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Review

Amino acid metabolism as drug target in autoimmune diseases

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ABSTRACT

In mammals, amino acid metabolism has evolved to control immune responses. Autoimmune diseases are heterogeneous conditions that involve the breakdown of tolerogenic circuitries and consequent activation of autoreactive immune cells. Therefore, critical enzymes along amino acid degradative pathways may be hijacked to keep in check autoimmunity. We examined here current knowledge of indoleamine 2,3-dioxygenase 1 (IDO1) and arginase 1 (ARG1), the main enzymes catabolizing tryptophan and arginine, respectively, in organ-specific and systemic autoimmune diseases as well as in the development of autoantibodies to therapeutic proteins. At variance with neoplastic contexts, in which it is known to act as a pure immunosuppressive molecule, ARG1 exhibited a protective or pathogenetic profile, depending on the disease. In contrast, in several autoimmune conditions, the bulk of data indicated that drugs capable of potentiating IDO1 expression and activity may represent valuable therapeutic tools and that IDO1-based immunotherapeutic protocols could be more effective if tailored to the genetic profile of individual patients.

1. Introduction

Amino acid metabolism is essential for the conservation of nitrogen and for maintaining physiologic concentrations of amino acids, which cannot be stored up when in excess from the diet. Over the evolution, some amino acid catabolic pathways have become critical checkpoints of immunity [60,124]. The novel function allows the control of adaptive immune responses to self and exaggerated inflammatory outcomes. These immunoregulatory effects rely on the depletion of specific amino acids in the microenvironment and/or generation of biologically active metabolites. Each degradative pathway is characterized by a rate-limiting enzyme, whose expression is normally subjected to strict regulation. To date, the most studied are indoleamine 2,3-dioxygenase 1 (IDO1) and arginase 1 (ARG1), which limit the catalytic rate in L-tryptophan (Trp) and L-arginine (Arg) metabolism [115], respectively. IDO1 and ARG1 normally work in different cells and are induced in response to distinct signals. However, transforming growth factor β (TGF-β), an immunosuppressive cytokine, can promote the sequential activation of ARG1 and IDO1 thus inducing a potent immunoregulatory phenotype in dendritic cells [116], immune cells capable of orchestrating immunity versus tolerance [16].

Although in neoplasia several data would classify both enzymes as inhibitors of anti-tumor immunity (thus favoring the so-called 'tumor escape' phenomenon), a thorough analysis of the role, either protective or pathogenetic, of Trp and Arg metabolism in distinct autoimmune diseases as well as in the development of autoantibodies to therapeutic proteins has not been addressed so far. Notably, the use of checkpoint inhibitors, which are yielding an unprecedented advance in cancer

Abbreviations: AhR, arylhydrocarbon receptor; Arg, L-arginine; CD, Crohn's disease; EAE, experimental autoimmune encephalomyelitis; FVIII, factor VIII; GCN2, general control nonderepressible 2; GD, Graves' disease; HT, Hashimoto's thyroiditis; IBD, inflammatory bowel disease; IDO1, indoleamine 2,3-dioxygenase 1; IDO2, indoleamine 2,3-dioxygenase 2; IFN-γ, interferon γ; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; ITIM, immunoreceptor tyrosine-based inhibitory motif; Kyn, L-kynurenine; MS, multiple sclerosis; NAD^+, nicotinamide adenindinucleotide; NMDA, *N*-methyl-D-aspartic acid; NO, nitric oxide; NOS, nitric oxide synthase; Orn, ornithine; PBMC, peripheral blood mononuclear cell; RA, rheumatoid arthritis; RRMS, relapsing-remitting multiple sclerosis; SNP, single nucleotide polymorphism; SOCS3, suppressor of cytokine signaling 3; SS, Sjögren syndrome; SSc, systemic sclerosis; T1D, type 1 diabetes; T2D, type 2 diabetes; TDO, tryptophan 2,3-dioxygenase; TGF-β, transforming growth factor β; Th, T helper; TNBS, trinitrobenzene sulfonic acid; Treg, regulatory T; Trp, L-tryptophan; UC, ulcerative colitis; VNTR, variable number of tandem repeat

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immunotherapy [125], is often accompanied by the development of autoimmune disorders [26,27], further suggesting that the other way around (i.e., potentiation of checkpoint mechanisms) could be of therapeutic value in autoimmunity. Because recent studies in patients with autoimmune diabetes underscored the importance of inter-individual genetic variation in IDO1 expression and function [131], we here interrogated literature for any known role of amino acid metabolism in distinct autoimmune disorders and also searched for genetic hints that might link dysfunctional amino acid metabolism with dysregulated immune responses to self.

2. Trp metabolism

2.1. Biology of relevant enzymes

Trp is the rarest essential amino acid found in food and its metabolism has evolved to be a primary regulatory node in the control of immune responses [60,124]. Three distinct enzymes, namely, tryptophan 2,3-dioxygenase (TDO), IDO1, and the IDO1 paralogue indoleamine 2,3-dioxygenase 2 (IDO2) catalyze the same rate-limiting step of Trp catabolism along a common pathway, known as the kynurenine pathway (Fig. 1), which leads to Trp starvation, production of metabolites collectively known as kynurenines, and synthesis of nicotinamide adenine dinucleotide (NAD^+) [157]. The kynurenine pathway is responsible for the metabolism of the majority of Trp intake.

2.1.1. Structure, catalytic activity, and expression

TDO is widely distributed in both eukaryotes and bacteria. In contrast, IDO1 has been found only in mammals and yeasts to date, whereas IDO2 is present not only in mammals but also in lower invertebrates. The genomic structures of IDO1 and IDO2 genes are well conserved in mammals and are present in a tandem arrangement on the same chromosome. Thus IDO1 and IDO2 may have derived from the duplication of a common ancestral gene, which was likely endowed with properties more similar or perhaps identical to the present IDO2. For this reason, IDO2 has also been referred as "proto-IDO1" [60,190].

IDO1 and IDO2 proteins are structurally quite similar (43% homology) and are both characterized by a heme-containing, monomeric structure of 42–45 kDa. In humans, IDO1 is encoded by the *IDO1* gene (*Ido1*, previously *Indo*, in the mouse) that contains 10 exons and spans 15 kb in chromosome 8, 8p12-p11 (8A2 in the mouse). The enzyme transforms Trp into N-formylkynurenine, a product that is rapidly converted by formamidase to L-kynurenine (Kyn), which in turn can either enter the bloodstream or be further metabolized to downstream kynurenines (Fig. 1), such as 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and quinolinic acid. Quinolinic acid can be further transformed into NAD^+. Lateral branches of the kynurenine pathway lead to the formation of other terminal products, including kynurenic acid, xanthurenic acid, anthranilic acid, and cinnabarinic acid, which represents a condensed form of two 3-hydroxyanthranilic acid molecules (Fig. 1).

Crystallization studies of human IDO1 have unveiled the presence of two folding domains in the enzyme [170]. While the smaller domain contains nine α-helices and two β-sheets, the larger one is composed of 15 helices and contains the catalytic pocket. A broad number of interactions including salt bridges, hydrogen bonding, and hydrophobic interactions spans the interface between the two domains and stabilizes the structure of the enzyme. Besides Trp, IDO1 recognizes a broad variety of indole-containing substrates, including the neurotransmitter serotonin, and it is expressed in many tissues and cells, including endothelial cells, fibroblasts, macrophages, myeloid derived suppressor cells, and dendritic cells [34]. Normally expressed at low basal levels, IDO1 is rapidly induced by the cytokine interferon γ (IFN-γ) [63,174]. TDO displays 10% homology with IDO1, contains only the large

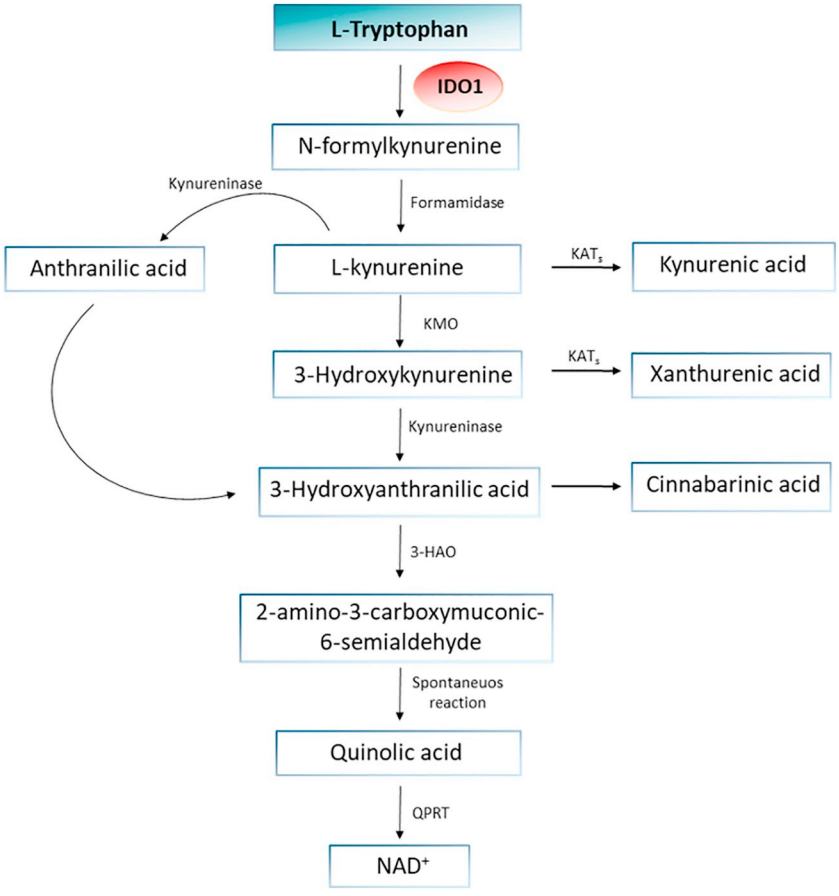

Fig. 1. The kynurenine pathway. The kynurenine pathway is initiated by the transformation of L-tryptophan into N-formylkynurenine by indoleamine 2,3-dioxygenase 1 (IDO1). N-formylkynurenine is rapidly degraded by formamidase to yield L-kynurenine, a common intermediate to kynurenic acid, 3-hydroxykynurenine and anthranilic acid via kynurenine aminotransferase (KATs), kynurenine 3-monooxygenase (KMO), and kynureninase, respectively. 3-Hydroxykynurenine is converted into 3-hydroxyanthranilic acid, which is in turn metabolized into 2-amino-3-carboxymuconate-6-semialdehyde. This metabolite can spontaneously rearrange into quinolinic acid, which is used for the synthesis of nicotinamide adenindinucleotide (NAD^+) via quinolinate phosphoribosyltransferase (QPRT). Additional lateral branches of the kynurenine pathway lead to the formation of other terminal products, including xanthurenic acid and cinnabarinic acid.

