvironment is not suitable for T lymphocyte functions and indeed a number of reports indicate that tumor-infiltrating lymphocytes (TILs) have defects in both signal transduction compartment and killing effector systems [7]. These findings have been obtained mostly in non-vaccinated tumor-bearing hosts, but it is reasonable to assume that the same constrains might apply to lymphocytes activated in the host by active immunotherapy or adoptively transferred after *in vitro* expansion, once they reach the tumor site.

Altered metabolism in the tumor microenvironment has a profound impact on anti-tumor immunity and, more generally, on T cell function. Tumor cells or host cells under tumor cell control can impair the immune system function by altering the metabolism of simple molecules such as amino acids (L-tryptophan and L-arginine) and unsaturated fatty acids (arachidonic acid). In this review, we discuss emerging evidence supporting a key role for the tumor-associated metabolism in controlling immunity and suggest novel therapeutic strategies in cancer immunotherapy.

2. Arachidonic acid metabolism

Lipids and their products – mainly generated as cleavage products of phospholipids in cellular membranes – can act both as pro-inflammatory and anti-inflammatory signals, modulating gene expression profile of cytokines and immune regulatory factors [8]. Arachidonic acid, a 20-carbon unsaturated fatty acid, is associated with phospholipids of the cell membrane and is cleaved by phospholipase A_2 activity. Arachidonate is the precursor of a large variety of immune active lipids, since the 15-lipoxygenase, 5-lipoxygenase and cycloxygenase pathways produce lipoxins, leukotrienes and prostaglandins (PG), respectively.

Cyclooxigenase (COX) has three isoforms, playing distinct roles in immunity. COX-1 is diffusely expressed in lymphoid cells in embryonic thymus and affects the transition from $CD4^-CD8^-$ to $CD4^+CD8^+$ T cells [9]. Also COX-3, an incompletely characterized and controversial COX-2-like variant that is inhibited by the drug acetaminophen, which has little effect on the other two COX enzymes, was found to be expressed in macrophages and is thought to produce mainly anti-inflammatory prostanoids that may modulate immune functions [10]. However, among the different isoforms, COX-2 is the most important regulator of the immune system in tumor-bearing hosts. COX-2 expression is induced by growth factors, cytokines, or tumor promoters and, indeed, the enzyme is over-expressed in a wide variety of tumors, such as in adenoma epithelium, in multiple intestinal neoplasia mouse model, in replication error repair positive human tumors, in human colorectal cancers, and in some pre-malignant lesions [9,11]. COX-2 activity is believed to enhance angiogenesis [12], suppress apoptosis and cause tumorigenesis [13] and, in addition, it seems to play a crucial role in suppressing tumor immunity. In a mouse model, inhibition of COX-2 or treatment with anti-PGE2 mAb led to marked lymphocytic infiltration of the tumor and reduced tumor growth [14]. COX2-overexpressing tumors strongly induce dendritic cells (DCs) to produce IL-10 and TGF-β, which in turn activate $CD4^+$ T regulatory (Treg) type 1 lymphocytes, as potentially relevant immunoevasive mechanism [15]. *In vitro*, COX-2/PGE_2 induced expression of the Treg cell-specific transcription factor, Foxp3, and increased Treg cell activity [16,17]. In addition, PGE_2 generated by COX2 can directly inhibit lymphocyte function by increasing the levels of cAMP [18]. In agreement with these data, administration of COX-2 inhibitors was shown to reverted tumor-induced immunosuppression and induce TIL recruitment *in vivo* [14,19,20].

Lipid mediators can act both as autocrine and paracrine signals and, depending on the site of exposition and target cell maturation, the same metabolite can produce opposite effects. For instance, PGE_2 can cooperate with inflammatory cytokines such as TNF-α, IL1 and IL-6 to address DC maturation, trough an over-expression of CCR7, or oppositely it can assume inhibitory behavior in lymphoid organs inducing DCs to produce IL-10, IL-12 and down-modulating their CCL3 and CCL4 expression [21]. The reasons for these opposite PGE_2 actions of are not known but might be related to the DC maturation stage, the prevalent subtype of expressed PGE receptors, and the interplay with either cytokines (IL-10) or mediators of the L-arginine metabolism, such as nitric oxide (reviewed in [22] and further discussed below).

