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Review article

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The roles of arginases and arginine in immunity

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Abstract

Arginase activity and arginine metabolism in immune cells have important consequences for health and disease. Their dysregulation is commonly observed in cancer, autoimmune disorders and infectious diseases. Following the initial description of a role for arginase in the dysfunction of T cells mounting an antitumour response, numerous studies have broadened our understanding of the regulation and expression of arginases and their integration with other metabolic pathways. Here, we highlight the differences in arginase compartmentalization and storage between humans and rodents that should be taken into consideration when assessing the effects of arginase activity. We detail the roles of arginases, arginine and its metabolites in immune cells and their effects in the context of cancer, autoimmunity and infectious disease. Finally, we explore potential therapeutic strategies targeting arginases and arginine.

Sections

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Introduction

Arginases (ARGs) are inducible manganese (Mn^{2+})-containing enzymes that catalyse the final step of the urea cycle in the liver, converting L-arginine into L-ornithine and urea, to detoxify ammonia in mammals. Genetic ARG deficiency in humans has profound effects on physiology, resulting, for example, in developmental delay and cognitive problems, as a result of the build-up of ammonia. In addition to their metabolic activity, ARGs can also modulate immune responses in various ways[1,2]; for example, the production of L-ornithine, which is a precursor of proline and polyamines, has effects on cell proliferation, metabolic adaptation, inflammation and extracellular matrix deposition[2]. Mammals express two ARG isoforms, ARG1 and ARG2 (refs. 1,3). ARG1 regulates immune functions in myeloid-derived suppressor cells (MDSCs), activated neutrophils, tumour-associated macrophages (TAMs) and $CD4^+$ T cells. ARG2 is involved in the regulation of T cell effector functions and T cell memory development[4–6]. Furthermore, as our understanding of the roles of ARGs evolves, new evidence suggests that the functions of ARGs are context dependent, as well as cell-type dependent. In this Review, we discuss established and emerging concepts, detailing the differences in cellular restriction and compartmentalization of ARGs between rodents and humans, as well as how ARGs and their metabolites contribute to immune dysfunction in various diseases. We also consider therapeutic approaches targeting ARGs or L-arginine levels to modulate the immune response.

ARG isoforms, regulation and distribution

ARGs are catalytically well-conserved enzymes across species. In addition to their function in the urea cycle in the liver, ARGs are expressed in immune cells, where they fuel the synthesis of proline and polyamines through the production of L-ornithine from L-arginine. Proline is the primary building block for collagen formation[7,8] and is required for extracellular matrix deposition and remodelling[9], whereas polyamines (such as putrescine, spermine and spermidine) are involved in cell proliferation, migration, differentiation and epigenetic control[10]. Ornithine transcarbamylase (OTC) and carbamoyl phosphate synthase 1 convert L-ornithine to L-citrulline (for recycling back to L-arginine), whereas ornithine decarboxylase and ornithine aminotransferase (OAT) convert L-ornithine to polyamines and proline, respectively[11] (Fig. 1). Most immune cells rely on L-arginine import[12,13] or engage autophagy[14] to replenish the intracellular L-arginine pool. Other cell types, such as osteoclasts, use argininosuccinate synthase 1 (ASS1) and argininosuccinate lyase (ASL) to recycle L-arginine from L-citrulline[15]. Exceptions are mouse macrophages and human primary, in vitro-activated $CD4^+$ and $CD8^+$ T cells[16], which can import L-citrulline[17,18] and use it for L-arginine synthesis mediated by ASS1 and ASL.

Extrahepatic ARGs are postulated to compete with nitric oxide synthase (NOS) for the common substrate L-arginine. ARGs have been suggested to prevent the synthesis of nitric oxide (NO) by NOS through several mechanisms: by decreasing L-arginine availability for NOS[19,20]; by uncoupling NOS to produce superoxide, peroxynitrite and NO scavenger[21]; by repressing the translation and stability of the inducible NOS protein (NOS2)[22]; by blocking the activity of NOS2 by producing urea[23] and by sensitizing NOS to its natural inhibitor, asymmetric dimethyl-L-arginine[24]. However, models to study the crosstalk between ARGs and NOS often do not account for the steady-state level of the L-arginine pool, which is dependent on both L-arginine supply and use. A recent study that revisited the concept of competition between NOS and ARGs for L-arginine suggests that ARGs can decrease the extracellular L-arginine pool if supply is finite, which ultimately affects NOS activity[25]. However, when there is a continuous supply of extracellular L-arginine, ARGs cannot affect NOS activity, even when L-arginine concentrations are far below the optimal level for NOS. Thus, the regulation of L-arginine pathways should not be seen as a simplified view of two enzymes sharing a common substrate.

The import of amino acids such as L-arginine across the plasma membrane is mediated by transporters of the solute carrier (SLC) superfamily[26], which are categorized into transporters for neutral, basic or acidic amino acids. Extracellular L-arginine is transported across the cell membrane through the y^+ system of cationic amino acid transporters[27], including SLC7A1, SLC7A2 and SLC7A3, with SLC7A1 being responsible for L-arginine uptake in human T cells[5]. Other members of the G-protein-coupled receptor (GPCR) family, such as GPCR6A, are also involved in L-arginine import[28]. The interplay among the extracellular pool of L-arginine, its cytosolic usage and transport into mitochondria defines the net intracellular pool of L-arginine.

ARG1 and ARG2 catalyse identical enzymatic reactions and have the capacity for reciprocal metabolic compensation. For example, deletion of ARG1 typically results in the compensatory overexpression of ARG2 (ref. 29). However, there are also marked differences between the two enzymes in terms of their subcellular localization, cell-type specificity, isoforms, regulation of expression and implications for disease pathogenesis.

ARG1

In humans, three splice variants of ARG1 have been identified: isoform 1, which comprises 322 amino acids and is mainly expressed in the liver; isoform 2, which comprises 330 amino acids and is expressed in immune cells and erythrocytes; and isoform 3, which comprises 236 amino acids, the function and distribution of which are not yet defined. By contrast, only one ARG1 isoform has been described in mice.

The expression of ARG1 is regulated by pro-inflammatory and anti-inflammatory cytokines. The net effect of these cytokines on ARG1 induction, at both the mRNA level and selection of splice variants, is the result of a tightly regulated balance of transcription factors. For example, T helper 2 (T_H2) cell cytokines, such as IL-4 and IL-13, stimulate the activation of signal transducer and activator of transcription 6 (STAT6), which, together with either CCAAT enhancer-binding protein-β (C/EBPβ) or Krüppel-like factor 4, drives the expression of *Arg1* in mice[30,31] (Fig. 2a). STAT6 also epigenetically modifies the *Arg1* locus, by inducing histone H3 lysine 4 (H3K4) methylation and Jumonji domain-containing protein 3 (JMDJD3)-dependent demethylation at H3K27 (ref. 32), thereby increasing access to the promoter regions and enhancing *Arg1* transcription. The haematopoietic transcription factor PU.1 also induces *Arg1* expression by binding to a specific site near the STAT6–C/EBPβ element in the *Arg1* promoter. A second PU.1 binding site, located 700 bp upstream of the transcription start site, regulates *Arg1* expression in a liver X receptor-α (LXRα)-dependent manner[33]. The transcription factor LXR contributes to the regulation of *Arg1* expression in a ligand-activated manner, mainly through the binding of oxysterols, but LXRα itself does not bind the *Arg1* promoter[33]. Instead, LXRα seems to drive the expression of *Arg1* by promoting the formation of a PU.1–interferon-regulated factor 8 (IRF8) complex and inducing its binding to a site upstream of the transcription start site[33].

In mice, the nuclear receptors peroxisome proliferator-activated receptor-δ (PPARδ) and PPARγ mediate the increase in macrophage ARG1 levels and activity induced by modified low-density lipoproteins (LDLs)[34] (Fig. 2b). In addition, PPARγ and PPARδ facilitate IL-4-induced *Arg1* expression, as the lack of these receptors completely abolishes the