catalytic domain, has a homotetrameric structure, and cleaves only Trp. In mammals, TDO is mainly expressed in the liver, where is induced by glucocorticoids and Trp, but has also been recently identified in mucous membranes, epididymis, and brain [67]. The enzymatic activity of IDO2 is characterized by a very high Km value in vitro, approximately 500–1000 fold higher than that of mammalian IDO1 [60,190]. IDO2 is mainly expressed in liver, epididymis, and kidney [50].

2.1.2. Effects

After its discovery in the '50s [72], IDO1 has been considered an effector molecule capable of mediating a survival strategy of depriving bacteria and tumor cells of the essential amino acid Trp [140,174]. In the late '90s, Munn and Mellor performed a pioneering experiment demonstrating that placental IDO1 activity is crucial in preventing allogeneic fetal rejection due to maternal T-cell immunity in mice [122]. More recently, IDO1 has been regarded as the most versatile component of immunoregulatory loops in a variety of pathophysiological conditions. A bulk of data points to IDO1 as one of the main causes of immune unresponsiveness in neoplasia [17], where it is highly expressed not only by dendritic cells within tumor-infiltrating lymph nodes but also by neoplastic cells themselves. In contrast, IDO1 appears to be defective in autoimmunity [61,131] and allergy [63].

IDO1's immunoregulatory effects involve both Trp deprivation and the production of kynurenines, mainly by dendritic cells, that exert a series of effects on cells of the immune system, among which the conversion of effector T helper (Th) cells into regulatory T (Treg) lymphocytes [47] and blockade of the conversion of Treg into pro-inflammatory type 17 Th (Th17) cells [15]. In vitro, 3-hydroxyanthranilic acid and quinolinic acid induce selective apoptosis of mouse thymocytes and type 1 Th (Th1) but not Th2 cells. 3-hydroxykynurenine and 3-hydroxyanthranilic acid inhibit the proliferation of T lymphocytes, whereas Kyn also blocks natural killer cell proliferation [62].

HeLa cells expressing recombinant human TDO are capable of inhibiting the growth of bacteria (i.e., *Staphylococcus aureus*), parasites (*Toxoplasma gondii*), and viruses (*herpes simplex virus*) [154]. In addition, TDO^+ cells can inhibit T-cell proliferation and production of IFN-γ. Although these data would suggest that TDO could be responsible for the immune tolerance spontaneously occurring in liver transplantation, evidence for an immunoregulatory role played by TDO in vivo is still scarce [154].

At variance with the other two enzymes, IDO2 appears to be more pro- than anti-inflammatory. In fact, in an experimental model of arthritis, mice null for *Ido2* display decreased joint inflammation relative to wild type mice owing to a reduction in pathogenic autoantibodies [109].

2.2. Mechanisms of immune regulation

Trp deprivation determines elevation in uncharged tRNA, which in turn activates an integrated stress response mediated by the general control nonderepressible 2 (GCN2) kinase, phosphorylation of the translation initiation factor 2 (eIF2a), and consequent reduced protein synthesis. GCN2 activation can trigger cell-cycle arrest, differentiation, compensatory adaptation, or apoptosis, depending on the cell type [134]. In T lymphocytes, GCN2 activation induced by $IDO1^+$ dendritic cells leads to proliferative arrest and anergy [123]. Maneuvers capable of increasing uncharged tRNA levels and activating the GCN2 kinase decrease Th17 cell differentiation [171]. The combination of Trp starvation (experimentally obtained with a low Trp-containing medium) and low concentrations of kynurenines (Kyn, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and quinolinic acid) down-regulates the T cell receptor ζ-chain and induces a Treg phenotype in naïve T cells [48]. However, only recently some of the mechanisms whereby kynurenines exert their immunoregulatory effects have been clarified.

Kyn, the direct IDO1 product, and kynurenic acid act as agonists of the arylhydrocarbon receptor (AhR), a ligand-activated transcription factor that was first discovered as a receptor for 2,3,7,8-tetrachlorodibenzodioxin, an industrial pollutant, and thought to act as a detoxification mechanism for polyaromatic hydrocarbons. AhR has been now recognized as a promiscuous receptor capable of recognizing both xenobiotic and endogenous molecules, and, when activated in immune cells (either dendritic cells or T lymphocytes), mediates immunoregulatory responses favoring Treg cell generation and production of anti-inflammatory cytokines [80,167]. Furthermore, in dendritic cells, AhR activation by Kyn upregulates expression of IDO1, thus creating an immunoregulatory circuitry [18,80]. An alternative mechanism activated by kynurenines implies the inhibition of the PDK1 kinase and canonical, inflammatory pathway of NF-κB by 3-hydroxyanthranilic acid, an effect involved in the suppression of experimental asthma [73].

IDO1 does not merely degrade Trp and produce kynurenines, but it also acts as a signal-transducing molecule, an effect that leads to long-term expression of IDO1 in dendritic cells and immune tolerance in vivo [135]. IDO1's signaling function relies on the presence of two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the small domain of IDO1 [4,129]. In response to transforming growth factor β (TGF-β), IDO1 ITIMs are tyrosine phosphorylated by Src kinases, thus creating docking sites for binding and activation of SHP-1 and SHP-2 tyrosine phosphatases. In turn, SHP-1 and SHP-2 dephosphorylate the IRAK kinase, shifting the balance of NF-κB signaling from the canonical, inflammatory pathway to the noncanonical, anti-inflammatory one. Interestingly, IDO2 contains only one functional ITIM and does not transduce signals [130]. In the presence of the pro-inflammatory cytokine interleukin 6 (IL-6), phosphorylated IDO1 ITIMs bind the suppressor of cytokine signaling 3 (SOCS3), which recruits an ubiquitinating complex that drives the proteasomal degradation of IDO1 [129]. Thus, depending on the cytokine milieu of the microenvironment of dendritic cells, the IDO1 protein can increase over the short (IFN-γ) or the long (TGF-β) term, or rapidly degraded by the proteasome (IL-6) [64]. Recent findings identified a functional interplay between arginine metabolism and the IDO1 signaling [115,116], as described below.

3. Arg metabolism

3.1. Biology of relevant enzymes

Arg is a multifunctional amino acid involved in several physiological processes, including protein synthesis, regulation of immune responses, and tissue repair. Arg is classified as a conditionally essential amino acid for healthy humans. In fact, although it can be endogenously synthesized, in case of catabolic stress and pathologic conditions (including infections, trauma, and cancer) Arg becomes limited, making it necessary to rely on exogenous supplements [20]. The majority of Arg biosynthesis occurs via the "intestinal-renal axis": by using dietary glutamine and glutamate, epithelial cells of the small intestine synthesize citrulline, which is released into the circulation, extracted by the proximal tubules of the kidney and then converted into Arg. In addition, the "citrulline-nitric oxide cycle" ensures immune cells a constant provision of Arg for the synthesis of nitric oxide (NO). Specifically, activated macrophages recycle back citrulline into Arg via the sequential action of argininosuccinate synthase and argininosuccinate lyase, providing fuel for more NO production [78].