3. Tryptophan metabolism

Tryptophan is metabolized by two enzymes controlling oxidative cleavage of the 2,3 double bound in its indole ring. Tryptophan 2,3-dioxygenase (TDO) is constitutively expressed in liver and not regulated by immune mediators, whereas indoleamine 2,3-dioxygenase (IDO) activity is regulated by and directly regulates a wide panel of immunological signals [23,24]. IDO is involved in maintaining maternal tolerance against fetus during pregnancy [25] and regulates the severity of several experimental autoimmune diseases [26–28]. However, $IDO^{-/-}$ mice do not develop spontaneous autoimmune disorders or lymphoproliferative disorders, indicating that the enzyme is not critical in maintaining systemic tolerance to self-antigens.

IDO exerts its immunosuppressive activity either by local depletion of tryptophan or accumulation of its toxic catabolites (kinurenins) which cause T cell arrest in G_1, anergy and death by apoptosis [29–31]. Interestingly, elevated levels of tryptophan catabolites have been associated with cancer progression. IDO is indeed expressed in primary human tumors and systemic administration of specific IDO inhibitor resulted in partial rever-

sion of the tumor-induced immunosuppression in mouse tumor models [32].

IDO is under the transcriptional control of Bin1, which restrains IDO expression acting on activator of transcription 1 (STAT1)- and NF-kB-dependent transcription, and is often mis-spliced or attenuated in cancers of breast, prostate, colon, brain and other organs [33]. The array of cells that express IDO in physiological manner or under an aberrant tumor control is wide. IDO expression was described in monocyte-derived macrophages upon activation with IFN-γ, monocyte-derived DCs that co-express CD123 and CCR6, and cells bearing the surface markers CD14−, CD83+, CD80+, CD86hi, HLA-DRhi [23]. CpG-reach oligonuclotides (CpG-ODN), which bind toll like receptor 9 molecule, have been implicated in a type I and type II interferon-independent induction of IDO [34]. Moreover, IDO is induced in different cells through a pathway activated by IFN-γ and signal transducer and STAT1 and IFN-regulatory factor 1 (IRF1) and synergized by LPS, IL-1 and TNF-α, suggesting that IDO expression may represent a general inflammation-induced counter-regulatory mechanism [35]. Mouse myeloid CD8+ DCs are able to upregulate IDO [36] and, in addition, a subpopulation of plasmacytoid DCs (CD123+/CCR6+/CD19+ cells) that constitutively express IDO in tumor-draining lymph nodes, but not in normal lymph nodes and spleen, has been described in mouse tumor models and tumor patients [37]; it was advanced that this small population of IDO+ pDC is able to redefine tumor-draining lymph nodes as a tolerogenic milieu [38]. However, in both healthy subjects and multiple sclerosis patients, the mere IDO expression is not immunosuppressive as shown by the fact that CD123+CCR6+ IDO-expressing DCs do not prevent activation of both resting and activated T cells [39]. In terms of anti-cancer therapy, regression of established tumors was obtained by combining a cytotoxic chemotherapeutic drug with an IDO inhibitor [33].

Ligation of CD80 by cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) in mouse and human DCs can trigger IDO, in part through the autocrine release of IFN-γ [40,41]. The main function of CTLA-4 in mediating the regulatory function of CD4+CD25+ Treg cells appears to be linked to the binding of CD80 and consequent IDO activation in DCs [40]. Moreover, another membrane receptor for the CD200 ligand (CD200R) can trigger IDO expression and the tolerogenic tryptophan catabolism in spleen plasmacytoid DC subset (CD11c+B220+120G8+), through a pathway requiring the presence of an intact receptor for type I IFN [42].

