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## Commentary

## Aminoacyl-tRNA synthetases, therapeutic targets for infectious diseases

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## ABSTRACT

Despite remarkable advances in medical science, infection-associated diseases remain among the leading causes of death worldwide. There is a great deal of interest and concern at the rate at which new pathogens are emerging and causing significant human health problems. Expanding our understanding of how cells regulate signaling networks to defend against invaders and retain cell homeostasis will reveal promising strategies against infection. It has taken scientists decades to appreciate that eukaryotic aminoacyl-tRNA synthetases (ARSs) play a role as global cell signaling mediators to regulate cell homeostasis, beyond their intrinsic function as protein synthesis enzymes. Recent discoveries revealed that ubiquitously expressed standby cytoplasmic ARSs sense and respond to danger signals and regulate immunity against infections, indicating their potential as therapeutic targets for infectious diseases. In this review, we discuss ARS-mediated anti-infectious signaling and the emerging role of ARSs in antimicrobial immunity. In contrast to their ability to defend against infection, host ARSs are inevitably co-opted by viruses for survival and propagation. We therefore provide a brief overview of the communication between viruses and the ARS system. Finally, we discuss encouraging new approaches to develop ARSs as therapeutics for infectious diseases.

## 1. Introduction

Humans come into contact with a range of infectious microbes, including bacteria, viruses, and parasites. Diverse safeguard systems are available to detect and defend against invading pathogens. Upon recognition of pathogen-associated molecular patterns, cells transmit intracellular signals to trigger immediate proinflammatory and antimicrobial responses [1]. These innate immune responses are the first line of defense in the early phase of infection. Toll-like receptors (TLRs), Nod-like receptors, and retinoic acid-inducible gene-I (RIG-I)-like receptors are representative families of innate immune receptors that distinguish microbial products as ‘non-self’ molecules [2].

TLRs can be grouped into two types depending on their cellular locations: cell surface TLRs (1, 2, 4, 5, and 6) and endosomal TLRs (3, 7,

8, and 9) [3,4]. Surface-exposed TLRs mainly detect bacterial products, while TLRs located in intracellular compartments recognize nucleic acids [1]. RIG-I mediates antiviral signaling by sensing viral RNA and subsequently interacting with the central antiviral signaling protein, mitochondrial antiviral signaling protein (MAVS) [5]. This signaling cascade ultimately leads to the transcription of type I interferons (IFNs) and other antiviral molecules [5,6]. All these innate immune responses orchestrate the early host response to infection, which subsequently activates and shapes the adaptive immune response [1,7]. The adaptive immune system controls the late phase of infection and generates immunological memory through T cell- or B cell-mediated (humoral) mechanisms [8,9].

Well-integrated defense systems will provide effective and long-lasting protective immunity [2]. The induction, maintenance, and

**Abbreviations:** ARS, aminoacyl-tRNA synthetase; TLR, toll-like receptor; RIG-I, retinoic acid-inducible gene-I; MAVS, mitochondrial antiviral signaling protein; IFN, interferon; ITCH, E3 ubiquitin-protein ligase Itchy homolog; MSC, multi-tRNA synthetase complex; AIMP, ARS-interacting multifunctional protein; WT, wild-type; BMDC, bone marrow-derived dendritic cell; Oas, 2'-5'-oligoadenylate synthase; HCV, hepatitis C virus; PTM, post-translational modification; GAIT, IFN- $\gamma$ -activated inhibition of translation; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase kinase; ERK, extracellular-related kinase; MITF, microphthalmia-associated transcription factor; PCBP2, poly(rC)-binding protein 2; T<sub>H</sub>1, T-helper type 1; LPS, lipopolysaccharide; TCID<sub>50</sub>, a median tissue culture infective dose; PBMC, peripheral blood mononuclear cell; BMDM, bone marrow-derived macrophage; TLS, tRNA-like structure; eEF1A, eukaryotic elongation factor 1 alpha; MDA5, melanoma differentiation-associated protein 5; HIV, human immunodeficiency virus; TGF- $\beta$ , tumor growth factor- $\beta$ ; IFN- $\alpha$ , interferon- $\alpha$ ; IL-1 $\beta$ , interleukin-1 $\beta$ ; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; EMAPII, endothelial monocyte-activating polypeptide II; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; IP-10, interferon-inducible protein 10; CRP, C-reactive protein; HMGB1, high mobility group box 1; AA-AMP, aminoacyl-adenylate