The major Arg-degrading enzymes are the isoforms of NO synthase (NOS1–3) and arginase 1 and 2 (ARG1 and ARG2). The three NOS isozymes catalyze the same reaction but have different distribution and regulation [19]. NOS1 (also known as neuronal; nNOS) and NOS3 (known as the endothelial form; eNOS) are constitutive enzymes, whereas NOS2 (referred as inducible NOS; iNOS) is induced by pro-inflammatory cytokines and microbial products. The metabolism of Arg by NOS generates NO, a relatively stable gas that diffuses through the lipid membrane and acts as signaling molecule. NO stimulates cytosolic guanylate cyclase, so to generate cyclic GMP and thus promote smooth

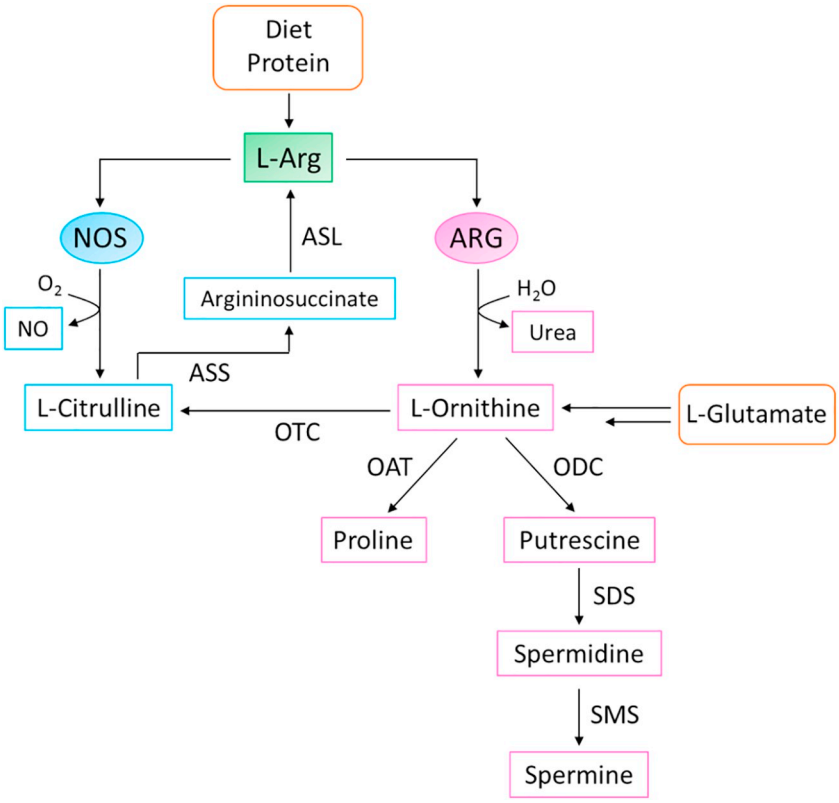

Fig. 2. The arginine metabolic pathway. L-Arg, derived from dietary supplements and protein turnover, can be degraded by two major enzymes, namely NOS and ARG. NOS catalyzes the conversion of L-Arg into NO and citrulline, which is recycled back into L-Arg by the sequential action of argininosuccinate lyase (ASL) and argininosuccinate synthetase (ASS). ARG catalyzes the degradation of L-Arg into L-Ornithine and urea. L-Ornithine acts as a substrate of ornithine decarboxylase (ODC), ornithine aminotransferase (OAT), and ornithine transcarbamylase (OTC) to yield putrescine, proline, and L-citrulline, respectively. Putrescine is sequentially converted into spermidine and spermine, through the action of spermidine synthase (SDS) and spermine synthase (SMS).

muscle cell relaxation. NO can also generate reactive nitrogen species and favor post-translational modifications by protein nitrosylation. Moreover, due to its cytostatic and cytotoxic activity, NO release is crucial for the initial phase of the immune reaction against intracellular pathogens and malignancies. Of note, to limit the potential toxicity of NO for host tissues, a counter-regulatory mechanism, i.e., mediated by Arg degrading enzymes (ARG), is activated in order to compete with iNOS for the same substrate and to control its mRNA translation.

The ureohydrolase ARG is a trimeric metalloenzyme that catalyzes the conversion of Arg into ornithine (Orn) and urea (Fig. 2). For its catalytic activity, ARG requires divalent cations and, although several reports have shown that Co^{2+}, Ni^{2+} and Fe^{2+} can fulfill this requirement, the Mn^{2+} ion is widely considered the physiological activator of the enzyme [11]. Resolution of the ARG crystal structure has revealed the presence of a 15 Å-deep active site in each monomer of the homotrimer, with the binuclear Mn^{2+} cluster located at the bottom of this cleft [11]. In particular, the metal ions make hydrogen bonds with histidine and aspartate residues, ensuring protein stability and thus ARG activation.

ARG appeared first in bacteria and then spread through the evolutionary route in yeasts, plants, vertebrates, and invertebrates [45]. It has been suggested that the transfer of ARG from ancestral species to eukaryotic cells occurred via mitochondria. Two ARG isoforms exist, catalyzing the same chemical reaction, yet encoded by different genes and characterized by distinct cellular and tissue distribution. ARG1 is a cytosolic enzyme abundantly present in liver, whereas ARG2 is expressed in mitochondria of cells from kidney, brain, small intestine, and pancreas. Murine myeloid cells, including macrophages, dendritic cells, and myeloid derived suppressor cells, express ARG1 in response to Th2-type cytokines, cyclic AMP, toll-like receptor agonists and several tumor-derived soluble factors, such as IL-6 and TGF-β [120,128]. In humans, ARG1 is constitutively present in an inactive form in the granular compartment of polymorphonuclear neutrophils, where becomes activated upon extracellular release during inflammation [150].

ARG activity results into two main physiological functions, i.e., detoxification of ammonia (being a key enzyme of the urea cycle) and production of Orn, which is the biosynthetic precursor of proline and polyamines [138]. Proline is produced through the activity of ornithine aminotransferase and is used for the synthesis of collagen, whereas polyamines (i.e., putrescine, spermidine, and spermine) derive from Orn decarboxylation catalyzed by ornithine decarboxylase, followed by spermidine and spermine synthases. Polyamines are small polycations involved in a variety of physiological functions, including cell growth and proliferation, neuronal development and regulation of immune responses [116,138].

3.2. Mechanisms of immune regulation

Arg metabolism has emerged as a critical regulator of immune responses, as it modulates the production of antimicrobial effectors and directs the activity of T cells [124]. Indeed, activated lymphocytes are auxotrophic for Arg since they need to accumulate nutrients as much as possible in order to get out from quiescence and make copies of themselves. Therefore, in case of limited Arg in the microenvironment, T lymphocytes lose CD3ζ expression and become unable to proliferate [149]. In addition to the effects of Arg starvation, the metabolites originated from its breakdown, particularly polyamines, are endowed with immunoregulatory properties, including attenuation of proinflammatory cytokines' production and activation of Trp catabolism [36,115,116]. Specifically, spermidine, through activation of the Src kinase, promotes the phosphorylation of IDO1 and thus favors the initiation of immunoregulatory signaling events in dendritic cells, creating a self-sustaining circuitry responsible for the maintenance of a long-term immune tolerance [116].

Although generally recognized as a pro-inflammatory mediator, NO can also promote several immunoregulatory functions, including modulation of cytokine production, regulation of cell survival, and chemotactic responses [160]. NO, when produced in large quantities, can promote T lymphocyte apoptosis by activating a caspase-independent pathway and can alter the T cell function/activation by

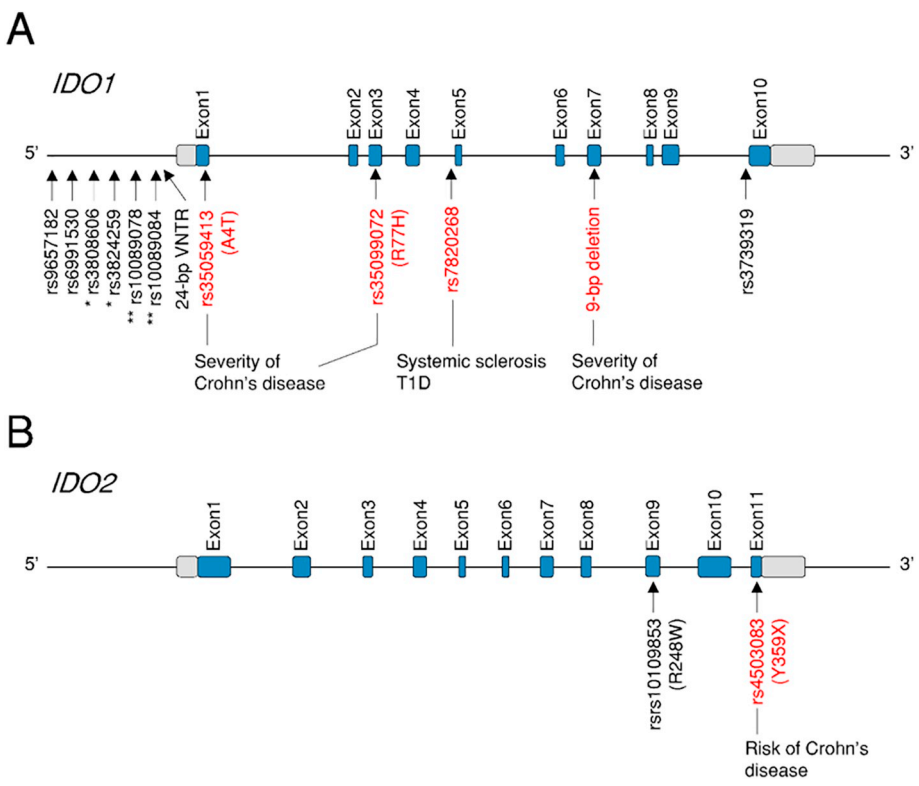

Fig. 3. Gene structure of human (A) *IDO1* and (B) *IDO2*, and the association of SNPs with autoimmune diseases. Exons and untranslated regions are depicted in dark blue and light gray, respectively. Asterisks denote SNPs that are in strong linkage disequilibrium. In the case of nonsynonymous SNPs, the amino acid substitution is indicated. The SNPs that have been associated with the risk or phenotype of autoimmune disease are identified.

repressing the production of IL-2 [160]. Moreover, in the tumor microenvironment, NO can decrease antigen presentation by dendritic cells to T cells, resulting in the suppression of immune responses [102]. However, NO, by direct binding of heme, exerts inhibitory effects on IDO1 catalytic activity [176] and therefore the co-presence of iNOS and IDO1 may have unpredictable outcomes.

Due to the variety of physiological responses regulated by Arg metabolites and Arg itself, an improper activation of this metabolic pathway may become damaging in several contexts. For instance, an increased production of proline and polyamines may result into thickening and fibrosis of blood vessels and airways, as well as neurodegeneration and growth of tumors. Adverse effects of abnormal degradation of Arg could be thus pathologically relevant in hypertension, diabetes, inflammation, cancer, and also autoimmune diseases.