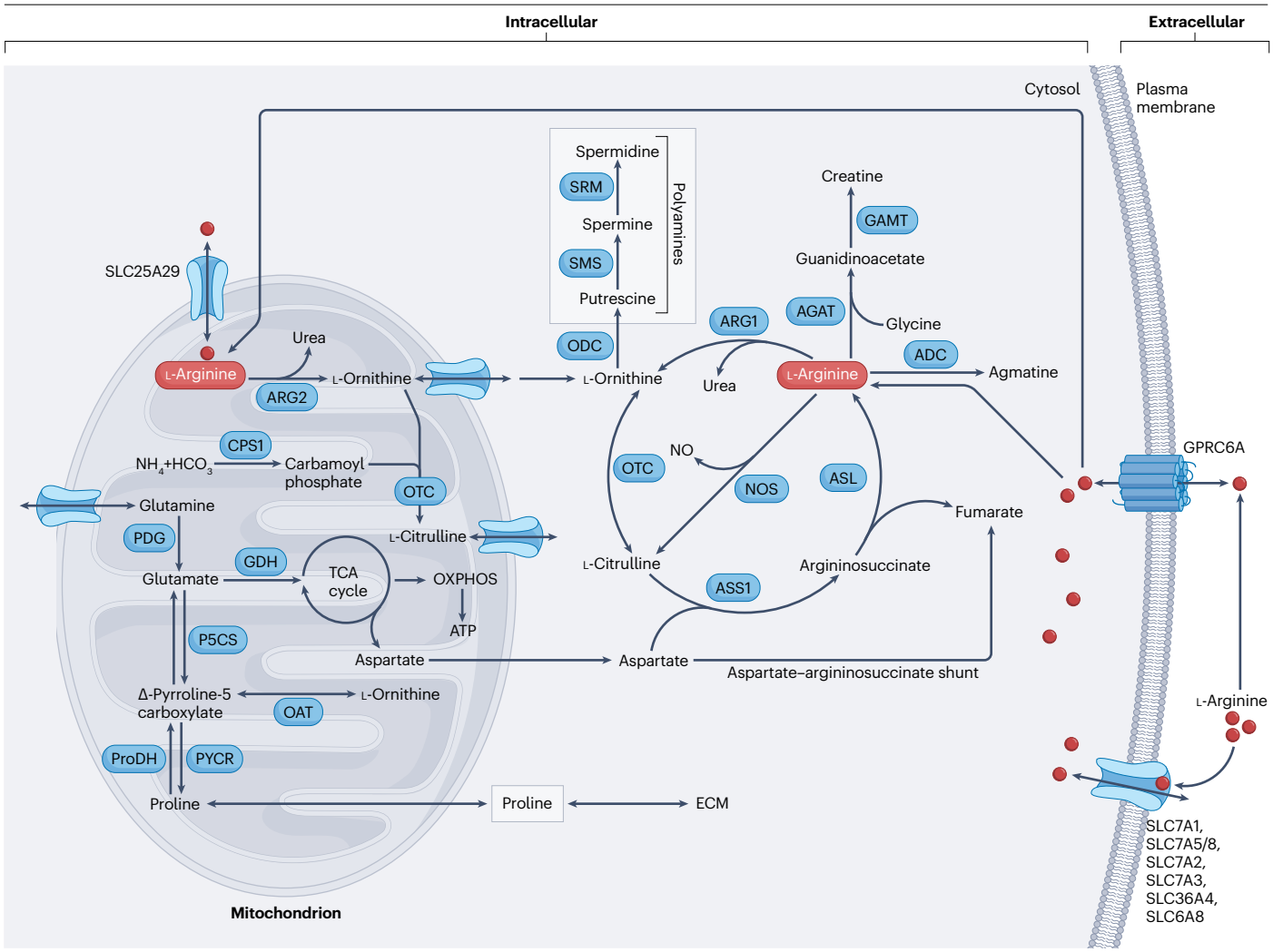


Fig. 1 | Biochemical pathways connecting ARG1, ARG2 and L-arginine and with other metabolic pathways. L-arginine can be transported into cells by two families of transporters, namely, the solute carrier (SLC) transporter superfamily and G protein-coupled receptor 6A (GPRC6A). L-Arginine is hydrolysed to L-ornithine and urea by arginase 1 (ARG1) in the cytosol or transported into the mitochondria, where it becomes the substrate of ARG2. In cancer cells, L-arginine is transported into mitochondria by SLC25A29, although it is not known whether this is also the case for immune cells. In the cytosol, L-ornithine is then converted into the polyamines putrescine, spermine and spermidine, by the sequential actions of ornithine decarboxylase (ODC), spermine synthase (SMS) and spermidine synthase (SRM). Polyamines are important in cell proliferation, cell migration, epigenetic control and cell differentiation. L-Ornithine can also be converted into L-citrulline, which, combined with aspartate, produces argininosuccinate through the activity of argininosuccinate synthase 1 (ASS1). Finally, argininosuccinate is used to regenerate L-arginine (de novo synthesis) and fumarate through the activity of argininosuccinate lyase (ASL). Inside the mitochondria, in addition to the actions of ARG2, L-ornithine can also be generated from glutamine, through the activities of phosphate-dependent glutaminase (PDG), pyrroline-5-carboxylate synthase (P5CS) and ornithine δ-aminotransferase (OAT). Mitochondrial L-ornithine can be shuttled to the cytosol to fuel polyamine synthesis or to reconstitute the pool of L-arginine through the actions of ornithine transcarbamylase (OTC), ASS1 and ASL. Alternatively, through the actions of OAT and pyrroline-5-carboxylate reductase (PYCR), mitochondrial L-ornithine generates proline, the building block of collagen, which is essential for extracellular matrix (ECM) deposition. Conversely, the ECM can be degraded to generate proline and L-ornithine through the activities of proline dehydrogenase (ProDH) and OAT in the mitochondria. Glutamine fuels the mitochondrial tricarboxylic acid (TCA) cycle through the activities of PDG and glutamate dehydrogenase (GDH), which sustains oxidative phosphorylation (OXPHOS) for ATP generation. TCA cycle activity generates aspartate, which is exported into the cytosol, where in combination with L-citrulline and catalysed by ASS1, it generates argininosuccinate; this is a substrate for ASL, the activity of which produces L-arginine and fumarate. This cycle is known as the aspartate–argininosuccinate shunt, connecting the TCA cycle with the urea cycle. Also in the mitochondria, ammonium (NH_4) and carbonic acid (HCO_3) are converted into carbamoyl phosphate by carbamoyl phosphate synthetase 1 (CPS1). Carbamoyl phosphate in combination with L-ornithine is then converted into L-citrulline. In the cytosol, L-arginine is converted into agmatine by agmatine decarboxylase (ADC). In addition, in combination with glycine, L-arginine is converted into creatine by the sequential actions of arginine:glycine amidinotransferase (AGAT) and guanidinoacetate *N*-methyltransferase (GAMT). In the cytosol, L-arginine can also be metabolized into L-citrulline and nitric oxide (NO) by nitric oxide synthase (NOS).

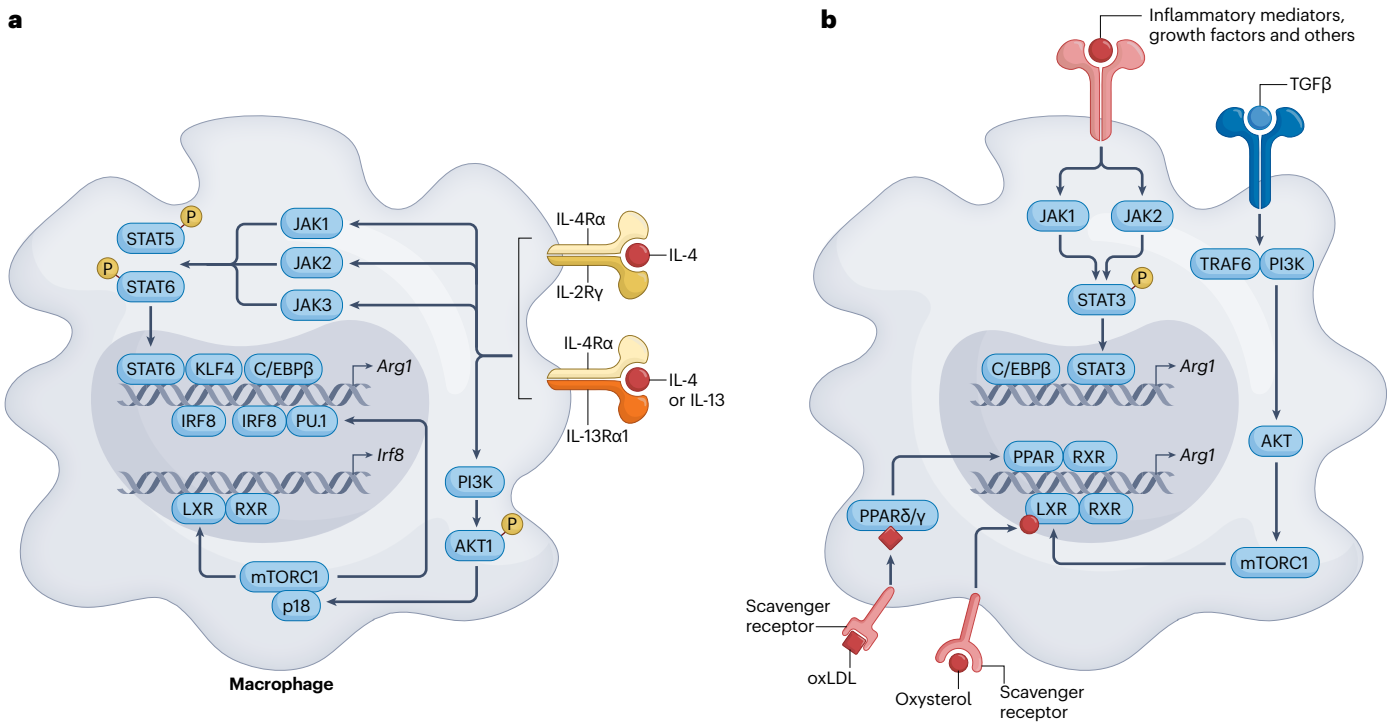


Fig. 2 | Signalling pathways contributing to *Arg1* transcription in macrophages. a, In macrophages, *Arg1* is transcriptionally induced by T helper 2 cell cytokines, such as IL-4 and IL-13. These cytokines signal through receptors composed of IL-4Rα and IL-2Rγ chain, which is specific for IL-4, and IL-4Rα and IL-13Rα1, which binds both IL-4 and IL-13. Signals mediated by these receptors activate Janus kinase 1 (JAK1), JAK2 and JAK3, which in turn phosphorylate signal transducer and activator of transcription 6 (STAT6) and STAT5. Together with CCAAT enhancer-binding protein-β (C/EBPβ) or Krüppel-like transcriptional factor 4 (KLF4), phosphorylated STAT6 binds to the promotor of the *Arg1* gene. Signalling from the same receptors can also activate phosphoinositide 3 kinase (PI3K), which in turn phosphorylates the serine/threonine kinase AKT1 and the mechanistic target of rapamycin complex 1 (mTORC1)–p18 complex. mTORC1 and p18 activate the nuclear transcription factor liver X receptor (LXR), which contributes indirectly to the regulation of *Arg1* expression. LXR binds to the interferon-regulated factor 8 (IRF8) promoter region as a heterodimer with retinoid X receptor (RXR). IRF8 alone or in combination with PU.1 binds to the *Arg1* promoter. **b**, Transcriptional upregulation of *Arg1* can also occur through signals originating from pro-inflammatory cytokines (such as IL-6, colony-stimulating factor 2 (CSF2) and CSF3), growth factors (vascular endothelial growth factor and epidermal growth factor), tumour necrosis factor, interferons, inflammatory mediators (adenosine, Toll-like receptor (TLR) ligands and prostaglandin E_2 (PGE_2)) and the regulatory cytokine IL-10. In these conditions, JAK1 and JAK2 activation result in the recruitment and phosphorylation of STAT3. Phosphorylated STAT3 then binds to specific regions of the *Arg1* promoter together with C/EBPβ. Peroxisome proliferator-activated receptor-δ (PPARδ) and PPARγ — as a heterodimer with RXR — also contribute to the activation of *Arg1* transcription by transducing signals from oxidized low-density lipoprotein (oxLDL). Transforming growth factor-β (TGFβ) was also shown to directly affect *Arg1* expression through the mobilization of LXR by a mechanism dependent on tumour necrosis factor receptor-associated factor 6 (TRAF6), PI3K, AKT and mTORC1 and involving oxysterol binding.

effect of IL-4 (refs. 34,35). To promote gene activation, PPARs bind as heterodimers with retinoid X receptor (RXR) to a peroxisome proliferator response element in the regulatory regions of their target genes[36]. The PPAR heterodimer can be activated by ligands for either PPAR or RXR. For example, the RXR agonist 9-*cis*-retinoic acid increases *Arg1* transcript levels through the *Arg1* peroxisome proliferator response element[34].

Nutrient sensing and metabolic adaptation mediated by mammalian target of rapamycin complex 1 (mTORC1) can also influence the expression of *Arg1* in mouse macrophages. In general, mTORC1 activation, by transforming growth factor-β (TGFβ) and IL-4 or IL-13, positively regulates *Arg1* expression. However, in a mouse model of myeloid lineage-specific constitutive mTORC1 activation, macrophages, in a nutrient-starved environment, were refractory to IL-4-induced polarization with subsequent repression of *Arg1* transcriptional induction[37]. These findings highlight a key role for the mTORC1 pathway in positively regulating *Arg1* and suggest how nutrient sensing and metabolic status might rewire macrophage function.

ARG1 expression can also be upregulated by the anti-inflammatory cytokines IL-10 (ref. 38) and TGFβ, which induce a C/EBPβ isoform that directly binds to the *Arg1* promoter[39]. Finally, the pro-inflammatory cytokines IL-6 and tumour necrosis factor[40,41] can upregulate *Arg1* expression in a STAT3-dependent manner[31,42]. In addition to cytokines, the metabolic milieu can affect *Arg1* expression — for example, lactic acid in the tumour microenvironment (TME) generated by tumour cells as a by-product of aerobic or anaerobic glycolysis increases ARG1 expression in TAMs by stabilizing hypoxia-inducible factor 1α (ref. 43). Thus, *Arg1* transcription can be initiated by different signals to favour homeostasis and repair in the course of inflammatory processes.