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termination of host immune responses to infection must be tightly controlled [10]. Turning off the immune system is as important as turning it on to avoid unwanted immune responses. Several positive and negative regulatory mechanisms control the immune response through a complex signaling network [11–13]. A number of endogenous proteins that either enhance or limit the magnitude and duration of the inflammatory response regulate this system. These non-immune proteins serve as a second safeguard system by fine-tuning host immune responses and ensuring cellular homeostasis. For example, a mitochondrial import receptor protein, Tom7, positively regulates MAVS signaling by serving as a critical adaptor linking MAVS to downstream signaling molecules that activate antiviral immunity [14]. Alternatively, cells have developed diverse strategies to halt antiviral signaling through MAVS [15]. Several E3 ligases, including E3 ubiquitin-protein ligase Itchy homolog (ITCH), Smurf1, Gp78, and TRIM25, have been shown to accelerate MAVS degradation after viral infection [16–18]. Collectively, cells employ multiple mechanisms to regulate immunity against microbial invaders, with the help of non-immunogenic proteins to prevent uncontrolled cellular damage.

Aminoacyl-tRNA synthetases (ARSs) are essential components for translation in every living species. Although the role of ARSs in protein synthesis is well recognized, it has taken scientists decades to acknowledge their roles beyond translation. There is an apparent propensity for new sequences and domains to be added to ARSs during evolution. The emergence of such new domains is consistent with the involvement of ARSs in a broad range of biological functions in addition to protein synthesis and correlates with the increasing biological complexity of higher organisms.

Interestingly, the activity of many ARSs in higher eukaryotes appears to be regulated by their presence in the cytoplasmic multi-tRNA synthetase complex (MSC), which is assembled in most cases via the appended domains and consists of nine ARSs [EPRS (which consists of ERS and PRS), DRS, IRS, KRS, LRS, MRS, QRS, and RRS, according to the standard nomenclature in which the ARSs are designated by the single-letter amino acid code followed by “RS”] and three auxiliary ARS-interacting multifunctional proteins (AIMPs), AIMP1, AIMP2, and AIMP3 [19]. MSC has been proposed to function as a depot system to perform specific regulatory functions via release of its components [19].

Recent studies identified novel molecular and cellular processes through which ARSs control host immune responses against infections [20–22]. EPRS contributes to innate antiviral immunity and promotes viral clearance [20]. The MSC protein AIMP1 also has a critical role in innate and adaptive antiviral immunity against influenza A virus infection [21]. In addition, secreted WRS acts as an early warning signal of host infections [22]. Together, these findings suggest that ARSs may act as cellular sensors as well as immunoregulators in the context of infection.

In this review, we will provide an overview of the current understanding of the mechanisms through which the ubiquitous, non-immunogenic ARSs are appropriated to regulate cell signaling upon infection. We also briefly discuss current strategies targeting pathogenic ARSs for antibiotics development. We hope that this review will stimulate emerging research in the field of ARSs in infectious diseases, including clinical applications for ARSs in anti-infective pharmacology.

## 2. Immunoregulatory roles of mammalian ARSs against infection

### 2.1. Multi-omics profiles reveal that ARSs respond to infection

Profiles of the transcriptional and proteomic changes in infected cells provide key information about cellular factors that may be central to host immune responses [23]. Multiple lines of evidence support a role for ARSs in the infected host. Our group conducted an RNA sequencing analysis of human bronchial epithelial cells infected with influenza A virus at 8 h and 24 h post-infection [20]. We particularly

noted that the MSC components *IRS*, *RRS*, *EPRS*, *AIMP1*, and *AIMP3* were up-regulated at 8 h, while the expression of *KRS* gradually increased up to 24 h [20]. With respect to non-MSC ARS genes, early induction was observed for *YRS*, *TRS*, and *SRS*, whereas the expression of the *WRS* gene was increased at both 8 h and 24 h post-infection (GEO accession code for RNA-Seq data: GSE75699).