4. Genetic variation of IDO1 and ARG1

4.1. Genetic basis of susceptibility to autoimmune diseases

Although humans are identical at most of the three billion base pairs in their genome, inter-individual variation is present in approximately 0.01% of the genome [59]. The most common genetic variation is the single nucleotide polymorphism (SNP), in which two alternative bases occur at appreciable frequency (> 1%) within a population. Another less frequent type regards the variable number of tandem repeats (VNTR), consisting of sequence repeats ranging from a single to thousands of base pairs. While many genetic variants are “silent”, functionally significant effects are likely to occur when SNPs are associated with amino acid substitutions in the gene product, when a deletion/insertion results in a frameshift in the coding region or when the SNP directly affects gene transcription, RNA splicing, or mRNA stability and translation. Only 1.5% of SNPs are thought to be located in coding regions, with the functions of nearly all the SNPs located outside gene coding or regulatory regions remaining undefined.

With the development of high-throughput sequencing technologies, hundreds of genomic susceptibility loci for autoimmunity have been identified, many of which overlap across different autoimmune disorders [66]. However, and despite progress in fine-mapping causal genes and identifying variants, the genetic mechanisms that trigger autoimmunity remain largely unknown. This may be due to the fact that genomic regions implicated in these risk loci are large, encompassing multiple potential candidate genes within each locus, and containing many SNPs that often have small effect sizes. Moreover, most risk variants that are likely to be causal fall in non-coding regions of the genome and are enriched in distant regulatory elements, known to be particularly active in immune cell types.

Before the genomics era, initial attempts aimed to disclose the genetic variation contributing to autoimmune diseases were mainly based on the study of familial clustering and captured a few loci with large effect sizes. More recently, high-throughput genome-wide sequencing technologies has led to the identification of hundreds of common variants with small to moderate effect sizes, and the study of rare variants has yielded a number of critical mechanistic insights into autoimmunity [66]. These new approaches are ultimately expected to contribute to the elucidation of the mechanisms and pathways whereby genetic variation influences gene regulation and immune function. Here, we will specifically discuss recent advances in our understanding of genetic variation in amino acid metabolism, particularly Trp, and its causal involvement in autoimmune disease (Fig. 3).

4.2. Genetic variation in amino acid metabolism and autoimmunity

4.2.1. IDO1

There is a large amount of inter-individual variation in the expression of enzymes involved in Trp metabolism, namely IDO1, across different patient settings [131], which may affect therapeutic responses and susceptibility to drug side effects. In light of the normal physiological functions of IDO1, its involvement in autoimmune diseases, which are typically characterized by impaired immune tolerance, has been investigated. Initial targeted sequencing of samples from African-American and Caucasian origin identified several genetic variants in the exons and intron-exon junctions of the *IDO1* gene [10]. Two of them (a

9-bp deletion in exon 7 and the nonsynonymous SNP rs35099072 underlying the amino acid substitution R77H) were particularly relevant since they impaired the protein expression and almost completely abrogated the enzyme activity. Despite these marked functional consequences, the allelic frequencies of these two *IDO1* variants were approximately 1% and were exclusively observed in the African-Americans. These findings ultimately highlighted the negative selection throughout evolution of coding variants that severely impair IDO1 function given their likely extremely detrimental effects to human health.

The low frequency of nonsynonymous SNPs in *IDO1* limits the ability to detect risk associations, as observed in a genetic association study of risk and phenotype of Crohn's disease [87]. However, despite rare, these SNPs were associated with a severe clinical course and with reduced enzyme activity during active disease. In contrast, common variants with modest effect sizes could explain the wide range of inter-individual variation in enzyme expression and function. For example, a common VNTR consisting of a 24-bp repeat motif was identified upstream of the transcription start site of *IDO1* [162]. Although this VNTR did not influence basal or cytokine-induced promoter activity in gene reporter assays, it was nonetheless shown to contain novel *cis*-acting elements, including a putative binding site for LEF-1, a transcription factor widely implicated in Wnt/β-catenin signaling [153]. Strikingly, the presence of this VNTR correlated with altered Trp serum concentrations and partial loss of enzyme activity in females, but not in males. Whatever the mechanism(s), these findings added a further layer of complexity to the molecular processes involved in the regulation of IDO1 activity, since the risk and progression of several autoimmune diseases, including autoimmune diabetes [177] and multiple sclerosis [193], have been shown to be under hormonal control. This specific 24-bp VNTR remains however to be evaluated as a genetic factor regulating susceptibility to autoimmune disease.

Common genetic variation in *IDO1* has only recently been exploited in genetic association studies, given its appealing role as a strong candidate gene plausibly implicated in autoimmune diseases. Although the robustness of the association tests was precluded by the insufficient sample size, the intronic SNP rs7820268 in *IDO1* was nonetheless reported at higher frequencies in patients with systemic sclerosis compared to healthy controls [175]. The SNP was correlated with impaired function of $CD8^+$ T regulatory cells, and this lack of suppressive activity was proposed as one potential mechanism contributing to the disease. Contrasting results were instead obtained in mesenchymal stromal cells derived from Crohn's patients, in which rs7820268 failed to modulate the suppressive function of these cells at inhibiting T-cell proliferation after IFN-γ pre-licensing [35]. Taken together, these findings likely suggested cell- or disease-specific effects of rs7820268 on the regulation of immune cell function. Further supporting this, rs9657182, but not rs7820268, was associated with IFN-α − induced depression in patients with hepatitis C [161], thereby also implying individual, SNP-mediated and context-specific mechanisms of IDO1 regulation.

The functional relevance of rs7820268 and its involvement in autoimmune disease was recently confirmed in a large, two-stage genetic association study comprising independent discovery and validation cohorts of pediatric T1D patients [131]. Mechanistically, the defective Trp catabolism detectable in children with T1D was found to profoundly rely on the presence of rs7810268. The actual molecular mechanism(s) influenced by rs7810268 remain unknown, but the fact that IDO1 activity is regulated by proteasomal degradation may suggest that, apart from a direct effect on IDO1, the SNP may also be linked with other variants in genes coding for proteins involved in proteasomal degradation, many of which reside on the same IDO1 chromosome. Collectively, these data highlighted the rs7820268 SNP as a critical regulator of IDO1 function in autoimmune disease and further stress the need to consider the genetic profile of patients when devising therapeutic strategies aimed at correcting defective IDO1 activity.

4.2.2. IDO2

In contrast to *IDO1*, the coding sequence of the *IDO2* gene is characterized by the presence of two common nonsynonymous SNPs, including rs10109853 (R248W) and rs4503083 (Y359X) [112]. These genetic variants, particularly the stop codon SNP leading to a truncated IDO2, have been associated with severely impaired enzyme activity. Of note, the minor allele of rs4503083 was associated with protection from Crohn's disease [87], supporting a function for IDO2 as a pro-inflammatory mediator in an autoimmune environment, at least in this specific disease setting. Of interest, IDO2 was found to be required for IDO1-mediated regulation of T-cell function [113], although the precise mechanism(s) whereby these enzymes synergize or play antagonistic roles during inflammation remains to be explored. In another study, no association was detected between those functional SNPs and multiple sclerosis (MS) [2], despite Trp catabolism was proposed as a critical homeostatic mechanism to control MS-associated neuroinflammation [29]. In this context, it will be interesting to explore whether additional, common regulatory variants in IDO2 may have more relevant effects than the most-studied nonsynonymous variants.

4.2.3. Other enzymes

Besides *IDO1* and *IDO2*, other members of the kynurenine pathway have been implicated in autoimmune diseases. In particular, the fine-mapping of the chromosome 1q43 region that encompasses the gene encoding kynurenine monooxygenase (Fig. 1) revealed an association with MS [107], although no functional validation was provided and the mechanism(s) whereby this variant may be associated with the disease remains unknown. In contrast, functional variants have instead been described in the genes encoding TDO, aminoadipate aminotransferase, kynureninase and aminocarboxymuconate-semialdehyde decarboxylase [21]. However, their implication in autoimmune disease and consequences to the immune system in the context of autoimmunity have yet to be determined.

Genome-wide association scans with high-throughput metabolic profiling provide unprecedented insights into how genetic variation influences metabolism and complex disease. The unbiased characterization of hundreds of loci embedded in their metabolic context allows the investigation of complex traits at a previously unexplored resolution leading to the identification of metabolomic quantitative trait loci (mQTL) [56]. Such approaches are expected to provide critical insights into the role of inherited variation in blood metabolic diversity and identify potential new opportunities for drug development and for understanding disease pathogenesis. For example, a recent genome-wide study exploring human metabolism revealed significant associations at dozens of metabolic loci and their biochemical connectivity with > 400 metabolites in human blood [158]. Interestingly, among the associations detected, two common variants in *IDO1* and *TDO2* were associated with the concentrations of 4-hydroxytryptophan and Trp, respectively. These mQTL have however not been addressed in the context of human disease, and therefore their pathogenetic potential remains unclear. Another report has implicated genetic variation in *ARG1* in the regulation of circulating levels of Arg [24], a finding that was subsequently validated in an independent genome-wide association study [44]. To our knowledge, there is no published evidence on the contribution of common genetic variation in *ARG1* to autoimmune disease, with the exception of a recent study that identified the rs2781666 and rs2781665 variants in the *ARG1* promoter as risk factors to type 2 diabetes (T2D), even though the classification of T2D as an autoimmune disease remains debated [182]. Therefore, although there is now evidence that regulatory variation modulates the levels of circulating metabolites, including amino acids, no studies have investigated their association in the context of autoimmunity.