In mice, ARG1 is present in the cytosol of macrophages, monocytes and dendritic cells (DCs). However, these cell types in humans do not seem to have ARG1 activity in vivo; instead, neutrophils are the primary

immune cells in humans expressing ARG1 (ref. 44), mainly being present in tertiary granules[45]. Another difference between humans and mice pertains to the cellular compartment in which ARG1 expressed by immune cells exerts its functions. In general, in mice, L-arginine catabolism in immune cells occurs in the cytosol after the transport of the amino acid into the cell. In humans, by contrast, immune cell-expressed ARG1 typically degrades L-arginine in the extracellular environment upon neutrophil stimulation and the subsequent secretion of ARG1-containing tertiary granules[46,47].

ARG2

ARG2 is a mitochondrial enzyme expressed in various cell types in both mice and humans[48]. It is present in numerous tissues, including the kidney, prostate, small intestine, mammary glands, brain and retina[1]. In contrast to ARG1, there is no evidence that ARG2 exists in multiple isoforms. ARG2 has 58% sequence identity with ARG1 (ref. 49) and is nearly identical within the catalytic region. Similar to ARG1, ARG2 catalyses the production of L-ornithine. However, within mitochondria, the catalytic activity of ARG2 also increases levels of the tricarboxylic acid (TCA) cycle metabolites, fumarate and malate, by the upregulation of glucose and glutamine transporters, such as SLC1A5 and SLC2A1, thereby enhancing oxidative phosphorylation through increased activity of complex II of the electron transport chain[50,51]. This finding aligns with observations that activated human $CD4^+$ T cells, which predominantly use ARG2 rather than ARG1, upregulate several intermediates of the TCA cycle in response to increased L-arginine levels[5]. The specific mechanism through which ARG2 influences mitochondrial energy metabolism remains to be determined, as does the mechanism by which mitochondria take up L-arginine in immune cells under steady-state and disease conditions. In the mitochondria of cancer cells, SLC25A29 transporter activity was shown to control the L-arginine pool[52].

ARG2 expression is induced by IL-10 in mouse macrophages[50] and during the maturation of DCs. It is negatively regulated by microRNA-155 (miR-155)[53,54]. In miR-155-knockout mice, ARG2 levels are increased, resulting in the excessive catabolism and depletion of the semi-essential amino acid L-arginine, which impairs T cell proliferation[53]. ARG2 is also induced in fetal DCs during the gestation period to control the L-arginine pool. In response to allogeneic antigens, fetal DCs promote the formation of regulatory T (T_{reg}) cells and inhibit tumour necrosis factor production by T cells through ARG2 activity, which suggests that ARG2 contributes to DC-dependent tolerance in the developing fetus[55]. Along the same lines, $CD71^+$ erythroid cells exert a tolerogenic function through ARG2 activity in neonates, who are more susceptible to infections[56]. ARG2 activity might thus create an immunosuppressive and tolerogenic environment during pregnancy and early life. In T cells, ARG2 is induced during the canonical activation programme and presumably functions as a negative-feedback regulator to avoid exacerbated immune responses by depleting L-arginine[5,57]. T_{reg} cells also express ARG2, which is involved in inhibiting the proliferation of effector T cells to maintain tissue homeostasis[58]. In summary, ARG2 has an important role in mediating immunosuppression, by direct depletion of the L-arginine pool as well as the regulation of mitochondrial dynamics and bioenergetics through its metabolic by-products.

ARG substrates and metabolites

The effects of ARG activity depend on both substrate (L-arginine) consumption and product generation (for example, L-citrulline, L-ornithine and polyamines). As all these molecules have important implications for other metabolic and signalling pathways, their levels are tightly regulated in steady state. Changes in L-arginine, L-ornithine and polyamine levels are observed during cancer, inflammation and autoimmune disease.

L-Arginine

L-Arginine is a semi-essential amino acid that serves as a precursor for the synthesis of L-ornithine, polyamines, proline and NO among others (Fig. 1). Recently, L-arginine has also been shown to have roles in a wide range of cellular processes, such as DNA replication, epigenetic regulation and translation control (Box 1). These functions may have profound implications for immune responses, as well as for immune cell adaptation and plasticity[59]. The main sources of L-arginine in the circulation in steady-state conditions include food intake, direct synthesis from L-citrulline and protein catabolism.

L-Arginine is essential for several cellular processes, including T cell activation, early B cell maturation and osteoclast formation[15]. The absence of L-arginine leads to cell cycle arrest and reduced proliferation of T cells, owing to reduced expression of cyclin D3 and cyclin-dependent kinase 4 (Fig. 3). T cells also downregulate the CD3ζ chain in the absence of L-arginine, which may impair T cell receptor (TCR) signalling[60,61]. However, the inability of T cells to proliferate in the absence of arginine is not caused only by the low-level expression of CD3ζ, because stimulation with phorbol myristate acetate, which bypasses the TCR, fails to restore T cell proliferation[62]. A likely mechanism by which L-arginine scarcity is sensed involves the kinase general control nonrepressible 2 (GCN2). In response to amino acid deficiency, uncharged tRNAs accumulate, leading to the activation of GCN2, which phosphorylates eukaryotic initiation factor 2α. This results in a general suppression of protein synthesis, including CD3ζ (ref. 22). T cells lacking GCN2 had normal CD3ζ levels in the absence of L-arginine and retained the ability to proliferate[63]. Alternatively, L-arginine scarcity sensed by GCN2 might lead to the phosphorylation of F-box protein 22 (FBXO22), which in turn accumulates in the cytoplasm and inactivates (by ubiquitylation) the mechanistic target of rapamycin (mTOR) at Lys2066 in a K27-linked manner, thereby disrupting translation and leading to decreased cytokine production and expression of activation markers, such as CD25 and CD69 (ref. 64) (Fig. 3). Moreover, the development of an immune synapse is adversely affected by L-arginine deficiency. This is correlated with the impairment of post-translational changes in cofilin, an actin-binding protein that is dephosphorylated upon TCR engagement with the antigen-presenting cell. In the absence of cofilin dephosphorylation, CD2 and CD3 do not accumulate in the evolving immune synapse[65].

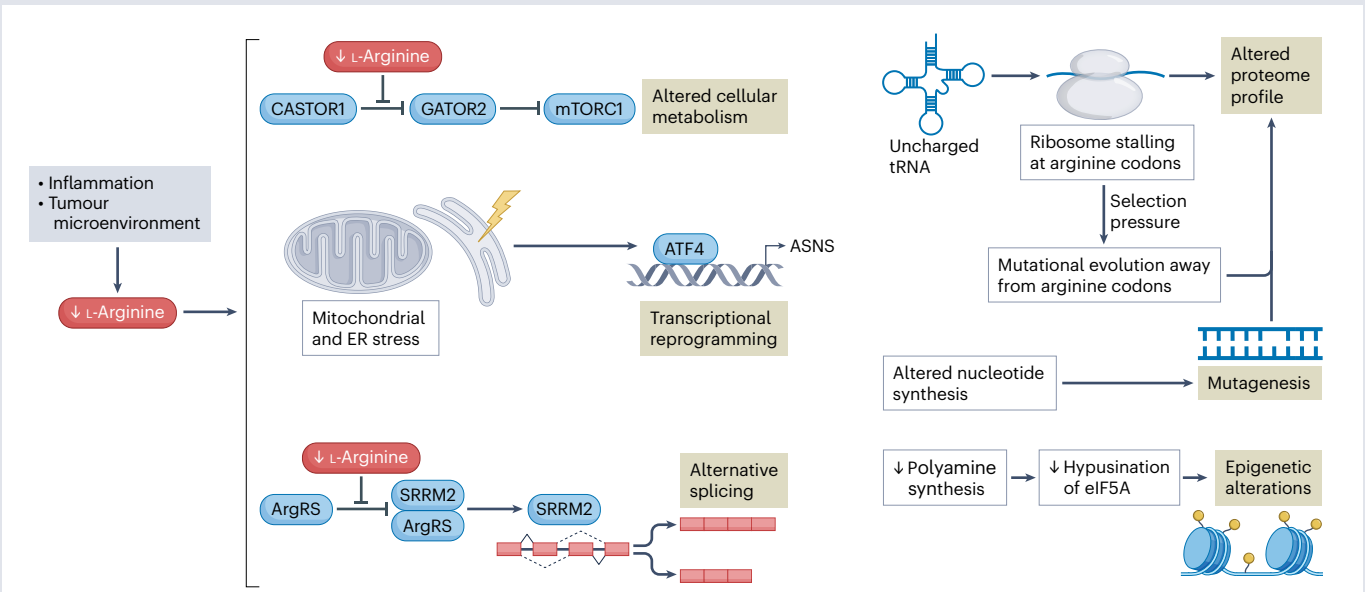
L-Citrulline

L-Citrulline is a non-proteinogenic amino acid that serves as a substrate for L-arginine synthesis. Unlike L-arginine, L-citrulline is not substantially metabolized by the liver[66]. After absorption in the gut, it largely bypasses hepatic metabolism and enters the systemic circulation. L-Citrulline is taken up by the kidneys, where it is converted into L-arginine by the enzymes ASS1 and ASL. This process effectively increases the systemic levels of L-arginine. Because of this difference in the hepatic metabolism of L-arginine and L-citrulline, L-citrulline supplementation is often used to increase systemic L-arginine levels more effectively than direct supplementation of L-arginine itself, which is metabolized by the urea cycle in the liver[67,68].

Some immune cells, although not most, can also convert L-citrulline into L-arginine intracellularly as a rescue strategy to cope with limited amounts of extracellular L-arginine. For example, activated $CD4^+$ and

Box 1 | L-Arginine at the intersection of translational, genetic and epigenetic programmes

In recent years, novel roles of L-arginine have been described with implications for genetic mutation, epigenetic modifications, splice variant selection and protein synthesis (see the figure). Although these findings have been inferred from tumour cells, similar mechanisms could be relevant for immune cells. There is a tightly regulated link between protein synthesis and nutrient availability[231,232]. In a steady-state condition, L-arginine levels are sensed by arginine-tRNA synthetase (ArgRS; also known as RARS1) and serine/arginine repetitive matrix protein 2 (SRRM2), a protein with splicing function that is retained in complex with ArgRS in the nucleus. A decrease in L-arginine levels, as occurs during inflammation and in the tumour microenvironment, reduces the levels of nuclear ArgRS, which allows for the splicing function of SRRM2 to result in changes to the proteome of cells. For example, alternative splice variants of fibroblast growth factor receptor 3 (FGFR3) and pleckstrin homology domain containing family G member 5 (PLEKHG5) are generated[233]. In addition to having a direct effect on protein composition, L-arginine deprivation also affects the genetic codon usage. Mechanistically, L-arginine limitation causes a rapid reduction of arginine-tRNAs, which results in the stalling of ribosomes over arginine codons. Such selective pressure against the translation of arginine codons induces a proteomic shift towards genes containing low levels of arginine codons[59]. These observations imply that over time, cancer cells and, potentially, immune cells growing in an L-arginine-deprived environment might induce mutations by selective pressure on the codon usage such that they lose L-arginine codons and undergo a proteomic shift. Decreased levels of polyamines and hypusinated eukaryotic initiation factor 5A (eIF5A) downstream of L-arginine deficiency cause a marked reduction in levels of H3K27 acetylation on genes such as *Tbx21* and *Gata3* that are necessary for T cell fate commitment. Amino acid levels are also detected by the mechanistic target of rapamycin complex 1 (mTORC1), the master regulator of cellular metabolism. A decrease in L-arginine levels leads to mTORC1 repression by a CASTOR1–GATOR2-mediated mechanism[234]. Considering the crucial role of mTORC1 activation in coordinating a wide range of immune cell functions[235], these findings open a new avenue of investigation in which ArgRS might integrate with mTORC1 pathways in sensing L-arginine levels during cancer and inflammation. Finally, the depletion of extracellular L-arginine in arginine-auxotrophic cancer cells causes mitochondrial and endoplasmic reticulum (ER) stress, which leads to transcriptional reprogramming. For example, L-arginine starvation induces ATF4-mediated upregulation of asparagine synthetase (ASNS), which depletes cancer cells of aspartate and disrupts their malate–aspartate shuttle, which is required for the tricarboxylic acid cycle, oxidative phosphorylation and ATP synthesis. Thus, low levels of L-arginine affect the energy state of the cell[236].