4. Arginine metabolism

Tryptophan is not the only amino acid whose metabolism is increased in tumor-conditioned microenvironment and numerous reports suggest also a role for the activation of L-arginine (L-Arg) metabolizing enzymes during tumor growth and development. L-Arg is a conditionally essential amino acid that is mainly metabolized by the enzyme arginase (ARG), which produces the downstream compounds urea and L-ornithine, and nitric oxide synthase (NOS) that cleaves L-Arg into nitric oxide (NO) and L-citrulline. Two different isoforms of ARG have been

identified in mammals, and in spite of a large sequence identity and similar enzyme activity they have different distribution among tissues. ARG1 is constitutively present in the liver as part of the urea cycle and is induced in different mouse myeloid cells by exposure to Th2-type cytokines (IL-4/IL-13), TGF-β, IL-10, hypoxia, PGE2, and cAMP. ARG2 is expressed by various cell types including renal cells and enterocytes [43].

Three different isoforms of NOS are known (reviewed in [44]) but, in cancer metabolism, the most interesting one is the inducible isoform (NOS2), that is expressed by various cell types of the immune system and is transcriptionally upregulated by pro-inflammatory cytokines (e.g. interferons, IL1, IL-2, and TNF-α), bacterial lipopolysaccharide, and hypoxia, whereas it is downregulated by steroids, anti-inflammatory cytokines (e.g. TGF-β, IL-10), and NO itself [45].

Increased ARG activity has long been detected in patients with colon, breast, lung and prostate cancer and the elevated serum ARG activity has been considered a marker of disease progression in colorectal and breast cancer, and thus proposed as tool to monitor disease progression [46–53]. The ARG enzymatic activity is suspected to be necessary to sustain the high demand of polyamines necessary to tumor growth [54] and suppress anti-tumor immune response through negative effects on TILs [55].

Expression of NOS2 in malignant cells or within the tumor-infiltrating leukocytes has been described, both at the mRNA and protein level, in breast carcinoma, colon carcinomas, ovarian cancer, melanoma, head and neck cancer, esophagus, lung, prostate, bladder and pancreatic carcinomas, brain tumors, Kaposi's sarcoma, mesothelioma, and hematological malignancies (reviewed in [44]).

ARG and NOS are over-expressed in prostate cancers as compared to hyperplasic prostate, with the intriguing observation that the tumor cells themselves rather than myeloid infiltrating cells could be the main source of the enzymes [55]. We recently investigated whether the alteration of L-Arg metabolism in tumor explants could be responsible for the induction of TIL dysfunctions. By culturing small tumor samples in medium containing a combination of ARG- and NOS-specific inhibitors, TIL recover their functions suggesting the presence of a predominant but fully reversible immunosuppressive mechanism based on the altered L-Arg metabolism in prostate cancer [55].

Among various leukocyte populations comprising the tumor stroma, machrophages, primarily derived from circulating monocytes, are certainly among the most represented. Macrophages, recruited by colony stimulating factor-1 (CSF-1) and CC chemokine ligand-2 (CCL2) often secreted by cancer cells, accumulate in the tumor site where they adversely impact both on local inflammation outcome and whole patient prognosis [56,57]. In the great majority of clinical studies, tumor-associated macrophage (TAM) density correlated with a poor prognosis, probably because TAMs are frequently dysfunctional, scarcely immunogenic or actively immunosuppressive [57,58]. In addition to macrophages, a heterogeneous mixture of myelo-monocytic cells at various maturation stages is expanded nearly in all tumor-bearing mice and in many cancer patients. These cells have been defined immature myeloid cells or