5. Amino acid metabolism in autoimmune diseases

5.1. Organ- and tissue-specific autoimmune diseases

5.1.1. Type 1 diabetes

Type 1 diabetes (T1D) results from the breakdown of immune tolerance that leads to the selective destruction of β-cells in the pancreas, responsible for insulin secretion, with a consequent severe impairment of glycaemia control. A long preclinical phase, characterized by immune cell infiltration in the pancreatic islets of Langerhans, precedes hyperglycaemia and disease onset. The circumstances driving this immune dysfunction are still unclear [13].

The IDO1 enzyme plays multiple roles in the regulation of immunity, promoting immune tolerance in pathophysiological conditions [60,62,108,188]. The enzyme, therefore, may restrain the autoimmune process that drives to T1D onset [132]. However, the inflammatory context that characterizes the prediabetes stage heavily affects IDO1 protein expression and function, impairing its role as guardian of immune tolerance in the pancreas. In dendritic cells of nonobese diabetic (NOD) mice during the prediabetes phase, a defective Trp catabolism seems to predispose to the onset of T1D [49,61]. Conversely, the preservation of adequate levels of the IDO1 enzyme, by either attenuating its accelerated turnover under inflammatory conditions (i.e., IL-6) or by forcing IDO1 expression in NOD plasmacytoid dendritic cells by cell transfection, can prevent T1D onset in NOD mice and reinstall immune tolerance to pancreatic autoantigens [117,136]. Moreover, transplantation of IDO1-expressing islets can prolong the islet graft survival [6]. In the human disease, peripheral blood mononuclear cells (PBMCs) from the majority of children with T1D express very low levels of IDO1 protein in response to the potent inducer IFN-γ. The IDO1 defect correlates with a higher IL-6 receptor (IL-6R) expression, as compared to matched controls, and it can be rescued by in vitro incubation of PBMCs with tocilizumab, an IL-6R blocker currently in use for juvenile arthritis. Tocilizumab also controls hyperglycemia in diabetic NOD mice and the therapeutic effect requires an intact IDO1 expression, since in *Ido1*$^{-/-}$ NOD mice tocilizumab does not provide any therapeutic effect [131]. A recent study by Anquetil et al. [9] also reported a deficient IDO1 expression in human β-cells of T1D patients as compared to healthy controls, who are characterized by a very high level of the IDO1 protein. Perhaps most interestingly, a progressive loss of IDO1 expression was observed in insulin-producing islets during the course of T1D, with a significant decay of IDO1 in pancreata at a time just preceding β-cells destruction [9]. In light of these promising results, restoration of IDO1 immunoregulatory mechanisms may be clinically beneficial in patients with T1D [132]. In addition, the screening of IDO1 activity — for example in PBMCs of pediatric T1D patients — may be exploited as a biomarker to identify, at the disease onset, those patients that will better respond to the tocilizumab-based therapy. Although no literature data currently exist in autoimmune diabetes, IDO2 appears to be overexpressed in human pancreatic tumors [183], suggesting that a dysregulation of the IDO1 paralogue may be possibly involved in a pancreas-specific autoimmune disease such as T1D.

Differently from Trp metabolism, the role of Arg catabolic pathways in T1D is still obscure. Based on the observations that blood cells from NOD mice just before diabetes onset display a reduced production of Orn (the main catabolic product of the ARG1 enzyme) and upregulation of citrulline (generated by NOS), NOD mice were treated with nor-NOHA, an ARG1 inhibitor. However, nor-NOHA did not exacerbate the disease, as expected, but rather reduced the incidence of autoimmune diabetes [75].

Ketosis-prone diabetes collects different phenotypes with varying degrees of impaired β-cell function and autoimmunity. Several patients with ketosis-prone diabetes were found positive to islet autoantibodies [i.e., to glutamic acid decarboxylase (anti-GAD65) and insulinoma-antigen 2 (anti-IA-2)]. In a case-control study conducted on a small number of ketosis-prone diabetes patients, Mulukutla et al. found that Arg bioavailability is reduced during hyperglycemic clamps and this correlates with reduced insulin secretion [119]. Intravenous Arg supplementation improved insulin secretion to concentrations similar to that of control participants. Compelling studies are needed for a better understanding of the role of Arg deficiency in β-cell functions and for elucidating the causes of Arg deficiency in ketosis-prone diabetes patients.

5.1.2. Multiple sclerosis

MS is a multifaceted chronic disease that affects the central nervous system, eventually leading to a fatal neuronal demyelination and axonal degeneration. Although MS pathogenesis is still not fully understood, a complex network of immunopathological, inflammatory, and oxidative parameters appears to be involved in the development and advancement of the disease. The inflammatory component of MS is clearly characterized by the presence of infiltrating macrophages and activated microglial cells around the characteristic neuronal lesions [41,184], while the autoimmune component arises from the Th1 and Th17 lymphocyte entry into the central nervous system [74]. Considering the role of other cells, such as monocytes, and other pathways that can further compromise oligodendrocyte health and contribute to the pathology of MS, the most accepted theory describes MS as a neurodegenerative disease with an autoimmune component [169].

In this scenario, the activation of Trp catabolism − a main source of several potent immunomodulatory as well as neuroactive intermediates − in microglial and peripheral immune cells, could play a crucial role in the pathogenesis and disease course of MS. In fact, in addition to the well characterized role of IDO1 as a pivotal player in the development and maintenance of immune tolerance, the activation of the kynurenine pathway leads to the production of neurotoxic metabolites, represented by quinolinic acid, an *N*-methyl-D-aspartic acid (NMDA) receptor agonist and excitotoxin, and 3-hydroxykynurenine (a free radical generator), and neuroprotective metabolites, i.e., picolinic acid and kynurenic acid. Thus dysregulation of one or more of the enzymatic steps in the kynurenine pathway may shift the balance towards the production of neurotoxic rather than neuroprotective metabolites [168]. Due to its complexity, multiple, and often controversial, literature data on the role of Trp catabolism in the pathogenesis of MS have been reported so far. In one hand, in the experimental autoimmune encephalomyelitis (EAE) model, significantly elevated levels of quinolinic acid and Kyn/Trp ratio in rat sera, correlating with disease severity, were observed [53]. In the other hand, IDO1 expression was demonstrated to play a role in the remission of acute EAE. Moreover, administration of 1-MT, the standard inhibitor of IDO1 catalytic activity, exacerbated the clinical course of EAE in mice [86,152]. Therefore, those data suggested that enzymes and metabolites of the kynurenine pathway could be involved in regulating the MS disease course. Importantly, administration of 3-hydroxyanthranilic acid alleviated the EAE course by an immunoregulatory mechanism promoted by dendritic cells [187].

Studies evaluating Kyn levels in cerebrospinal fluid and plasma from MS patients have shown conflicting results, probably ascribable to the complexity of the multiple disorders grouped under the general definition of MS [97]. The first evidence of the potential involvement of the kynurenine pathway in MS reported decreased levels of Trp in plasma and cerebrospinal fluid of MS patients [114]. This was confirmed in subsequent studies in the cerebrospinal fluid of relapse-remitting MS (RRMS) patients, showing higher levels of neurotoxic quinolinic acid and decreased levels of neuroprotective kynurenic acid during the relapsing and acute phase, with kynurenic acid becoming elevated during the remission phase as compared with healthy controls [71,147]. A study analyzing changes in IDO1 activity and expression in PBMCs of RRMS patients showed high IDO1 expression in the relapsing but not in the remitting phase of the disease, suggesting that the activation of Trp catabolism may be associated with disease relapsing and appearance of clinical signs. In the authors' opinion, IDO1 activation could represent a self-protective response to alleviate auto-aggressive inflammation in the

central nervous system [99]. Other authors found that not only IDO1 but also ARG1 expression are increased in PBMCs of MS patients undergoing clinically isolated syndrome, possibly providing a sustained homeostatic mechanism to control MS-associated inflammation [29]. However, recent data demonstrated that, in monocytes from PBMCs of MS patients, a significantly reduced expression and activity of both IDO1 and ARG1 as compared to healthy controls subjects is observed. Moreover, in addition to changes in Trp and Arg catabolism, the authors also found an impaired catabolism of leucine, isoleucine and glutamine [126].

The considerable amount of conflicting data concerning the metabolism of amino acids, in particular Trp and to a lesser extent Arg, in EAE and MS would suggest an urgent need of further investigations in order to establish the pathogenetic/protective role of each amino acid metabolism in the multiple and different variants and phases characterizing this neurodegenerative and autoimmune disease. In this regard, not just IDO1 and ARG1 but also the other enzymes along Trp and Arg metabolic pathways may represent attractive therapeutic targets for the development of a personalized immunotherapy in MS. In fact, it might be interesting to note that an increased expression of the *Ido2* gene was found in EAE mice effectively treated with an Ahr activating agent [81].

5.1.3. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a disease characterized by inflammation of the capsule surrounding the joints, hyperplasia of synovial cells, oedema, and fibrosis [51]. The pathology frequently causes destruction of articular cartilage and joint ankylosis. In addition, RA can cause subcutaneous nodular lesions and can result in inflammation in the lungs, pericardium, pleura, and sclera [144]. Although the etiology of the disease is still unknown, it is widely assumed that autoimmune processes play a major role in the initiation and/or perpetuation of the disease.

Patients with RA manifest significantly decreased and increased levels of circulating Trp and Kyn, respectively, when compared to healthy individuals [156]. Further evidence of kynurenine pathway activation has been provided by the finding of reduced baseline levels of Trp, 3-hydroxykynurenine and 3-hydroxyanthranilic acid, accompanied by increased levels of Kyn and xanthurenic acid in RA patients as compared to healthy subjects [54]. However, other studies reported a defective expression and activity of IDO1 in RA. This defect was explained on the basis of methylation of an NF-AT binding site occurring within the gene promoter of cytotoxic T lymphocyte antigen 4, which causes down-regulation of cytotoxic T lymphocyte antigen 4 expression in Treg cells from RA patients. As a consequence, Treg cells are unable to induce expression and activation of IDO1, which in turn results in a failure to activate the kynurenine pathway [40].