CD8$^+$ T cells, which constitutively express ASL, can upregulate ASS1 to convert L-citrulline into L-arginine, which restores T cell proliferation in the context of L-arginine deficiency[16]. Similarly, macrophages and multinucleated, macrophage-derived cells such as osteoclasts can recycle L-citrulline for endogenous L-arginine synthesis to enhance NO production. This L-citrulline–NO cycle is crucial for the control of mycobacterial and *Listeria monocytogenes* infections[18,69]. Macrophages constitutively express ASL and they can induce ASS1 expression in response to Toll-like receptor (TLR) agonists and interferon-γ (IFNγ)[70]. The induction of ASS1 leads to the depletion of intracellular L-citrulline, which, in this context, functions as a checkpoint for innate immune responses[69].

L-Ornithine

L-Ornithine is a precursor for the synthesis of polyamines and proline and also has immunomodulatory properties. For example, L-ornithine can suppress the function of T cells. During chronic infection of mice

with lymphocytic choriomeningitis virus, a type I interferon-dependent response rewires the expression of urea cycle enzymes within the liver[71]. This leads to reduced circulating levels of L-arginine and an accumulation of L-ornithine, which likely contributes to the suppression of virus-specific $CD8^+$ T cell responses and mitigates liver pathology[71]. In a recent work[72], macrophages synthesizing proline-rich collagen were shown to consume environmental L-arginine and to secrete L-ornithine, which compromised $CD8^+$ T cell function. Daily administration of L-ornithine impaired the immune control of breast tumours in a genetically engineered mouse model, whereas L-arginine administration improved tumour growth control. These findings suggest that ARG activity inhibits T cell function through a dual mechanism: by depleting the semi-essential amino acid L-arginine and by producing the immunosuppressive product L-ornithine. However, in tumours that produce ammonia as a result of the use of amino acids as a carbon source in a glucose-deprived TME, such as breast[73,74] and colon[75] cancers,

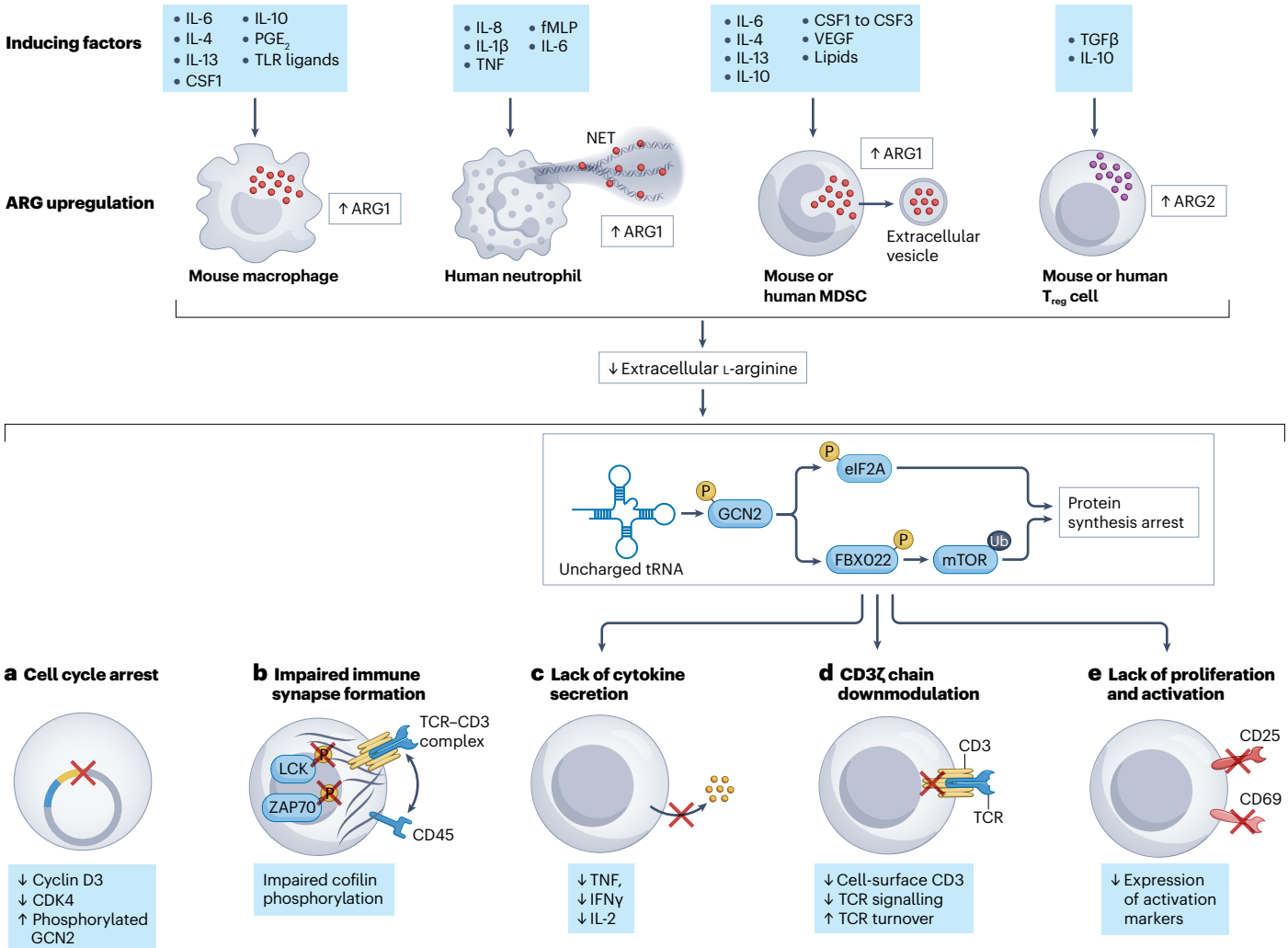


Fig. 3 | ARG1-mediated and ARG2-mediated immunoregulatory mechanisms. Upregulation of *ARG1* and *ARG2* in macrophages, neutrophils, myeloid-derived suppressor cells (MDSCs) and regulatory T (T_{reg}) cells is promoted by a range of overlapping and cell-type-specific factors, such as interleukins, colony-stimulating factors (CSFs), vascular endothelial growth factor (VEGF), tumour necrosis factor (TNF), Toll-like receptor (TLR) ligands, prostaglandin E_2 (PGE_2), lipids and the chemotactic peptide *N*-formylmethionyl-leucyl-phenylalanine (fMLP). As a result of arginase (ARG) upregulation, extracellular levels of L-arginine are decreased, which can affect the effector functions and differentiation of T cells in various ways. **a**, Decreased levels of L-arginine prompt T cells to arrest the cell cycle transition from G0 to G1 by the downregulation of cyclin D3 and cyclin-dependent kinase 4 (CDK4) and upregulation of phosphorylated general control nonrepressible 2 (GCN2). **b**, They also impair immune synapse formation (the clustering of T cell receptor (TCR)–CD3 complex and CD45) by blocking LCK-mediated and ZAP70-mediated phosphorylation of cofilin. **c**, The secretion of cytokines such as interferon-γ (IFNγ), IL-2 and TNF that are essential for T cell effector function and proliferation is decreased as a result of a general suppression of protein synthesis, involving the accumulation of uncharged L-arginine-specific tRNAs that signal to GCN2 to mediate the phosphorylation of eukaryotic initiation factor 2A (eIF2A). L-Arginine scarcity sensed by GCN2 also leads to the phosphorylation of F-box protein 22 (FBXO22), which in turn inactivates (by ubiquitylation) the mechanistic target of rapamycin (mTOR), causing protein synthesis arrest. **d**, GCN2 activation also induces CD3ζ downmodulation, lack of cell-surface CD3 complex, impaired TCR signalling and increased TCR turnover. **e**, Expression levels of CD25 and CD69, which are essential for the proliferation and activation of T cells, are decreased as a result of the suppression of protein synthesis. NET, neutrophil extracellular trap; TGFβ, transforming growth factor-β.

L-ornithine can enhance T cell function by decreasing levels of ammonia, which otherwise drives T cell exhaustion[75]; indeed, L-ornithine is a substrate for glutamine synthesis, which detoxifies ammonia[76].

Polyamines

Polyamines are a heterogeneous group of molecules, including putrescine, spermine and spermidine. They are involved in various biological processes, including cell proliferation, differentiation and survival, gene transcription, proteome profile, autophagy, post-translational modifications, epigenetic modifications and mitochondrial protein synthesis. Polyamines can bind to DNA and RNA, with effects on gene expression and protein synthesis. In addition, they can promote autophagy, modulate the activity of proteins and have antioxidant and anti-inflammatory properties[77,78].

Spermidine induces immunosuppressive properties in macrophages[79,80] by increasing ARG1 expression and by decreasing the expression of IL-6, IL-2, IL-17 and IL-12, which generally sustain activated and autoreactive T cells (Fig. 4). This results in milder disease in a mouse model of experimental autoimmune encephalomyelitis (EAE). Spermidine can also induce immunosuppressive properties in DCs by upregulating the expression of indoleamine 2,3-dioxygenase 1 (IDO1)[81], an enzyme that converts L-tryptophan into kynurenines. The immunosuppressive effect is caused both by the depletion of the essential amino acid L-tryptophan and through direct effects of kynurenines, which inhibit T cell proliferation[82] and increase the differentiation of T_{reg} cells[83]. In conventional DCs, a 'relay' pathway, characterized by the sequential activation of ARG1 and IDO1, can promote a potent immunoregulatory phenotype[84]. In this setting, spermidine