In agreement with our data, another group showed that *AIMP1* is up-regulated after influenza A infection. This transcriptome analysis revealed that *AIMP1* is closely related to antiviral immunity [21]. Microarray analysis of antigen-loaded wild-type (WT) or *AIMP1*<sup>-/-</sup> mouse bone marrow-derived dendritic cells (BMDCs) showed that *AIMP1* regulates a variety of immune processes through important antiviral genes including IFN-activated genes, IFN regulatory factor genes, and innate immune sensors such as 2'-5'-oligoadenylate synthase (*Oas*) family genes and *Ddx58*.

An integrated analysis of microarray and proteomics data showed that hepatitis C virus (HCV)-infected hepatocytes up-regulate the *WRS*, *SRS*, *AlaRS*, *TRS*, and *GRS* genes and express higher levels of *YRS* and *SRS* proteins [24]. In A549 cells inoculated with swine influenza virus, *WRS* was up-regulated at both the mRNA and protein levels [25]. Increased expression of *WRS* was also observed in cells infected with *Vibrio cholera*, human cytomegalovirus, and human hepatitis B virus [22].

A microarray analysis revealed upregulation of ARS genes (including the MSC members *Irs*, *Lrs*, *Mrs*, *Rrs*, and *Eprs*, as well as the free ARSs *Alars*, *Crs*, *Grs*, *Nrs*, *Srs*, *Trs*, *Vrs*, *Wrs*, and *Yrs*) in the brains of mice infected with flaviviruses, including Japanese encephalitis virus and West Nile virus, which are important causes of central nervous system diseases [26]. Note that this study performed the RNA analysis at 5–8 days post-infection, when clinical symptoms of encephalitis are already apparent [26]. All these data indicate that ARSs are responsive to infection and may be involved in immune regulation against infection.

In addition to these transcriptional and proteomic analyses, post-translational modifications (PTMs) in ARSs have been characterized in response to various stimuli [27]. These studies revealed their diverse and extended functions in biological and pathological processes [28,29]. Phosphorylation is the most frequent PTM occurring in ARS proteins, and it is thought to induce structural changes that may drive their dissociation from the MSC and/or allow for new interacting partners [30]. It is well established that the Ser886 and Ser999 residues of the EPRS WHEP domains are sequentially phosphorylated in response to IFN- $\gamma$  stimulation. IFN- $\gamma$  induces phosphorylation of EPRS at Ser886 by activating cyclin-dependent kinase 5 and indirectly induces phosphorylation at Ser999 via a distinct kinase [31]. This promotes the dissociation of EPRS from the MSC, and subsequently promotes its association with the IFN- $\gamma$ -activated inhibition of translation (GAIT) complex [32]. The heterotetrameric GAIT complex, including EPRS, binds to distinct 3'-untranslated regions of mRNAs involved in chronic inflammation to suppress their translation [33]. RNA virus infection-specific phosphorylation was also identified at the EPRS Ser990 residue [20]. This modification was critical for dissociation of EPRS from the MSC and activation of antiviral signaling [20].

Because infections cause massive production of diverse cytokines and activate multiple cellular signaling pathways, including the mitogen-activated protein kinase (MAPK) cascade, infectious signals may also indirectly induce PTMs of ARS proteins [5,34]. In the case of *KRS*, allergen stimulation of activated mast cells results in phosphorylation of *KRS* at Ser207 in a MAPK-dependent manner [35]. Signaling through MAPK kinase kinase (MEK)/extracellular-related kinase (ERK), but not p38 MAPK, was critical for the *KRS* phosphorylation. Ultimately, this phosphorylation triggered dissociation of *KRS* from the MSC and its translocation into the nucleus, where it controls the level of diadenosine tetraphosphate [36], an important signaling molecule in microphthalmia-associated transcription factor (MITF) transcriptional activity [37]. Therefore, *KRS* phosphorylation might be induced directly

by infection-related MAPK signaling or indirectly by infection-induced products like cytokines, which can activate multiple regulatory pathways.