In experimental models of RA, the role of IDO1 has been evaluated by comparing the progression of arthritis induced by immunization with type II collagen (collagen-induced arthritis) in IDO1 deficient (*Ido1*$^{-/-}$) versus wild-type mice. In *Ido1*$^{-/-}$ animals, there was an earlier onset of arthritis as compared to IDO1 competent animals of the same strain (i.e., C57BL/6). Clinical severity showed a similar progression in early arthritis but reached a plateau in wild type mice and continued to increase in *Ido1*$^{-/-}$ mice [39]. Moreover, administration of adenoviral vectors encoding IDO1 in mice with collagen-induced arthritis, as well as treatment with Kyn, significantly reduced both clinical and histological progression of arthritis [32,39]. The protective role of IDO1 in RA was also demonstrated in the experimental model of antigen-induced arthritis, in which the IFN-α treatment prevented arthritis by activating an IDO1/TGF-β–dependent anti-inflammatory program in plasmacytoid dendritic cells [30].

In contrast to IDO1, evidence for a pathogenic function of IDO2 in the establishment and development of autoimmune arthritis has been provided [109]. Using the KRN preclinical model of RA (i.e., mice transgenic for a T cell receptor recognizing an epitope of bovine RNase, bred onto the NOD background, and developing severe destructive arthritis), it was demonstrated that IDO2 is required for activation of CD4$^+$ Th cells, production of pathogenic autoantibodies, and subsequent development of arthritis. Severity of arthritis was significantly reduced in arthritic mice lacking IDO2 or treated with an anti-IDO2 antibody [38,110].

In the plasma of RA patients, a diminished global Arg availability and decreased levels of citrulline have been observed, whereas levels of ARG1 activity and its catabolic product Orn were elevated. Moreover, increased levels of endogenous inhibitors of NO production, namely, asymmetric and symmetric dimethylarginine, were detected [31]. However, no association between increased ARG1 activity and RA disease activity could be found, thus suggesting that ARG1 activity may not elicit a significant effect on RA pathogenesis per se but may influence the induction of subclinical endothelial dysfunction in RA patients.

Macrophages are centrally involved in the process of arthritis. In fact, they increase in the synovial membrane during the disease and exhibit a highly activated phenotype with up-regulated expression of pro-inflammatory cytokines [141], which contribute to the destruction of bone and cartilage in the acute and chronic phases of arthritis [82]. Only recently, it was shown that the c-Jun transcription factor regulates the activation state of macrophages and promotes arthritis via direct activation of cyclooxygenase-2 and indirect inhibition of ARG1 [68]. Similarly, alternatively activated macrophages expressing ARG1 may contribute to resolve the inflammation of arthritis [33].

In summary, while the role of IDO1 and ARG1 is still controversial, IDO2 appears to be clearly pathogenetic and may represent a valid therapeutic target for RA.

5.1.4. Inflammatory bowel diseases

Inflammatory bowel diseases (IBDs), such as Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing disorders of the gastrointestinal tract that are characterized by intestinal inflammation and epithelial injury [127]. The exact cause of IBD remains unknown. IBDs exhibit alternating phases of clinical relapse and remission and both long standing UC and CD have been associated with increased risk of intestinal cancers. Both innate and adaptive immune responses are involved in the pathogenesis of IBD.

Emerging evidence indicates that defective immunoregulatory mechanisms in IBD are associated with alterations in microbiota composition, more specifically with a reduced abundance of “anti-inflammatory” bacteria [94]. Gut microbes produce several indole derivatives, often different from those produced by humans, and some of them have been shown to balance gut mucosal reactivity via engagement of AhR [191]. An interesting study in 2001 reported that while a normal amount of Arg in the diet is not harmful for rats with chemically induced colitis, both absence of Arg and supplementation with high doses of Arg are deleterious [100]. More recently, supplementation of Arg has been shown to affect microbiota composition and activate intestinal innate immunity [148]. Therefore, in IBDs more than in other inflammatory/autoimmune diseases, the intimate interplay with microbiota should be considered in evaluating the effects of amino acid metabolism.

IDO1 mRNA is markedly induced in lesional colonic biopsies of IBD patients [185]. However, the role of overexpressed IDO1, whether pathogenetic or protective in IBD, is still unclear. Studies demonstrated that inhibition of IDO1 and/or lack of IDO1 expression augment colitis induced by trinitrobenzene sulfonic acid in mice [65,173]. Moreover, expression of IDO1 in gut CD103$^+$ dendritic cells has been correlated with an increase in Treg cells and decrease in Th1/Th17 cells in the intestine [105]. Along the same line, Zhao et al. recently reported that administration of *Bifidobacteria* greatly alleviates colitis by trinitrobenzene sulfonic acid in mice via up-regulation of IDO1 [192]. In contrast, IDO1 deficiency protected against *Citrobacter rodentium*- [69] and dextran sulfate sodium-induced colitis [159]. The pathogenetic role of IDO1 in experimental colitis has been attributed to the repression of

IL-10 production [111]. Although less extensively studied, IDO2 does not appear to be involved in the maintenance of normal homeostasis of colitis and in the exacerbation of IBD [159].

The role of Arg metabolism in IBD is also controversial. On one hand, both ARG1 and ornithine decarboxylase enzymes were found to be protective in *C. rodentium*-induced colitis, whereas deletion of iNOS significantly improved disease symptoms [58]. On the other, inhibition of ARG1 ameliorated dextran sulfate sodium induced colitis [3].

Thus, although a bulk of data would indicate IDO1 and ARG1 being critical players in IBD, no clear-cut definition of their role is available yet. This could be possibly due to the complex interplay of host immune responses with microbiota and/or the large variety of experimental models (i.e., chemically-versus bacteria-induced) used in IBD studies.

5.1.5. Psoriasis

Psoriasis is a chronic inflammatory disease of the skin characterized by poorly differentiated and hyperproliferative keratinocytes (epidermal hyperplasia) and extensive infiltration of various leukocytes, including T cells (mostly IL-17$^+$CD4$^+$), dendritic cells (mostly overproducing IL-23), macrophages, and neutrophils [155]. Although the etiology and pathogenesis of psoriasis remain primarily unknown, it is generally assumed that unbalanced immune molecular pathways and responses contribute to the disease process [42]. Both iNOS and ARG1 are overexpressed in the psoriatic epidermis of the majority of patients [1,23]. Moreover, significantly higher levels of Orn, one of the ARG1 products, are present in sera of patients with psoriasis [133]. Because high concentrations of NO can induce differentiation of keratinocytes [85], the high and sustained output of NO in psoriatic epidermis may attenuate the pathophysiological sequelae of psoriatic hyperproliferation. However, the concomitant high expression and activity of ARG1 would limit the availability of Arg and thus iNOS catalytic activity [1,23]. Therefore, ARG1 inhibitors have been proposed to be therapeutic in patients with psoriasis, at least in those overexpressing ARG1 and iNOS (i.e., approximately 70%).

In contrast to Arg metabolism, immune cells from patients with psoriasis are defective in inducing expression of IDO1 in response to inflammatory stimuli [93]. Moreover, in psoriasis-like dermatitis induced in mice by imiquimod, the intralesional injection of IDO1-expressing fibroblasts significantly improves the critical factors associated with psoriasis, including clinical appearance, skin erythema and scaling score, skin thickness, the number of infiltrated IL-17$^+$CD4$^+$ T cells, and IL-23 − producing dendritic cells [46]. Thus, at variance with ARG1, drugs capable of potentiating IDO1 expression and activity may represent valuable tools to control the disease.

5.1.6. Hashimoto's thyroiditis and Graves' disease

Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the most common forms — and the two extremes — of a wide spectrum of mixed thyroid autoimmune conditions that lead to thyrocyte death or hyperfunction, respectively [163]. HT, the most common cause of hypothyroidism, is characterized by gradual autoimmune-mediated thyroid failure with occasional goiter development [28]. The infiltration of hematopoietic mononuclear cells, mainly lymphocytes, can be detected in the interstitium among the thyroid follicles in most forms of the disease. In patients with GD, the production of anti-TSH − receptor antibodies promotes thyrocyte growth and unrestrained thyroid hormone secretion, resulting in hyperthyroidism with increased gland vascularity, mild lymphocytic infiltration, ophthalmopathy, and goiter [180]. Because of the quite distinct features between available experimental models and the human pathology, human studies (observational in nature) represent the primary source of information for understanding the pathogenesis of thyroid autoimmune diseases [163]. In patients with GD, the ratio of serum Kyn to Trp ratio as well as IDO1 expression in B cells and dendritic cells resulted increased as compared to healthy subjects [179]. However, in another study, lower levels of Kyn to Trp ratio in sera and expression of IDO1 in plasmacytoid dendritic cells (constituting a rare yet important dendritic cell subset in the induction of immune tolerance [172]), purified from PBMCs of HT and GD patients as compared to matched controls, were detected [90]. Although the causes of this discrepancy are not evident, factors such as genetic background, disease stage, and presence of leukocyte infiltration in the thyroid tissue could account the differences between the two studies. In GD but not HT patients, high expression of iNOS and eNOS was found in differentiated monocytes and thyroid follicular cells, respectively, suggesting a possible role for NO in the disease [96]. Although no insightful data for amino acid metabolism in HT and GD are available yet, it might be interesting to note that GD, similarly to other autoimmune diseases, significantly ameliorates during pregnancy and relapses postpartum [181]. This may be attributed to the fact that placenta syncytiotrophoblasts synthesize a number of immunologically active molecules, including IDO1, which suppress immune responses at the interface between mother and placenta. In contrast, no clinical change in HT occurs during pregnancy, although the dose of levothyroxine replacement treatment needs to be increased in the first trimester, as in all forms of hypothyroidism, due to the increased requirements of the fetus and mother [181].