myeloid suppressor cell but recently renamed myeloid-derived suppressor cells (MDSC). MDSCs represent an heterogeneous cell population that includes mature and immature myeloid cells such as granulocytes and monocytes/macrophages, as well as immature myelo-monocytic precursors and DCs [5,59]. There is considerable *in vitro* and *in vivo* evidence of a role for MDSCs in mediating suppression of T cell responses in tumor-bearing hosts. The mechanisms underling these effects are probably various and not completely defined but require cell-to-cell contact with antigen-activated T cells, release of both Th1 and Th2 cytokines, and activation of key intracellular enzymes [60]. In this context, a crucial mechanism is based on alteration the L-Arg metabolism. Depending on the tumor histology, disease status, and the genetic background of the host, MDSCs can express either or both NOS2 and ARG1 and tumor masses can profit by over-expression of these enzymes to restrain the function of TILs. Independently of NOS activity, ARG can deplete L-Arg from the microenvironment, thus inhibiting the expression of the ζ-chain of the TCR/CD3 receptor complex [61]. Likewise, NOS can exert its immunosuppressive action through NO production, thus affecting signaling pathways downstream of IL-2 receptor. In addition, when these two enzymes are co-expressed, L-Arg reduced availability results in the switching of NOS activity from the prevalent production of NO to the generation of superoxide and highly reactive nitrogen species (RNS), which can have multiple inhibitory activities on T cells [43]. Cell membranes offer no significant barrier to diffusion to some RNS, such as peroxinitrites, from different compartments, within or between cells [62,63]. We recently demonstrated the presence of high levels of nitrotyrosines in human prostatic TILs, suggesting a local production of peroxynitrites. Nitrotyrosines are, in fact, generated by the nitrosative reaction with peroxynitrites and represent a hallmark of their tissue production. Interestingly, by inhibiting the activity of ARG and NOS reduced tyrosine nitration and restoration of TIL responsiveness to tumor were achieved [64].

Recent evidence is unveiling multiple intersecting branches in metabolic events controlled by COX, ARG and NOS. Among the factors regulating ARG1 expression and function in tumor-infiltrating myeloid cells, for example, COX-2 might play an important role in virtue of its frequent overexpression in different human and mouse tumors, as previously discussed. In a mouse lung cancer model, signaling through the PGE$_2$ receptor in tumor-infiltrating myeloid cells was necessary for ARG1 induction and pharmacological interference by COX-2 inhibitors resulted in ARG1 downregulation and stimulation of an otherwise silent lymphocyte-mediated antitumor response [65]. The connection between COX-2 and NOS2 is even more strict since it was recently demonstrated that NOS2 physically associate to COX-2 and activates it by *S*-nitrosylation of critical cysteine residues [66].

5. Central role of amino acid control in T cell activation

Initially studied in yeasts, response to amino acid deprivation has been recently characterized in mammals. Amino acid starvation causes the accumulation of free, uncharged (deacylated) tRNAs that bind the histidyl-tRNA-synthetase-related domain of the "sensor" general control non-derepressible-2 (GCN2) kinase [67,68]. The conformational change induced by this binding results in activation of the kinase domain and phosphorylation of serine residues in the eukaryotic initiation factor (eIF)2α, one of the key steps in protein translation. Phosphorylation converts eIF2α from substrate to competitor of the subunit eIF2B, which is no longer able to recycle eIF2-GDP to the functional eIF2-GTP, leading to an arrest of protein synthesis [69]. The finality of this reaction is to limit consumption of amino acids in an already deprived environment but the side effect might be a temporary arrest of vital functions for the cells. In yeast, which are able to synthesize all the 20 amino acids, eIF2α phosphorylation also induces translation of GCN4 [70], a transcription factor binding to various promoters of genes involved in essential biosynthetic pathways that are activated by the general amino acid control (GAAC). So translation and transcription of specific genes might coexist with an impaired protein synthesis following GCN2 activation. GCN2 is member of a family of related kinases (including PERK, HRI and PKR) activated by different signals but sharing eIF2α as substrate and downstream events; for this reason, this pathway is also known as integrated stress response (ISR) [71].