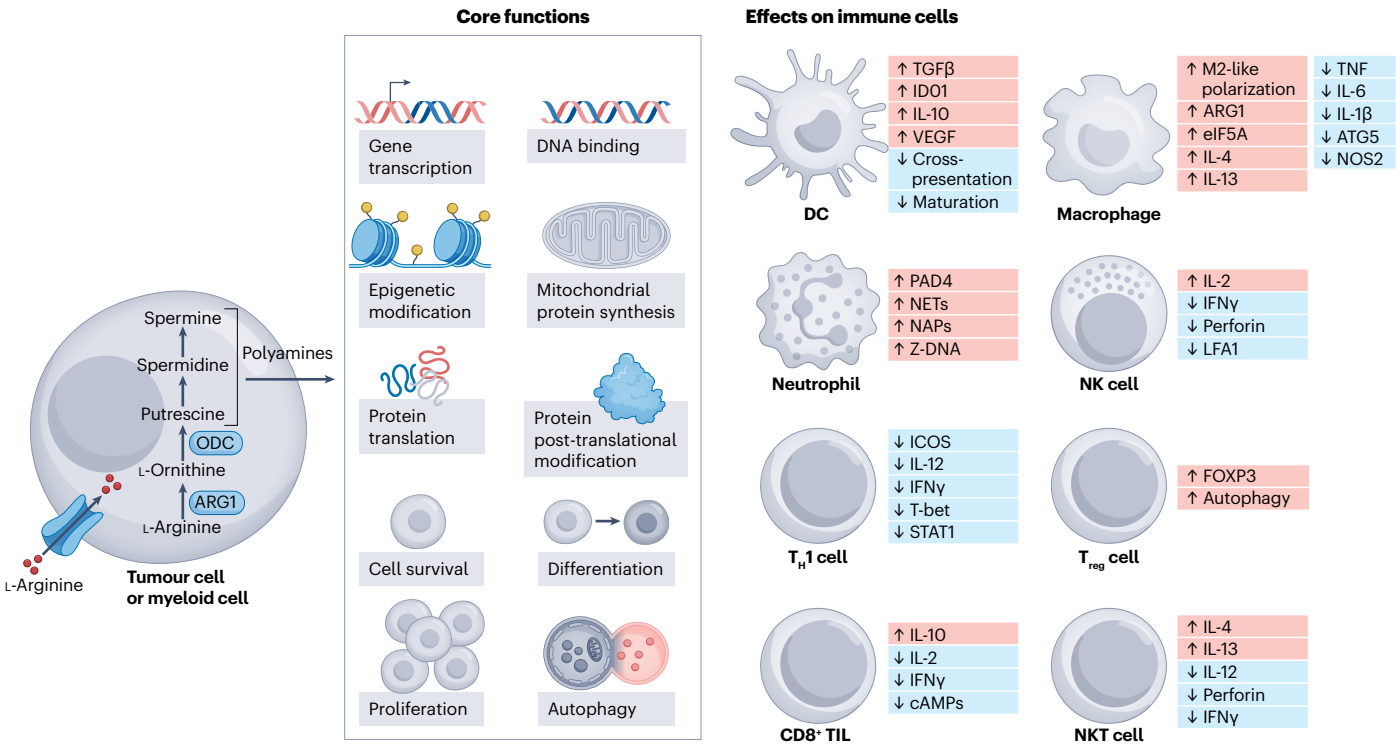


Fig. 4 | Immune-related functions of polyamines. In tumour cells and myeloid cells, activation of arginase 1 (ARG1) leads to the production of L-ornithine from L-arginine, from which polyamines (putrescine, spermidine and spermine) are produced by the rate-limiting enzyme ornithine decarboxylase (ODC). Polyamines regulate several core cellular functions, such as DNA binding, transcription, translation, epigenetic modifications, protein post-translational modification (through hypusination), proliferation, autophagy, mitochondrial protein synthesis and cell survival and differentiation. Through these functions, polyamines can affect the activity of many types of immune cell, such as dendritic cells (DCs), macrophages, neutrophils, natural killer (NK) cells, T helper 1 (T_H1) cells, regulatory T (T_{reg}) cells, $CD8^+$ tumour-infiltrating lymphocytes (TILs) and natural killer T (NKT) cells. For example, in DCs, polyamines stimulate the production of transforming growth factor-β (TGFβ), IL-10 and vascular endothelial growth factor (VEGF) and activate indoleamine 2,3-dioxygenase 1 (IDO1), while repressing antigen cross-presentation and maturation of these cells. In macrophages, polyamines drive M2-like polarization and expression of ARG1, eukaryotic initiation factor 5A (eIF5A), IL-4 and IL-13, while decreasing the expression of inflammatory mediators (tumour necrosis factor (TNF), IL-6 and IL-1β), nitric oxide synthase 2 (NOS2) and autophagy related 5 (ATG5). In neutrophils, polyamines favour protein arginine deiminase 4 (PAD4) expression and consequent neutrophil extracellular trap (NET) formation, increase levels of Z-formation DNA (Z-DNA) and, by binding to DNA phosphate groups, favour the accumulation of nuclear aggregates of polyamines (NAPs). In NK cells, polyamines promote IL-2 secretion and downmodulate expression of interferon-γ (IFNγ), perforin and the integrin lymphocyte function-associated antigen 1 (LFA1). Polyamines repress the acquisition of a T_H1 cell phenotype as they negatively regulate the expression of T-box expressed in T cells (T-bet), signal transducer and activator of transcription 1 (STAT1), IFNγ, IL-12 and inducible T-cell co-stimulator (ICOS, also known as CD278); by contrast, polyamines promote the acquisition of a T_{reg} cell phenotype by inducing the expression of forkhead box P3 (FOXP3) and promoting autophagy. In $CD8^+$ TILs, polyamines favour the contraction of the T cell response by promoting IL-10 expression and decreasing IFNγ, IL-2 and cAMP levels. Finally, in NKT cells, polyamines promote the release of immunomodulatory cytokines, such as IL-4 and IL-13, and constrain the expression of perforin, IL-12 and IFNγ.

produced downstream of the ARG1-dependent cascade triggers IDO1 phosphorylation and signalling activity[81]. Polyamines affect MDSCs through a mechanism involving the V-domain suppressor of T cell activation (VISTA). VISTA increases polyamine synthesis, which promotes mitochondrial respiration and the proliferation of MDSCs[85]. Cell-intrinsic polyamine synthesis is also important to sustain the proliferation of T cells[86], including the pathogenic T_H17 cells that drive EAE[87]. In this model, inhibition of ornithine decarboxylase (which converts L-ornithine to putrescine), resulting in a decrease in polyamine synthesis, alleviates EAE[87]. Thus, both spermidine supplementation and inhibition of spermidine synthesis can have immunoregulatory effects in different contexts.

Spermidine is a substrate for the post-translational modification of eukaryotic initiation factor 5A (eIF5A), referred to as hypusination. Hypusinated eIF5A promotes the expression of a subset of mitochondrial proteins of the TCA cycle and oxidative phosphorylation in macrophages, sustaining an alternatively activated, M2-like macrophage state[88,89]. In addition, polyamine metabolism and eIF5A hypusination can rewire the TCA cycle and affect epigenetic patterns in $CD4^+$ T helper cells, ultimately controlling their development and function; loss of polyamines or hypusinated eIF5A causes a marked reduction in levels of H3K27 acetylation on genes such as *Tbx21* and *Gata3* that are necessary for T cell fate commitment[88].

Polyamines can support anticancer immune responses by promoting autophagy, which is required for T cell function and survival[90]. However, the immunosuppressive effects of polyamines may contribute to multiple mechanisms by which cancer cells escape immune responses. In response to the inhibition of polyamine biosynthesis, T cells can rapidly restore the intracellular polyamine pool through a compensatory increase in extracellular uptake. Simultaneously blocking both polyamine synthesis and uptake efficiently depletes the intracellular polyamine pool, leading to inhibition of T cell proliferation and reduced inflammatory responses[91]. This indicates the potential therapeutic value of targeting the polyamine pool for managing inflammatory and autoimmune diseases[91].

Effects of ARG activity in immune cells

Extrahepatic ARG1 is found in many types of immune cell but mice and humans differ markedly in cell-type distribution. Although human and mouse alternatively activated, M2-like macrophages generated in vitro produce ARG1, it is currently debated whether ARG1 is expressed in vivo by human macrophages, possibly in a tissue-specific and cell subset-restricted manner. By contrast, mouse macrophage subsets expressing ARG1 have been identified in vivo in the context of cancer. Neutrophils are the main immune cell source of ARG1 in humans, but mouse $Ly6G^+$ neutrophils — apart from polymorphonuclear (PMN)-MDSCs and tumour-associated neutrophils — do not express ARG1 under steady state.

Macrophages

There is a large body of work on the effects of L-arginine metabolism in mouse macrophages, which can be generalized in terms of the opposing effects of NO-producing (NOS2-dependent), pro-inflammatory (M1-like) macrophages and L-ornithine-producing (ARG1-dependent), alternatively activated (M2-like) macrophages[92,93]. Nonetheless, the importance of L-arginine metabolism in human macrophages, as well as the physiological relevance of the M1–M2 dichotomy observed in vitro, is still a debated issue. Although single-cell RNA-sequencing data have enhanced our understanding of macrophage polarization as a continuum of context-dependent states, the M1 and M2 classification framework has, nevertheless, been useful in elucidating the regulation and functions of ARG1. However, basing the classification of activated macrophages on polarization of L-arginine metabolism is an oversimplification as activated macrophages may express neither ARG1 nor NOS2 (ref. 94) or may use both pathways concomitantly, as observed in mouse macrophages after stimulation with lipopolysaccharide[95].

Under resting conditions, little L-arginine is used by mouse myeloid cells owing to a lack of expression of high-affinity cell membrane transporters. In addition, in the absence of immune stimulation, myeloid cells do not express NOS2 and ARG1 (ref. 96). Thus, dietary L-arginine supplementation does not affect myeloid cell function in the absence of disease. L-Arginine transport into myeloid cells increases markedly after stimulation, mainly due to the upregulation of expression of high-affinity, cationic amino acid transporters[97], which are co-induced with the L-arginine-metabolizing enzymes[98].

T_H1 cell cytokines promote M1-like macrophage polarization, stimulating the expression of NOS2 and inhibiting the expression of ARG1, which is typically induced by T_H2 cell cytokines. This activity increases the pro-inflammatory and microbicidal properties of M1-like macrophages against intracellular pathogens and malignant cells[99,100]. Inflammatory cytokines such as IFNγ, IFNα, IFNβ and IL-1 activate transcription factors including NF-κB, AP1, IRF1 and STAT1 (ref. 101) that upregulate NOS2 expression, as well as the expression of L-arginine transporters and enzymes essential for synthesizing NOS2 cofactors[70].

M2-like macrophages predominantly catabolize L-arginine to L-ornithine in an ARG1-dependent manner. The T_H2 cell cytokines IL-4 and IL-13 promote ARG1 upregulation[31,41], as do autocrine cytokines such as IL-10, IL-6 and colony-stimulating factor 2 (ref. 42), as well as TGFβ, prostaglandin E2, catecholamines, cAMP and TLR agonists[102,103] (Fig. 2). The functions of M2-like macrophages are, in part, mediated by the synthesis of L-ornithine, proline and polyamines[104,105]. Overall, these biochemical pathways regulate humoral immunity, anti-parasite responses, allergy, fibrosis and wound repair processes, as reviewed elsewhere[106]. For example, in vitro pharmacological inhibition of ARG1 and macrophage-specific deletion of ARG1 indicate that ARG1-expressing macrophages are crucial for matrix deposition and wound healing[107], potentially as a result of increased proline synthesis and collagen production[108]. In line with this notion, the metabolism of L-arginine in wounds has a biphasic pattern: an early burst of microbicidal NO synthesis precedes a drop in L-arginine concentration and an increase in L-ornithine and proline synthesis to promote wound healing[109,110]. In breast cancer, collagen-producing TAMs were shown to restrict the antitumour $CD8^+$ T cell response by a dual mechanism: they generate a physically stiff matrix that is inaccessible to $CD8^+$ T cells and they deplete L-arginine that is required to support T cell proliferation[72].

The expression of ARG2 in macrophages may also increase L-arginine catabolism[111], although this is not mediated by the classic cytokines affecting macrophage function and ARG2 is not the predominantly active enzyme in these cells[112,113]. The roles of creatine and agmatine, which are both downstream metabolites of L-arginine, remain poorly defined in macrophages and it is not known whether immune cells produce their own agmatine or obtain it from extracellular sources. However, recent studies indicate that the uptake of creatine in macrophages promotes chromatin remodelling and subsequent IL-4–STAT6-dependent ARG1 expression, resulting in the suppression of M1-like polarization[114]. Similarly, agmatine promotes ARG1 expression in vivo[115] and suppression of the M1-like phenotype in vitro[116].