5.2. Systemic autoimmune diseases

5.2.1. Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a multi-organ system autoimmune disease with clinical and serological heterogeneity as well as dysregulated IFN response [189]. Serum anti-nuclear, anti-ds-DNA, and anti-Smith antibodies are important biomarkers of the disease. In patients with SLE, elevated Trp degradation, significantly associated with high production of IFN-γ, has been correlated with disease activity, suggesting that IDO1 could be a key element shaping the pathogenic nature of chronic inflammation [118]. However, treatment of MRL.FAS$^{lpr/lpr}$ mice, an experimental model of SLE, with 1-methyl-tryptophan (an IDO1 catalytic inhibitor) accelerated the disease, suggesting a protective effect by Trp metabolism [146]. Similarly to human cells, MRL.FAS$^{lpr/lpr}$ splenocytes express more IDO1 than conventional, healthy mice [146]. Therefore, taking into consideration that IDO1 could be a counter-regulatory mechanism (meaning that it is induced by the proinflammatory signals that it acts to suppress) [121], high IDO1 expression in SLE and possibly in other autoimmune diseases would act to attenuate harmful inflammation.

Myeloid derived suppressor cells are a heterogeneous population of cells that expand during cancer, inflammation and infection, and that have a remarkable ability to suppress T-cell responses [55]. ARG1 and iNOS are thought to have a role in myeloid derived suppressor cell-mediated T-cell suppression. In a recent study, a significant increase in myeloid derived suppressor cells, positively correlated with serum levels of ARG1 activity, was found in PBMCs from SLE patients [186]. SLE myeloid derived suppressor cells promoted differentiation of pro-inflammatory Th17 cells in vitro in an ARG1-mediated fashion. Moreover, in humanized SLE mice (obtained by injection of PBMCs from SLE patients with active disease into immunodeficient mice), depletion of myeloid derived suppressor cells or ARG1 inhibition mitigated lupus nephritis-like symptoms [186]. Thus, although the mechanism whereby ARG1 would act as inflammatory mediator still remains obscure, these data would challenge the paradigm indicating ARG1 as a pure immunosuppressive molecule. In another study, administration of myeloid derived suppressor cells from healthy mice to roquin$^{san/san}$ mice (another SLE experimental model) resulted in reduction of anti-ds-DNA antibodies and proteinuria accompanied by the expansion of regulatory B cells [137]. In this case, the therapeutic effects were iNOS-dependent and ARG1-independent. There might be important differences between human SLE and experimental models regarding NO effects. In fact, whereas deficiency of eNOS in MRL.FAS$^{lpr/lpr}$ mice increases renal lesions [57], human SLE is characterized by abnormally high levels of NO in T lymphocytes and reduction of the eNOS inhibitory protein [52].

Thus it appears that there is still much to investigate in SLE in order understand the role, protective versus pathogenetic, of enzymes catabolizing Trp and Arg.

5.2.2. Systemic sclerosis and scleroderma

Systemic sclerosis (SSc), also termed scleroderma, is a generalized disorder characterized principally by skin thickening, vascular disease, and immune dysfunction [89]. Among autoimmune diseases, SSc appears as a peculiar multifaceted disease, in which autoimmune phenomena coexist with vascular abnormalities and multi-visceral fibrosis [70,143]. Several genetic pre-clinical models (tight-skin mice, Fra-2 mice, TGFβ-R2Δk mice, and UCD200 chicken) have been used to study SSc in the last 20 years, although they rarely encompass the systemic nature of SSc, with simultaneous skin and lung fibrosis, vasculopathy, and autoimmunity. In SSc patients, the percentage of $CD11b^{+}IDO1^{+}$ cells in PBMCs has been found to be much lower than that of healthy subjects [88]. As mentioned above, the presence of the intronic SNP rs7820268 of the *IDO1* gene is prevalent in SSc patients and is associated with an impaired suppression activity of $CD8^{+}$ Treg cells [175]. Because the same SNP correlates with the risk of developing T1D and also results in defective IDO1 catalytic activity in PBMCs from pediatric patients with autoimmune diabetes [131], SSc patients may also be characterized by defective Trp metabolism impairing immunoregulatory mechanisms. Besides that, Kyn, the main product of IDO1, has been shown to exert anti-fibrotic effects in both in vitro and in vivo studies (i.e., wound models) by attenuating the fibrosis genetic program via activation of AhR [142]. In contrast, serotonin, also a Trp metabolite, promotes fibrosis in the same conditions. Interestingly, in fibroblasts, the use of an IDO1 inhibitor has been shown to favor the balance towards the Trp-serotonin pathway, further outlining the existence of a delicate equilibrium between distinct Trp metabolic pathways and potential pathologic consequences (such as fibrosis in SSc) deriving from alteration of this equilibrium [43].

In contrast to IDO1, ARG1 activity has been associated with induction of tissue remodeling and fibrosis [103]. High levels of ARG1, detected in the skin of SSc patients, could be caused in turn by the high expression of thymic stromal lymphopoietin, particularly in cutaneous perivascular areas and immune cells [37]. In tight-skin mice, administration of paquinimod, a quinoline derivative (by the way, quinolinic acid is a metabolite downstream IDO1 along the kynurenine pathway; Fig. 1), reduced skin fibrosis by skewing macrophage polarization from M2 (expressing ARG1) to M1 (expressing iNOS) [164]. Because administration of pirfenidone, an ARG1 inhibitor, showed therapeutic effects in patients with idiopathic pulmonary fibrosis [14], inhibition of ARG1 activity could represent a valuable option, at least for the fibrotic issue, also in SSc. However, ARG1 inhibition may skew Arg metabolism towards production of NO, considered to represent the sign of lung inflammation and initiation of interstitial lung disease in SSc in both patients and experimental animal models [76].

5.2.3. Sjogren's syndrome

Sjögren syndrome (SS) is a systemic autoimmune disease that manifests with sicca symptomatology of mucosal surfaces, mainly dry mouth and dry eyes. There is often systemic involvement (extraglandular manifestations) and lymphoma is a recognized complication. SS is one of the most prevalent autoimmune diseases (with an estimated 0.5 million to 3 millions affected persons in the United States, primarily perimenopausal women) [145]. In salivary glands, SS manifests in the form of focal T lymphocyte-rich infiltrates and xerostomia.

In a study aimed at evaluating the role of NO in SS [83], higher levels of nitrites were found in the saliva of SS patients as compared to healthy subjects. Excess NO in SS was produced by salivar gland acinar and ductal epithelial cells rather than immune cells. The authors postulated that NO might contribute to inflammatory damage and acinar cell atrophy in SS [83]. The systemic type I IFN signature, which can be assessed on the basis of expression of multiple IFN-inducible genes, has been found in more than one-half of patients with primary SS and identifies a subgroup of patients with more active disease [22]. In that subgroup, higher IDO1 activity (measured as Kyn to Trp ratio in sera and gene transcripts in $CD14^{+}$ monocytes) and increased $CD25^{high}Foxp3^{+}$ Treg cells were detected [101]. In addition, kynurenine monooxygenase (producing proapoptotic 3-hydroxykynurenine) and kynurenine aminotransferase I, III, and IV (producing neuroprotective kynurenic acid; Fig. 1) were up- and down-regulated, respectively. The authors hypothesized the occurrence of an imbalanced kynurenine pathway in SS. In another study, the in vitro treatment of PBMCs from SS but not RA patients with hCDR1, a tolerogenic peptide that ameliorates manifestations of experimental lupus [165], increased IDO1 expression and Treg cell numbers in a TGF-β − dependent fashion, suggesting that the IDO1 signaling rather than catalytic activity could be at work in this condition [166]. In contrast, the expression of pro-inflammatory cytokines was down-regulated. Massive up-regulation of IDO1 expression and conversion of Th17 into Treg cells was found in SS PBMCs incubated with human umbilical cord mesenchymal stem cells, endowed with tolerogenic properties [8]. Therefore, at variance with the previous study [101], data obtained with hCDR1 and mesenchymal stem cells may indicate IDO1 as an immunoregulatory and protective molecule in SS.

6. Amino acid metabolism in the development of neutralizing antibodies to therapeutic proteins

In about three decades, the use of therapeutic proteins has drastically changed the treatment of an increasing number of diseases. Many different types of biotherapeutics (such as antibodies, clotting factors, and hormones) were progressively added to the already available treatment options and this allowed for major progresses in the management of a number of different diseases. However, it rapidly became evident that many of these products carried the risk of the development of an unwanted immune response.

Immunogenicity of biopharmaceuticals can be a serious and unpredictable outcome of the administration of therapeutic proteins obtained by recombinant DNA and hybridism technologies [151] and has the potential to undermine the treatment efficacy. Several potential factors that might contribute to breaking host tolerance in response to biodrugs can occur. The immunogenic profile of therapeutic proteins and the risk to patients can be determined by numerous product- and patient-related factors. These proteins necessarily undergo numerous production steps during a complex manufacturing process, with each step potentially affecting not only the pharmacokinetics and pharmacodynamics of the prospective biodrug [25], but also its ability to trigger immunity to epitopes of the protein or processed peptides thereof [84].