Amino acids can also regulate the binding of mRNA to the 43S preinitiation complex, mediated by a heterotrimeric eIF4F complex. A key control step in this translation initiation pathway is the association between the eIF4E-mRNA to the preinitiation complex, which is precluded when eIF4E associates with the eIF4E binding proteins (4E-BP). Hyperphosphorylated 4E-BPs do not associate with eIF4E, thus allowing initiation of translation to occur [72]. Amino acids, and leucine in particular, increase threonin phosphorylation of at least two of the binding proteins, 4E-BP1 and 4E-BP2, through a signal transduction pathway involving the mammalian target of rapamycin (mTOR) but not the PI3-kinase. The serine/threoinine kinase mTOR phosphorylates the 70 kDa ribosomal protein (rp) S6 kinase (p70^{S6k1}), which in turn phosphorylates and activates the eIF4E binding proteins, rpS6, and eIF4B. The mTOR pathway is inhibited by rapamycin, a well-known immunosupressive drug, and integrates different inputs that promote translation and cell division, including hormones (insulin, IGF-1), growth factors, ATP and amino acids availability [73]. The interest in rapamycin has thus extended from immunology to oncology and the drug is being tested as anti-cancer agents, with the aim of blocking mitogenic signals. In mammals and yeasts, amino acid deprivation, rapamycin or the accumulation of poor nitrogen sources, such as urea (so far shown only in yeasts), can turn off the signal pathways controlled by mTOR [73].

One intriguing aspect of the GAAC and ISR is linked to the finding that phosphorylation of eIF2α and GCN2 activity is not enhanced *in vivo* by short-term food deprivation, raising the question about the physiological role of GCN2. Recent evidence seems to suggest that both GCN2 and mTOR might be critical to control T cell proliferation following antigen-mediated activation.

The combined effects of tryptophan deprivation and tryptophan catabolites induced by IDO activation in CD8$^+$ DC and plasmocytoid DC was shown to induce GCN2 kinase. In these

studies GCN2 activation was associated, both *in vitro* and *in vivo*, with proliferative arrest, anergy induction, impaired CTL effector function and down-regulation of the TCR ζ-chain in mouse CD8$^+$ T cells [30,31]. Prolonged activation of tryptophan catabolism by IDO has also a secondary effect, the conversion of naïve CD4$^+$ CD25$^-$ T cells into Foxp3$^+$, CD25$^+$, CD69$^-$, CTLA-4$^+$ *bona fide* Treg cells able to control autoimmune diabetes when adoptively transferred *in vivo* [30]. Generation of Treg cells was dependent on GCN2 and TGF-β. These results, suggesting that CD8$^+$ and CD4$^+$ T cells might sense thrytpophan deprivation in a different way, are extremely intriguing. As reported above, cell cycle limitation and initiation of specific differentiation pathways may coexists and explain the low proliferative rate of Tregs described in some studies.

Similar results were recently reported for L-arginine deprivation. In fact, consumption of L-arginine by ARG1 hyperexpression in tumor-conditioned macrophages can also mediate GCN2 kinase-dependent cell cycle arrest in G_0–G_1 phase and downregulation of the ζ-chain of the TCR/CD3 complex in antigen-activated T cells [61,74]. The anergic status of T cells is associated with the impairment of cyclin D3 and cyclin-dependent kinase 4 upregulation, decreased phosphorylation of retinoblastoma protein and a low expression and binding of E2F1. Despite the analogies between molecular dysfunctions induced by altered L-tryptophan and L-arginine metabolism, some important differences are emerging. For example, the loss of CD3 ζ-chain might be prominent for CD4$^+$ but not for CD8$^+$ T cells activated by the cognate antigens in the presence of tumor-derived MDSCs overexpressing ARG1 and ROS [75], suggesting that metabolic control by amino acid metabolisms may activate both common and selective pathways, depending on the T lymphocyte subset (i.e. CD4$^+$ or CD8$^+$) and, possibly, their functional status (i.e. naive, memory, or effector).