Box 2 | ARG1 in neutrophil extracellular traps

Neutrophil extracellular traps (NETs), which evolved to protect against microbial infection, are formed by a web-like structure of DNA that is decorated with antimicrobial effectors, proteins (such as myeloperoxidase, metalloproteinase 9 and cathepsins) and modified histones (such as citrullinated histone 3)[237]. Owing to their potent inflammatory functions, NETs also cause tissue damage and can promote and/or aggravate inflammatory diseases; thus, their induction, release and degradation must be tightly regulated[238]. In cancer, NET formation has been linked to the awakening of dormant tumour cells, chronic stress[239], increased risk of metastasis[240] and immunosuppression[126]. In individuals with cancer, neutrophils are constitutively activated by high levels of circulating inflammatory cytokines (such as IL-8 and tumour necrosis factor). In humans, full-length arginase 1 (ARG1) is released by activated neutrophils in NETs, where ARG1 is cleaved by the cysteine protease cathepsin S (CTSS) at the N-terminal region, generating molecular forms of 31 kDa and 25 kDa. These cleaved forms are active at physiological pH and have greater immunosuppressive function than full-length ARG1 (ref. 126). Antibody-mediated inhibition of CTSS or ARG1 relieves the immunosuppression caused by various ARG1 variants and restores T cell proliferation. NETs form stable, enzymatically active aggregates that preclude the spread of ARG enzymes into surrounding normal tissue but allow them to affect the local concentration of nutrients, metabolites and other mediators. Thus, ARG1 in NETs might rapidly decrease the local concentration of L-arginine below a threshold sufficient to inhibit effector T cell functions.

Neutrophils

Neutrophils are the first immune cells to enter damaged sites from the circulation; their rapid recruitment in large numbers is crucial for their protective function[117], having important roles in inflammation and cancer[118,119]. Similar to macrophages, neutrophils have wide phenotypic and functional diversity. This heterogeneity extends to the bone marrow, where granulocytic progenitor cells differentiate sequentially into precursor, immature and mature neutrophils, each of which is characterized by distinct functional capabilities and transcriptional programmes.

Neutrophils transcribe *ARG1* only during the early stages of differentiation in the bone marrow. Subsequently, neutrophils enter the circulation with ARG1 protein stored inside their tertiary granules, whereas *ARG1* mRNA is no longer detectable. However, in some settings, neutrophils and PMN-MDSCs may restart *ARG1* transcription to replenish their granules. For example, this was observed in tumour-associated neutrophils in non-small-cell lung cancer (NSCLC)[120]. In blood-circulating cells from individuals with head and neck cancer (HNC) or NSCLC, the upregulation of *ARG2* expression occurs in parallel with increased fatty acid and lipoprotein metabolism, which are associated with the suppressive functions of PMN-MDSCs[121]. However, the functional relevance of ARG2 in neutrophils needs to be demonstrated, given that ARG1 is the more prevalent of the two isoforms in both neutrophils and PMN-MDSCs. *ARG1* and *ARG2* transcription could thus be regulated by different signals.

Endoplasmic reticulum (ER) stress was associated with altered lipid metabolism and ARG-dependent immunosuppression in neutrophils from individuals with NSCLC or HNC[122,123]. In individuals with NSCLC, as well as in a mouse model of pancreatic ductal adenocarcinoma (PDAC) and a transplantable mouse model of lymphoma, immunosuppressive neutrophils were characterized by their low density and increased expression of genes associated with the ER stress response, such as C/EBP-homologous protein (CHOP), X-box-binding protein 1 (XBP1), binding-immunoglobulin protein (BIP) and AMP-dependent transcription factor (ATF4)[124]. Induction of ER stress in neutrophils upregulated the expression of lectin-like oxidized LDL receptor 1 (LOX1), a scavenger receptor involved in lipid metabolism, and in individuals with NSCLC or HNC, $LOX1^+$ neutrophils had higher levels of ARG1 expression than $LOX1^-$ neutrophils and were associated with immunosuppressive activity[122]. Interestingly, ARG1 released from activated neutrophils can induce the apoptosis of cancer cells by causing ER stress downstream of L-arginine deprivation[125].

ARG1 inside neutrophil tertiary granules is inactive at physiological pH, becoming active only upon its release into the extracellular environment and activation by components of neutrophil primary granules[47]. Although ARG1 exocytosis can be induced by various stimuli, only those causing the release of both primary and tertiary granules drive the activation of extracellular ARG1 at physiological pH[47]. For example, some stimuli result in ARG1 exocytosis and its inclusion in neutrophil extracellular traps (NETs) (Box 2); within NETs, cathepsin S, which is stored in primary granules and released during the process of NETosis, cleaves ARG1, which increases its enzymatic activity and its ability to consume L-arginine in the extracellular environment[126].

T cells

Metabolic adaptation of T cells in the TME is essential for an effective antitumour response. In the TME, degradation of L-arginine by extracellular ARG1 that is released by various elements of the tumour, such as neutrophils and macrophages, deprives T cells of an essential amino acid, therefore interfering with T cell survival and effector function[95,127]. In contrast to myeloid cells, in which ARG1 is the main enzyme for L-arginine hydrolysis, in T cells, ARG2 has been identified as the isoform responsible for the metabolism of L-arginine, which supports $CD4^+$ T cell activation, survival and acquisition of the effector memory phenotype[5]. In vitro, proteomic and genetic deletion studies have shown that the L-arginine–ARG2 axis regulates glycolysis and mitochondrial respiration, as well as T cell survival, by altering the activity of transcriptional regulators such as BAZ1B, PSIP1 and translin[5]. However, it remains unknown how L-arginine alters the function of these regulators and how this results in increased T cell survival in vivo.

Although ARG2 is the main isoform in T cells, recent evidence shows that during an influenza virus infection, lung $CD4^+$ T cells upregulate ARG1 (ref. 6). Ablation of *Arg1* in mouse $CD4^+$ T cells accelerated virus-specific T_H1 cell-mediated effector responses, resulting in efficient viral clearance[6]. ARG1-generated L-ornithine ensures optimal glutamine flux into the TCA cycle. In the absence of ARG1, glutamine is used instead for ornithine and polyamine generation. This glutamine shunt reduces TCA cycle activity, which minimizes tissue damage caused by the T_H1 cells while maintaining effective viral clearance. Interestingly, the function of ARG1 in $CD4^+$ T cells cannot be compensated by ARG2, suggesting that they are not functionally redundant[128]. Concerning ARG2 expression, mouse *Arg2*-deficient T cells have higher intracellular levels of L-arginine and increased

survival, whereas their proliferation is unaffected. Notably, *Arg2*-deficient T cells have increased antitumour activity in vivo[57]. This implies that the canonical induction of ARG2 in activated $CD8^+$ T cells functions as a negative-feedback mechanism to inhibit T cell effector function. Thus, increased levels of L-arginine favour T cell survival and antitumour effector function by maintaining a memory phenotype. Mechanistically, L-arginine steers the acquisition of a memory phenotype in T cells by inhibiting cellular glucose uptake and shifting to mitochondrial oxidative metabolism through the concerted activities of BAZ1B, translin and PSIP1 transcription factors[5].

Role of ARGs and L-arginine in disease

Variations in ARG activity alter L-arginine bioavailability, which in turn leads to an imbalance in the synthesis of downstream metabolites, such as polyamines and proline. The ensuing dysregulation of protein synthesis can affect several biological responses, including, but not limited to, fibrosis, cell proliferation and metabolic adaptation in immune cells during cancer, autoimmune disease and infectious disease. L-Arginine bioavailability not only depends on ARG activity but is also affected by a deficiency in L-arginine transporters or a lack of L-arginine-specific tRNA.

Cancer

ARG expression is upregulated across many tumour types, leading to increased L-arginine catabolism. This creates an immunosuppressive environment, through scarcity of L-arginine (which is required for T cell proliferation), and fuels the synthesis of polyamines, proline and collagen, which contribute to tumour growth[129,130]. For example, in a cell-autonomous manner, mutation of the tumour suppressor protein p53 has been shown to increase ARG1 levels and polyamine synthesis necessary for tumour growth[131]. In various types of cancer, ARG levels increase with tumour grade and stage, which correlates with a worse prognosis[132]. ARG1 is mainly produced by myeloid cells but can also be present in cancer cells, whereas ARG2 is found in cancer cells and cancer-associated fibroblasts[133]. MDSCs tend to be more abundant in the peripheral blood of individuals with cancer than in tumour-free individuals[134,135], and ARG1 levels are increased in the plasma and increase further with tumour stage[134,136]. This may be caused in part by increased levels of IL-8, which induce neutrophils to exocytose ARG1 (ref. 136). Cancer cells can also release ARG1-containing extracellular vesicles, which can be isolated from the plasma. These vesicles are transported into the draining lymph nodes, where they are taken up by DCs and inhibit antitumour T cell responses[137].

Numerous studies have associated the presence of ARG1-expressing MDSCs in tumours or ARG expression in general with reduced T cell infiltration of tumours and unfavourable prognosis[61,133,138–141]. Additional studies have provided evidence for a causal link between ARG1 expression and immunosuppression in tumours. For example, genetic deletion of *Arg1* in TAMs in an autochthonous mouse model of pancreatic cancer increased $CD8^+$ T cell infiltration and delayed invasive disease[29]. Genetic deletion of *Arg2* in cancer cells in an orthotopic transplant model of obesity-driven PDAC suppressed tumorigenesis[142]. Furthermore, ARG inhibitors or L-arginine supplementation can restore T cell functions in different tumour models[143], as discussed later in the text.

Autoimmunity and inflammation

The management of autoimmune disease typically involves immunomodulatory therapies that aim to reduce inflammation. Given that increased levels of L-arginine enhance T cell activity, reducing L-arginine availability is a potential strategy to treat T cell-mediated autoimmune disorders. For example, psoriasis is an autoimmune disorder of the skin that is largely driven by T cells. In a mouse model of induced psoriasis-like dermatitis, administration of ARG1 led to a notable decrease in skin acanthosis and inflammatory markers[144], which suggests that L-arginine depletion has therapeutic potential for psoriasis. Following spinal cord injury, T cells can infiltrate the central nervous system and cause autoimmune-like neuroinflammation. In a mouse model of spinal cord injury that triggers neuroinflammation, systemic administration of recombinant ARG1 reduced T cell infiltration in the spinal region and improved locomotor recovery[145]. Rheumatoid arthritis is a chronic autoimmune disease characterized by the destruction of synovial joints, involving B cells and T cells, as well as osteoclasts. L-Arginine levels are increased in the synovial fluid of individuals with rheumatoid arthritis and positively correlate with increased levels of the pro-inflammatory cytokines IL-1β, IL-6 and IL-8 (ref. 146). An L-arginine-free diet or blocking the cellular uptake of L-arginine using D-arginine suppressed arthritis progression in a mouse model of collagen-induced arthritis[146]. In addition, the administration of recombinant ARG1 to these mice led to a reduction in symptoms, likely by hindering osteoclast formation as well as potentially attenuating T cell responses[15]. These examples illustrate how L-arginine depletion can reduce T cell activity and autoimmune symptoms.