6.1. Factor VIII and hemophilia

The development of an immune response to exogenous therapeutic proteins is still a serious complication in the treatment of many diseases. In particular, anti-drug antibodies constitute a major concern in the therapy of people affected by bleeding disorders when using plasma-derived or recombinant clotting factor concentrates [139]. Following administration of factor VIII (FVIII) concentrates, patients with severe hemophilia A can develop anti-FVIII neutralizing antibodies in approximately 20–40% of cases [79]. These neutralizing antibodies are commonly referred to as FVIII inhibitors.

FVIII inhibitors are polyclonal antibodies that can target multiple antigenic sites within the A2, A3, and C2 domains of the FVIII protein [95]. When such immune response occurs, it essentially eliminates all pro-hemostatic effects of the treatment and causes a substantial increase in morbidity and mortality for patients [12]. Patients who develop antibodies to FVIII or other coagulation factors can present with severe bleeding that can be difficult to treat. This also precludes the

prophylactic administration of factors that has shown to be effective in preventing the long-term consequences of intra-articular bleeding, known as hemophilic arthropathy [98]. Overall, the development of neutralizing antibodies in hemophilia A and B can transform these disorders from a treatable to refractory condition, which represents the major complication of the treatment.

A potential link between Trp catabolism by IDO1 and the immune response to FVIII concentrates has been shown in different experimental settings. Liu and colleagues demonstrated that the co-delivery of FVIII and IDO1 coding genes could prevent the development of FVIII inhibitors leading to an enhanced therapeutic response [91]. In this report, sleeping beauty transposons harboring both IDO1 and FVIII genes were administered by hydrodynamic injection in hemophilic mice. This treatment led to a significant reduction in inhibitor development and revealed that the reduction in inhibitor titers was correlated with higher plasma Kyn concentrations. These findings indicated that modulation of Trp catabolism pathway attenuates inhibitor formation in a gene therapy model of hemophilia A. In 2011, Allacher et al. showed a suppressive effect of high-dose CpG oligodeoxynucleotide on inhibitor production in a preclinical hemophilia A model [7]. In particular, the treatment inhibited FVIII-specific memory responses at high concentrations, both in vitro and in vivo. The authors suggested that a possible mechanistic explanation for the observed effects could be the induction of IDO1 in immune cells, and more specifically in dendritic cells [178]. In a different setting, it was later shown that the administration of IL-2/anti−IL-2 monoclonal antibody complexes together with weekly injections of low dosage FVIII protein could induce long-term tolerance to FVIII. Measurement of Kyn levels during these experiments demonstrated a significant increase in mouse plasma synchronized with Treg cell expansion in the tolerized mice, suggesting that the immunoregulatory effects were correlated with the activation of the Trp degradation pathway catalyzed by IDO1 [92]. However, although interesting and of potential clinical relevance, a direct involvement of IDO1 and Trp catabolites in suppressing inhibitor development was clearly demonstrated only a few years after these initial observations.

In a large, multi-center, cross-sectional study of IDO1 induction, expression, and function was investigated for any relationship with inhibitor occurrence in severe hemophilia A patients [104]. In this study, multivariable logistic regression results, adjusted for other known variables potentially influencing the development of FVIII inhibitors, confirmed that a defective IDO1 induction was associated with an inhibitor positive status. In addition, in the same study, induction of Trp catabolism in hemophilic mice promoted long-term tolerance to FVIII, even after repeated treatment with the recombinant human FVIII protein. As a consequence, a reduced number of FVIII-specific antibody-secreting cells was found in the spleen of treated mice together with an enhanced Treg cell response. These effects, obtained using CpG oligodeoxynucleotide in FVIII knockout mice, were abolished in FVIII/IDO1 double knockout mice.

Overall, evidence for the role of Trp catabolism in the development of drug-specific antibodies in hemophilia has reinforced the concept that IDO1 is a critical component in the complex system that allows long-term control of acquired immune tolerance in the adult life.

7. Clinical perspectives and personalized medicine

Most of the studies demonstrating a relationship between alterations of amino acid metabolism and immune system functions have been carried out in vitro or in experimental animal models. However, several of the collected data seem to indicate that they can be transferred to humans and an accurate direct or indirect modulation of amino acid metabolism can play a relevant role in the prevention or treatment of human autoimmune diseases.

One of the best examples in this regard is given by the use of molecules able to influence the oxidative catabolism of Trp through modulation of IDO1 activity in patients with T1D by means of administration of tocilizumab, a humanized monoclonal antibody specific for IL-6R. IL-6 has a well-documented role in chronic inflammatory and autoimmune diseases. In patients with T1D, a higher IL-6R expression [77] was found to be associated with very low levels of IDO1 [131], as a consequence of the IL-6 − dependent proteasomal degradation of the IDO1 protein [129]. In fact, addition of tocilizumab to PBMCs of children with T1D restored IDO1 levels in one third of study samples, thus indicating the potential efficacy of tocilizumab for T1D immunotherapy. A phase II study (NCT02293837), specifically planned to evaluate the therapeutic effect of tocilizumab on the maintenance of C-peptide levels − as indicator of preservation of β-cell life − in adults (18–45 years of age) and children (6–17 years of age) with new onset T1D (i.e., within 100 days of study enrollment) is presently ongoing in the United States sponsored by the National Institute of Allergy and Infectious Diseases. However, the evidence that addition of tocilizumab to PBMCs restores IDO1 activity in vitro only in a portion of patients with T1D will indicate that IL-6R blockade cannot be considered potentially effective for all patients with T1D but that the tocilizumab treatment should be personalized. It is likely that tocilizumab is effective only in those patients with reduced IDO1 activity (associated with the presence of the SNP rs7820268), and a screening test for measuring IDO1 activity in PBMCs from T1D patients before initiation of tocilizumab administration could be predictive of the final efficacy of this drug, leading to a personalized form of drug therapy. A second potential approach for T1D prevention and therapy based on modulation of IDO1 activity and Trp catabolism is the use of a chimeric vaccine composed of proinsulin and the cholera toxin B subunit. The vaccine upregulated IDO1 biosynthesis in human dendritic cells, suggesting the possibility of developing a new safe and effective immunotherapeutic strategy for the prevention of autoimmunity in T1D [106].

Another example of the potential use of amino acid metabolism modulation to reduce the impact of autoimmunity in humans is given by the use of a monoclonal antibody against IDO2 for RA treatment. IDO2 induces autoreactive B and T cell responses that lead to RA and a recent study demonstrated that administering a cell-penetrating monoclonal antibody against IDO2 it is possible to simultaneously inhibit immune responses and reduce inflammation in a preclinical model of RA [110]. Unfortunately, no studies in humans are available yet. The availability of small compounds with drug properties and inhibiting IDO2 activity may speed up the transition of IDO2 basic research to the clinics.

Finally, ARG1 inhibitors can be potently used to treat psoriasis. However, also in this case, a personalized therapy seems needed as these substances may be effective only in those psoriatic patients with demonstrated overexpression of ARG1 and iNOS [23] and therefore an appropriate selection of potential responder patients should be performed prior treatment. However, the coupling of enzyme expression with variations in relevant genes (known for *IDO1* and also *IL6*) may better guarantee a successful immunotherapy of autoimmune diseases based on modulation of amino acid metabolism. Nevertheless, additional efforts are required in many cases to identify the actual causative alleles, their functional consequences and the biological mechanisms through which they influence disease pathogenesis. Indeed, despite the undeniable evidence gathered so far, the development of strategies translating insights on the genetic basis of autoimmunity into improved patient outcomes is a major challenge, given the relatively small effect size of the identified variants, which is typically not discriminatory enough to inform clinical decision-making. A key step towards this improved understanding will be to define in vivo and ex vivo cellular and molecular immune traits implicated in the development of autoimmunity and that are influenced by genetic factors. In this regard, the longitudinal measurement of a wide range of immunophenotypes, such as signaling responses, immune cell abundances and serum levels of cytokines and other inflammatory mediators, using robust sample sizes may provide critical information to be exploited in the future in

personalized medical interventions in the context of autoimmunity [132]. An improved understanding of the genetic variation in amino acid metabolism is also expected to strongly influence the success of immunotherapeutic strategies, particularly if aiming at the manipulation of these and other related pathways. The application of systems biology and next-generation sequencing technologies has provided the exciting possibility to identify essential genes and pathways in autoimmunity at a level of complexity that was previously impossible. By exploring this novel and unbiased understanding of the genetic regulation of individual metabolic traits with mechanistic insights, personalized medical interventions in autoimmune diseases may become a reality in the near future.

8. Conclusion and future perspective

In several autoimmune disorders, an effective immunotherapy is still demanding. Trp and Arg metabolism represent critical immune checkpoint mechanisms clearly exerting immunoregulatory effects in health and neoplasia. The current review provided a comprehensive analysis of the known role of IDO1 and ARG1, the main enzymes involved in Trp and Arg metabolism, respectively, in the most prevalent organ-specific and autoimmune diseases as well as in the development of autoantibodies towards biodrugs. Although no uniform picture emerged, the bulk of data indicated IDO1 rather than ARG1 as suitable target to be potentiated in several autoimmune disorders and in therapies with recombinant proteins. In fact, although the studies might be still limited, ARG1 appeared to be often pathogenetic rather than protective, possibly owing to the enzyme capacity of inducing fibrosis and/or failing to promote immunoregulatory effects in pronounced inflammatory contexts. Therefore, further investigations of Arg metabolism in autoimmune disorders may provide important pieces to define its biology and functional meaning. Regarding IDO1, the development of drugs enhancing the catalytic and/or signaling function of the enzyme either indirectly (i.e., tocilizumab) or directly [5] may bring some fresh air in the autoimmunity disease space.

Disclosure

There is no conflict of interest to be discussed.

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