An important advancement in understanding the molecular basis of T cell costimulation was recently reported. Transmission of mitogenic signal from the costimulatory molecule CD28 seems to require an intracellular complex formed by the serine–threonine kinase aurora B, survivin (an inhibitor of apoptosis family protein), and mTOR [76]. This complex was shown to be responsible for the phosphorylation of mTOR substrates, expression of cyclin A, hyperphosphorylation of retinoblastoma protein and activation of cyclin-dependent kinases 1 and 2 and it
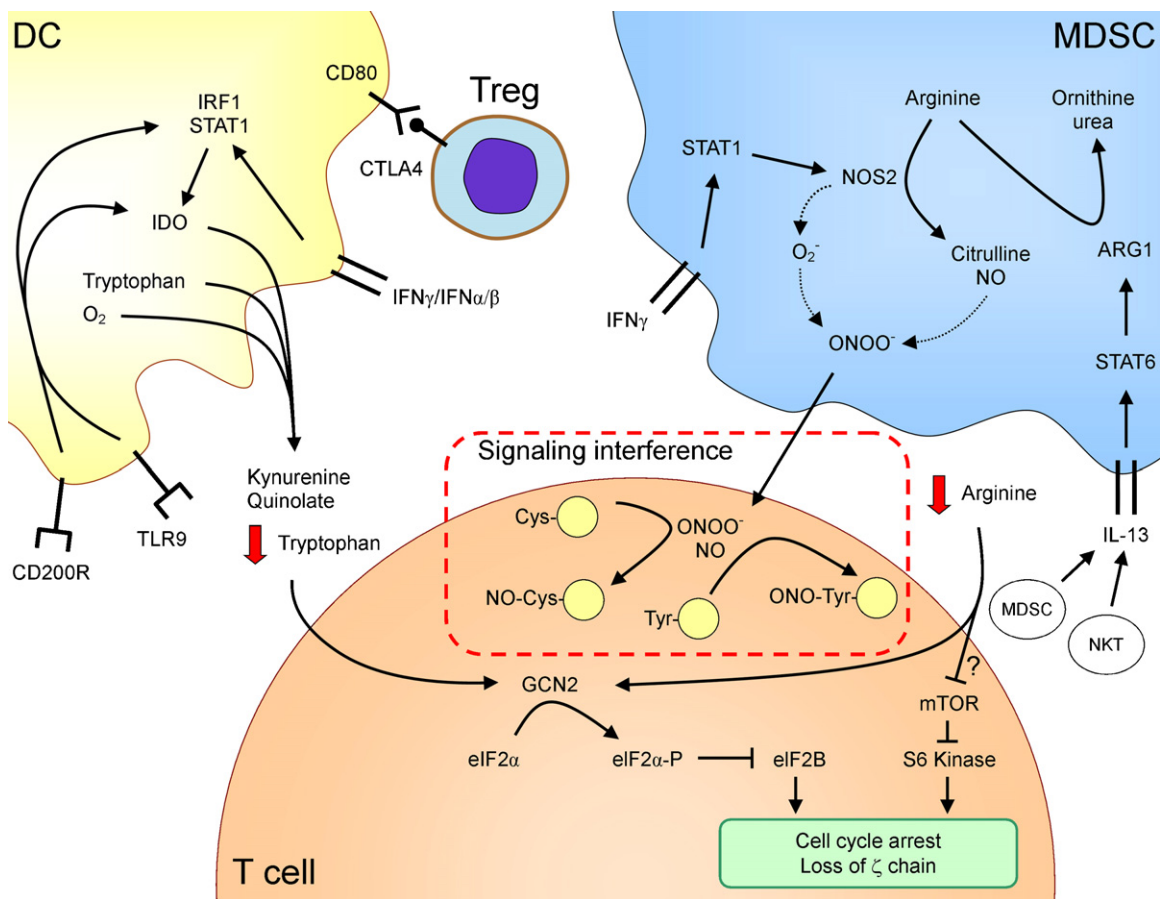