However, although ARG1 is generally considered to be a negative regulator of immune responses, it may paradoxically promote autoimmune disease under certain conditions. For example, in EAE, there is a marked upregulation of ARG1 in the spinal cord, which contributes to disease progression. Inhibition of ARG1 by systemic administration of amino-6-boronohexanoic acid (ABH) causes delayed onset and milder course of the disease[147]. A potential explanation for this is that the accumulation of L-arginine that occurs when ARG1 is inhibited serves as a substrate to produce NO, which in turn inhibits the differentiation of EAE-promoting T_H17 cells. Mechanistically, NO binds covalently to tyrosine residues on the transcription factor retinoic acid-related orphan receptor-γt (RORγt), through a process known as nitration, which inhibits the activation of the *Il17* promoter[148]. Alternatively, NO can inhibit T_H17 cell differentiation by reducing the expression of aryl hydrocarbon receptor, which is a positive regulator of T_H17 cell formation[149]. Inhibition of ARG1 also ameliorates disease symptoms in a preclinical model of systemic lupus erythematosus (SLE), a chronic autoimmune disease that affects various parts of the body. Individuals with SLE have an increased frequency of T_H17 cells, which likely contributes to disease progression[150]. MDSCs from individuals with SLE were found to promote T_H17 cell differentiation in an ARG1-dependent manner and to exacerbate disease progression in mouse models[151]. These findings suggest that the roles of L-arginine and ARGs in autoimmune disease are context dependent and that, in T_H17 cell-mediated autoimmune diseases, L-arginine depletion strategies may not be indicated.

Infectious disease

Although an early inflammatory response is a hallmark of sepsis, immunosuppression occurs both at early and late stages of the disease. Initially aiming to resolve or limit the negative consequences of excessive inflammation, immunosuppression can prevail in some individuals. Survivors of sepsis frequently experience long-term clinical outcomes and can manifest a metabolic syndrome characterized by persistent inflammation and immunological suppression[152,153]. The rapid

activation and proliferation of immune cells (such as myeloid cells and T cells) during inflammation induce a massive demand for L-arginine that exceeds supply, leading to hypoargininaemia, which is a common observation in adults and children with sepsis. A decrease in the L-arginine-to-L-ornithine ratio suggests that increased ARG1 activity is a key determinant of L-arginine consumption[154,155]. Furthermore, in individuals with sepsis, ARG1-expressing low-density neutrophils are found in the circulation, with their numbers proportional to disease severity. They actively metabolize L-arginine and suppress T cell proliferation[156] causing immune dysfunction.

A low L-arginine-to-L-ornithine ratio has also been reported in individuals with COVID-19 or children with multisystem inflammatory syndrome, reflecting an increase in ARG activity[157]. Indeed, ARG1 levels are increased in the plasma of individuals with COVID-19 (refs. 158,159), and peripheral blood contains increased numbers of monocytes and neutrophils, often associated with disease severity[160,161]. The immunosuppressive activity of circulating myeloid cells in individuals with COVID-19, including a $CD14^+$ fraction of cells, partially depends on the expression of ARG1 (ref. 162). Interestingly, increased numbers of $STAT3^+ARG1^+CD14^+$ immunosuppressive cells have also been described in individuals with PDAC[40,163], which is suggestive of a common deviation in emergency granulopoiesis that leads to the emergence of atypical $ARG1^+$ monocytes in humans in both infection and cancer. Neutrophil activation and NETosis are involved in the pathology of lung immunothrombosis and severe acute respiratory syndrome in individuals with COVID-19 (ref. 164); the contribution of NET-associated, activated ARG1 has not yet been addressed in this context but ARG1 was among the most highly upregulated proteins in lung tissue from individuals with COVID-19 with increased venous thromboembolism, when compared with postmortem lung tissue from individuals with influenza[165].

In parasitic infections, ARG1 controls T cell proliferation in a tissue-specific manner[166]. *Schistosoma mansoni* is a blood fluke whose eggs can lodge within host tissues, mainly in the liver. T_H2 cell-driven immune responses to these eggs can lead to chronic inflammation, which results in schistosomiasis and fibrosis. In this context, ARG1-expressing macrophages are crucial mediators of immune downmodulation. A failure to adequately activate immunosuppressive mechanisms, such as in mice with a macrophage-specific knockout of *Arg1*, causes severe disease owing to uncontrolled inflammation in the liver and gut[167]. Conversely, deletion of *Arg1* in macrophages had no effect on T_H2 cell proliferation when the worm eggs were lodged in the lung[106]. This discrepancy might be explained by the fact that in response to *S. mansoni*, the damaged liver itself releases ARG1 from hepatocytes, which further reduces L-arginine levels to below a crucial threshold that inhibits T cell proliferation.

A direct involvement of ARG1 in the pathogenesis of microbial and parasitic infections is supported by a range of experimental data. Tissue-resident, dermal macrophages are permissive for *Leishmania major* replication and retain ARG1 expression even in the presence of a prevalent anti-parasitic T_H1 cell response, a factor that likely contributes to chronic disease[168]. Conditional *Arg1* knockout in haematopoietic and endothelial cells protected these mice from lethal disease, without affecting the ongoing T_H2 cell and T_{reg} cell response against the parasite[169]. In the blood of individuals infected with *L. major*, low-density neutrophils are the main population of $ARG1^+$ cells[170,171], and these might impair protective T_H1 cell responses against parasites[172,173]. Pathogens can also enforce an ARG1-rich environment to alter the immune landscape and modulate the response to other microorganisms. For example, *S. mansoni* infection induces ARG1-expressing inflammatory granulomas that can increase disease severity upon co-infection with *Mycobacterium tuberculosis*[174].

Helicobacter pylori, *L. major* and *S. mansoni* can regulate both their own ARGs and the host ARGs. In a model of progressive visceral leishmaniasis, *Leishmania donovani* parasites directly induced STAT6 signalling and downstream ARG1 expression in peritoneal macrophages and fibroblasts, independently from the effects of T_H2 cell cytokines[175,176]. Furthermore, *Leishmania* genes encoding ARG and L-arginine transport proteins are transcribed during macrophage infection; the parasite can increase the expression of these genes without the need for host STAT6, which was instead required to modulate the size and permissiveness of the host monocyte reservoir for parasite proliferation[177]. Also, mycobacteria can induce ARG1 expression in macrophages independently of the STAT6-mediated pathway, in an autocrine manner by inducing IL-10 production[38] or by signalling through the TLR–MYD88–C/EBPβ axis[178]. *H. pylori* ARG is encoded by the constitutively expressed gene *rocF*; it can directly impair host T cell function by reducing CD3ζ chain expression and it cooperates with host ARG2 to produce polyamines that are immunosuppressive and induce macrophage apoptosis[179]. Several other microorganisms, such as *Escherichia coli*, *Salmonella Typhimurium*, mycobacteria, *Trypanosoma brucei* and *Candida albicans*, have developed strategies that interfere with host ARG isoforms[180] to favour their own proliferation and survival. For example, by driving host ARG1 induction, *S. Typhimurium* prevents NO production, which is otherwise detrimental to its survival[180]. In a different context, *T. brucei* activates host ARG1 expression in myeloid cells to facilitate the synthesis of polyamines necessary for its proliferation[181].

Targeting arginine metabolism and ARGs

Amino acid modulation might represent a novel approach to treat cancer and infectious diseases. L-Arginine has an important role in cancer cell metabolism and L-arginine depletion can induce cell death in various types of cancer cell by autophagy and apoptosis-related mechanisms[182,183]. ARGs are good candidates for the therapy of cancer and autoimmune disease given their roles in promoting immunosuppression. Recent advances in both ARG enzyme biochemistry and our understanding of the multifaceted roles of ARGs have led to renewed interest in exploring mechanisms of interference.

L-Arginine supplementation

Oral supplementation with L-arginine has some disadvantages. Dietary L-arginine undergoes first-pass metabolism via the gastrointestinal tract and liver, with an estimated loss of 38% in humans[184] and 75% in mice[185]. Furthermore, chronic dietary supplementation with L-arginine may cause gastrointestinal distress and diarrhoea. Finally, L-arginine supplementation may cause a sudden drop in blood pressure and can cause adverse outcomes in critically ill patients[186,187]. For this reason, L-citrulline supplementation could be used to increase plasma L-arginine concentration indirectly. T cells, which express low levels of ASS1, could benefit from L-citrulline-derived L-arginine released by other cells endowed with a functional ASS1 enzyme[128].

Reduced L-arginine availability inhibits the proliferation, activation and function of T cells[5], and plasma concentrations of L-arginine below 42 μM are associated with reduced clinical benefit from immune checkpoint inhibitors[188]. Therefore, supplementation of L-arginine to enhance antitumour immunity has been suggested as a strategy for cancer treatment. In preclinical mouse models, dietary L-arginine supplementation decreases the number of T_{reg} cells[189] and enhances

Glossary

Alternatively activated, M2-like macrophage
M2 macrophages have roles in reducing inflammation, promoting tissue repair and favouring tolerance to certain antigens. Primarily described from in vitro culture studies, they upregulate expression of mannose receptor (CD206), ARG1 and IL-10 in response to IL-4 and IL-13. However, the complexity of macrophages in vivo, which represents a continuum of cellular states with distinct features and functions, is not fully reflected by this phenotype.

Autophagy
The physiological process by which a cell degrades its own components by lysosomal digestion.

Emergency granulopoiesis
A well-regulated, de novo generation of granulocytes (for example, neutrophils, basophils and eosinophils) from progenitor cells in the bone marrow in response to inflammation.

Endoplasmic reticulum (ER) stress
The accumulation of unfolded proteins in the ER in response to oxidative stress, inflammation and metabolic shift.

Immune checkpoint inhibitors
Antibodies that bind to and inhibit regulatory molecules, such as PD1, PD-L1, CTLA4, TIM3 and LAG3, that are expressed by activated T cells and myeloid cells and that otherwise limit their activity.

Immune synapse
The tight juxtaposition of membranes of a T cell and an antigen-presenting cell (APC), including the collection of membrane proteins that become organized at the point of juxtaposition. These include the T cell receptor complex, CD4 or CD8, co-stimulatory receptors and integrins on the T cell, which bind to peptide–MHC complexes, co-stimulatory ligands and integrin ligands on the APC.

Mammalian target of rapamycin complex 1
(mTORC1). A serine/threonine protein kinase that responds to various signals, including growth factors, amino acids, energy status, stress and oxygen level, and controls cell survival, cell growth, cell cycle and cell metabolism, favouring the maintenance of homeostasis.

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous group of myeloid cells composed of both neutrophils (PMN-MDSCs) and monocytes (M-MDSCs) that have anti-inflammatory and immunosuppressive properties.

Neutrophil extracellular traps
(NETs). Web-like structures extruded from neutrophils, composed of DNA, modified histones, myeloperoxidase and other proteins of the neutrophil granules.

Osteoclasts
A multinucleated, bone-resorbing and remodelling cell type that forms at skeletal sites from the fusion of blood-derived haematopoietic precursors of the monocyte-macrophage lineage.

Oxidative phosphorylation
Defined also as electron transport-linked phosphorylation or terminal oxidation. The metabolic pathway by which cells oxidize nutrients, thereby releasing chemical energy to produce ATP.

Tertiary granules
Also known as gelatinase granules. They are characteristic of polymorphonuclear cells and contain arginase, proteases (such as cathepsin) and matrix metalloproteinases (such as MMP9).

Tricarboxylic acid (TCA) cycle
Also known as the citric acid cycle or the Krebs cycle. A series of biochemical reactions that release carbon and energy (in the form of ATP) through the oxidation of acetyl-CoA derived from nutrients.

Urea cycle
A series of biochemical reactions necessary to dispose of the excess nitrogen generated by the metabolism of amino acids and other nitrogen-containing molecules.

antitumour T cell responses, thereby slowing the progression of various tumours, especially when used synergistically with checkpoint inhibitors[190]. L-Arginine enhances the effector functions of activated T cells without affecting resting T cells[5]; hence, supplementation of L-arginine primarily enhances immunity against 'hot' tumours with pre-existing antitumour T cell responses. Nevertheless, supplementation of L-arginine comes with the potential risk of promoting tumour cell proliferation and tumour growth[191]. This caveat could be overcome by the targeted delivery of L-arginine to antitumour T cells. In this regard, strategies to increase the metabolic adaptability of chimeric antigen receptor (CAR) T cells to a nutrient-deprived TME have been developed. CAR T cells engineered to stably express ASS1 and OTC, allowing for the recycling of L-ornithine to produce L-arginine, have increased function and cell survival[192]. ARG inhibitors that systemically increase L-arginine concentrations are also more effective in hot tumours[188]. This suggests that although L-arginine can boost antitumour immunity, it does not initiate it. An alternative strategy to dietary L-arginine supplementation is the administration of engineered bacteria that colonize tumours and continuously generate L-arginine in the TME, which results in strong, long-lasting antitumour T cell responses when combined with checkpoint inhibitor therapy[193].

From a clinical perspective, whereas oral supplementation requires daily ingestion of large amounts of L-arginine, engineered bacteria delivering L-arginine or other key nutrients would need only infrequent administration.

The effects of dietary L-arginine supplementation on the immune system in humans are supported by a few studies. In healthy volunteers, short-term administration of L-arginine increased the number of circulating natural killer cells and increased the activity of ex vivo-stimulated lymphocytes and natural killer cells, whereas extended administration of L-arginine increased the $CD4^+$ T cell proliferative response[194]. A meta-analysis of 11 clinical trials involving 321 patients substantiated the finding that L-arginine supplementation enhances $CD4^+$ T cell proliferation and revealed a marked decrease in the incidence of infectious complications compared with non-supplemented controls[195]. In addition, a phase III clinical trial showed that oral supplementation with a dietary formula enriched in L-arginine, omega-3 fatty acids and ribonucleic acids improved the long-term survival of patients with HNC who were treated with chemo-radiotherapy[196]. However, given the combination of nutrients used in this study, it is challenging to isolate the specific contribution of L-arginine, as is also the case in the context of sepsis and infectious disease.

Increasing L-arginine levels via dietary supplementation has been used to treat patients with sepsis and COVID-19. Patients with sepsis in intensive care units who received an L-arginine-enriched diet had reduced bacteraemia and reduced mortality[197]. By contrast, enteral immunonutrition with a formula containing low levels of L-arginine was associated with excess mortality in patients with severe sepsis, mostly from pneumonia[198]. A meta-analysis reports a decrease in post-surgery infections associated with dietary supplementation with L-arginine plus other immunonutrients, including omega-3 fatty acids and nucleotides[199], but the specific contribution of L-arginine supplementation in sepsis remains inconclusive. Confounding factors in these studies are highly heterogeneous patient cohorts, variability in L-arginine dosage, combinations with other immunonutrients and different administration routes (enteral versus intravenous) of supplement formulations[200]. Properly designed studies of L-arginine or L-citrulline administration as a monotherapy are needed to define the clinical utility of this approach in critically ill patients. Individuals with COVID-19 also have lower plasma levels of L-arginine[201,202], and L-arginine supplementation in individuals with severe COVID-19 reduced the need for respiratory support, decreased the length of hospitalization and shifted the serum concentrations of cytokines towards an anti-inflammatory profile[203].

L-Arginine deprivation

L-Arginine deprivation can be used as a potential targeted therapy for the treatment of cancers that are either ASS1-deficient or express oncogenic genes, such as *KRAS*, that silence the expression of ASS1, making them auxotrophic for L-arginine[204]. Several types of human tumour, including melanoma, hepatocellular carcinoma, prostate cancer and lymphoma, are frequently deficient in ASS1 and OTC[182,205,206]. The mechanisms by which ASS1 and OTC are silenced in tumours are only partially understood. Previous work in lymphoma cell lines reported a methylation-dependent transcriptional silencing of *ASS1*, whereas melanoma cells repress the *ASS1* promoter via a hypoxia-inducible factor 1α (HIF1α)-dependent mechanism[207–209].

Modified arginine-degrading enzymes, such as PEGylated recombinant human ARG1 (rhArg1-PEG or BCT-100) and PEGylated arginine deiminase (ADI-PEG 20), have been developed and shown to be safe and effective in clinical trials for cancer (Supplementary Table 1), either as monotherapy or in combination with chemotherapy and radiotherapy.

L-Arginine deprivation can also be achieved using arginine decarboxylase, an enzyme that breaks down L-arginine into carbon dioxide and L-agmatine. L-Agmatine inhibits the proliferation of tumour cells and reduces intracellular polyamine content[210]. Furthermore, L-agmatine-induced inhibition of cell proliferation promotes apoptosis[210]. However, although arginine decarboxylase can inhibit cancer growth and induce the death of tumour cells in vitro[211], clinical development of the enzyme has faced challenges related to efficacy, safety and cost.

Targeting ARG activity

Classical approaches to target the function of ARGs have involved small-molecule chemical inhibitors[212]. ARGs have a small, hydrophilic, active site with stringent requirements for competitive inhibitors. The most potent inhibitors closely resemble the natural substrate and have a similar binding mode. For example, the discovery that *N*-hydroxy-L-arginine (NOHA), an intermediate of NO synthesis, inhibits ARG activity led to the development of the first micromolar ARG inhibitor, *N*-hydroxy-nor-arginine (nor-NOHA). The development of another class of ARG inhibitors was inspired by the observation that borate binds to Mn^{2+}, which is present in the active site of ARGs; boronic acid derivatives used as ARG inhibitors include ABH and *S*-(2-boronoethyl)-L-cysteine (BEC)[213]. NOHA and nor-NOHA have been evaluated in various experimental tumour models with positive results[214], but neither has reached clinical trials; ABH and BEC have not been advanced further owing to acute toxicity towards hepatocytes and their low oral bioavailability. Furthermore, many of the first-generation ARG inhibitors were slightly more active against ARG2 than ARG1. Virtual screening of potential ARG inhibitors using dynamic pharmacophore models of the molecular features required for ligand binding has opened up some new possibilities for boronates[215], suggesting that ligands containing bulky moieties might enter the active pocket of ARGs.

Subsequent developments of ARG inhibitors have focused on enhancing their inhibitory activity. For example, this was achieved by complementing the ABH molecule with fragments that enhance the interactions with ARG. This led to development of the oral ARG inhibitors, 2-amino-6-borono-2-(1-(2,4-dichlorobenzyl)piperidin-4-yl) hexanoic acid (CB-1158) and OATD-02, which have shown promising results in models of lung, colorectal, kidney, ovarian and mammary tumours, pancreatic adenocarcinomas, gliomas and melanomas, often together with immune checkpoint inhibitors[216–218]. These compounds are now undergoing clinical trials as single agents and in combination with either checkpoint inhibitor therapy or chemotherapy for the treatment of advanced and metastatic solid tumours (Supplementary Table 1). Although CB-1158 is thought to affect mainly extracellular ARG1, OATD-02 should target both ARG1 and ARG2 intracellularly.

An alternative, indirect approach to inhibiting ARG activity involves targeting type-5 phosphodiesterase (PDE_5). By increasing the cellular levels of cGMP, PDE_5 supports the transcription of several genes involved in cell cycle regulation. Pharmacological blockade of PDE_5 (for example, with sildenafil, tadalafil or vardenafil), in addition to promoting the apoptosis of cancer cells, downregulated MDSC-mediated immunosuppressive pathways, notably by blocking the transcription of ARG1 and NOS2, and restored antitumour immunity in both preclinical models and patients with cancer[219].

ARG1 and ARG2 have different roles in different pathological conditions and their distinct functions depend on cell type and environmental context; thus, there is a need to develop isoform-specific ARG inhibitors, which currently do not exist. Furthermore, a complete picture of the temporal and cell-specific activities of ARG1 and ARG2 across immune cell networks is still lacking. In addition to small-molecule inhibitors, other approaches being explored to interfere with ARG activity are monoclonal antibodies[220], which have the advantage of being isoform specific and likely less toxic owing to their extracellular activity in serum, tumour interstitial fluids and NETs that does not affect the intracellular pathways of the urea cycle and L-arginine metabolism. However, no ARG-specific antibody has yet reached clinical trials.

The main potential risk of using systemically acting ARG inhibitors is the induction of renal dysfunction and urea-cycle disturbance, which may cause hyperammonaemia. Therefore, biological monitoring of plasma ammonia levels as well as biomarkers of hepatic function will be required. The use of a cell-specific targeting approach and the selection of the ARG isoform to be targeted will be important for the development of less toxic approaches.

Immunomodulatory vaccines targeting ARGs are an alternative approach to modulating ARG-dependent immunosuppression, although their potential effects on immune homeostasis and

haematopoiesis will need to be considered. Preclinical studies showed that immunogenic peptides from the ARG1 protein activated ARG1-specific T cells, resulting in the suppression of ARG1-expressing malignant cells and inhibitory immune cells[221,222]. A phase I vaccination trial indicated that most ARG1-vaccinated individuals developed $CD4^+$ and $CD8^+$ T cell responses against at least one of the ARG1 epitopes[223]. ARG2, unlike ARG1, is mostly expressed in various solid tumour cells, such as acute myeloid leukaemia primitive cells and cancer-associated fibroblasts. Recently, ARG2-targeting vaccines were shown to suppress tumour growth by activating ARG2-specific T cells in a lung carcinoma model[224] (Supplementary Table 1).

Conclusions and future directions

ARGs have evolved from their original roles in nitrogen metabolism, production of the metabolic precursor L-ornithine and stress adaptation in prokaryotes to having roles in the regulation of innate and adaptive immunity in mammals. Species-specific evolution has further diversified the context in which the enzymes are activated. The functions of ARG1 and ARG2 are thus context dependent and non-redundant, requiring additional studies to clarify their involvement in specific immune responses and pathologies. This might be achieved by selectively editing ARG1 and ARG2 in immune cell subsets with targeted deleters. For example, inducible CRISPR–Cas9 systems in mice[225,226] will allow for the effects of ARG induction to be studied in a time-restricted and tissue-restricted manner. From a human standpoint, humanized mouse models provide a better platform than standard mouse models for drug development and treatment validation[227,228], as they offer greater plasticity in terms of the type of cancer that can be studied (for example, patient-derived xenografts and organoids) and the immune cell subsets involved (for example, immune-reconstituted mice can be genetically modified to express cytokines and growth factors that favour the expansion of a specific immune cell subset). In the future, the combination of spatial transcriptomic, spatial metabolomic and single-cell proteomic–metabolomic analyses[229,230] will assist in redefining the role of ARGs within tissues, which is likely underappreciated by the current exclusively transcriptomic approach.

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The authors declare no competing interests.

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