Fig. 1. GCN2 kinase may be crucially involved in the suppression of T cell responses by the tumor microenvironment. GCN2 kinase phosphorylates eIF2α, an event affecting negatively the function of eIF2B and leading to an arrest in protein synthesis and cell cycle progression. GCN2 induction can derive from tryptophan deprivation and/or tryptophan catabolites produced by IDO activity in DC subsets and plasmocytoid DC. Similarly, consumption of L-arginine by ARG1 expression in tumor-conditioned macrophages and MDSCs or in the tumor itself can also mediate GCN2 kinase-dependent cell cycle arrest in G_0–G_1 phase and downregulation of the ζ-chain of the TCR/CD3 complex in antigen-activated T cells. In addition, simultaneous expression of ARG and NOS results in the switching of NOS activity from the prevalent production of NO to the generation of various reactive species, including peroxynitrites (ONOO$^-$), which can have multiple inhibitory activities on T cells, as described in the text. Hypothetically, amino acid deprivation may also block the mTOR-S6 kinase pathway controlling cell division.

was necessary to allow G_1–S transition in antigen-stimulated T lymphocytes. The same signaling pathway is activated by IL-2 [76]. These data provide an explanation for the ability of rapamycin to block T cell proliferation by arresting the G_1–S transition and, possibly, for the effect of amino acid deprivation on T cell proliferation. However, this remains an intriguing hypothesis that will have to be tested experimentally.

6. Clinical perspectives

Immune effector mechanisms can be counteracted by evasive measures displayed by the tumors, and the knowledge of the interplay between tumors and cells of the immune system at the tumor–host interface will provide us with novel insights that might lead to a successful immunotherapy. All the metabolic restraints described above (summarized in Fig. 1) constitute potential checkpoints for tumor-specific T lymphocytes, which need to be considered in designing novel immunotherapeutic approaches. Considering the complex connection between individual metabolic pathways interfering with anti-tumor immune responses, multi-target therapies are the "obligatory choice" for cancer immunotherapy, at least at this stage of our knowledge. This type of approach has been recently investigated in several laboratories and provided promising results in mouse tumor models. NO-donating aspirin was shown to inhibit NOS and ARG activity, both *in vitro* and *in vivo*, and abrogate myeloid cell-dependent suppression *in vivo* [77]. More importantly, in some mouse tumor models, NO-aspirin was shown to synergize with a recombinant cancer vaccine in inducing prevention and even treatment of established tumors [77]. Although the mechanism of NO-donating aspirin action as a modulator of ARG and NOS activity has not been completely clarified yet, these data indicate that NO-releasing drugs might represent a potent immune adjuvant, acting by relieving the suppressive mechanisms negatively affecting T lymphocytes in tumor-bearing hosts.

Phosphodiesterase 5 (PDE5) inhibitors (sildenafil, tadalafil, and vardenafil) can also inhibit ARG and NOS activity in tumor-infiltrating myeloid cells and these compounds were recently tested as potential adjuvants for immunotherapy. PDE5 inhibition enhanced intratumoral T cell infiltration and reverted tumor-induced immunosuppressive mechanisms, thus allowing the spontaneous generation of an anti-tumor immune response that significantly delayed tumor progression in the absence of any immunotherapeutic approach [78]. PDE5 inhibitors are safe and already used in the clinic and this confers to the mouse data a direct link to the clinical translation.

This new approach to fight cancer metabolism will provide a novel class of adjuvants specific for cancer immunotherapy, a novel class of small molecules inhibitors altering tumor-induced tolerance [79]. In contrast to conventional adjuvants, characterized by broad action on the immune system and lack of selectivity, this approach is aimed at aiding and boosting the function of effector anti-tumor T lymphocytes, either spontaneously present or elicited in patients upon vaccination, without exposing patients at risk of systemic deregulation of the immune responses.

Conflict of interest

The authors have no conflicting financial interests.

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