Collectively, these changes in the expression and regulation of ARSs by microbial infections suggest that ARSs have substantial potential to regulate pathogenesis and anti-infective immunological responses. ARSs are ubiquitous and abundant proteins; therefore, transient changes in their expression may not significantly affect global protein synthesis. Alteration of TRS and AlaRS expression does not markedly alter global protein synthesis [38]. PTMs located in the additional domains of vertebrate ARSs do not affect aminoacylation of these enzymes [20,33]. However, PTMs of residues located in the catalytic core domain can influence the enzymatic activities of ARSs. Phosphorylation of MRS at Ser662, which is located in the critical tRNA-binding site, significantly down-regulates global translation by inactivating methionine-charging activity until damaged DNA is repaired [39]. By looking at the distinct transcriptional and protein expression profiles of ARSs, as well as the dynamics of their regulation by PTMs, novel, infection-specific roles for individual ARSs will be elucidated. Future studies should investigate whether changes in the expression and PTMs of each ARS upon infection correlate with its canonical tRNA aminoacylation activity and global protein synthesis as well as with its non-canonical functions.

## 2.2. The role of EPRS in antiviral immunity

Human EPRS, one of the members of the MSC, is the only bifunctional enzyme for protein synthesis. It comprises ERS and PRS, which are linked together by three WHEP domains [40]. During the past two decades, EPRS has become known for its non-canonical function as a translational silencer by forming the IFN- $\gamma$ -dependent GAIT complex [32,40,41].

Our group hypothesized that the cytoplasmic MSC may function as a surveillance system for infection. We found that the MSC immediately senses invasion by RNA viruses and activates EPRS to promote antiviral immunity via its infection-specific phosphorylation and release from the MSC [20]. EPRS knockdown in macrophages increased the replication of the RNA viruses influenza A virus and vesicular stomatitis virus, and reduced the production of antiviral cytokines. Conversely, ectopically expressed EPRS in macrophages significantly attenuated viral replication and increased antiviral IFN- $\beta$  production. Moreover, RNA viruses were much more lethal in EPRS<sup>+/-</sup> haploid mice due to the high rates of viral replication and weak antiviral cytokine responses. Intriguingly, infection with RNA viruses led to a previously unreported phosphorylation of EPRS at Ser990 that triggers the release of EPRS from the MSC. This phosphorylation was not induced by IFN- $\gamma$ , and thus the released EPRS was not involved in the GAIT system. Instead, the EPRS associated with poly(rC)-binding protein 2 (PCBP2), a well-known negative regulator of MAVS that mediates its degradation via ubiquitin ligase ITCH [13] after release from the MSC. Ultimately, the phosphorylated EPRS protected MAVS from PCBP2-mediated ubiquitination and degradation, and the stabilized MAVS activated antiviral immunity.

These data indicate that EPRS displays a phospho-code that controls its function in response to different stimuli. Furthermore, the role of EPRS in antiviral immune responses demonstrates the functional significance of the MSC as an immune regulatory system in the early stages of infection.

## 2.3. The role of AIMP1 in innate and adaptive antiviral immunity

AIMP1 is a non-enzymatic component of the MSC that mainly associates with RRS, QRS, and another non-enzymatic component, AIMP2, within the complex [42]. Extracellularly, AIMP1 activates innate immune cells such as macrophages and monocytes to produce inflammatory cytokines through MAPK signaling [43] and induces

maturation of BMDCs [44]. AIMP1 potentiates the link between innate and adaptive immunity by activating NK cells [45] and B cells [46] and by promoting IL-12-mediated T-helper type 1 (T<sub>H</sub>1) cell immunity [47]. AIMP1 expression in BMDCs directly promoted T<sub>H</sub>1 cell polarization, which is characterized by the secretion of IL-12 and IFN- $\gamma$  from antigen-presenting cells and T cells, respectively, and is associated with the generation of cell-mediated adaptive immune responses [48]. These adaptive responses were critical for effective clearance of intracellular infections as well as antitumor immunity [49,50]. Lipopolysaccharide (LPS) treatment of AIMP1<sup>-/-</sup> BMDCs impaired the secretion of the T<sub>H</sub>1 polarizing cytokine IL-12p70, but secretion of the proinflammatory cytokines IL-6 and IL-1 $\beta$  was not changed. AIMP1<sup>-/-</sup> BMDCs also expressed lower levels of costimulatory molecules and MHC class II following LPS treatment. The p38 MAPK signaling pathway was also significantly impaired in AIMP1<sup>-/-</sup> BMDCs which reduced T<sub>H</sub>1 polarization [21].

AIMP1 is also critical for innate and adaptive antiviral immunity against influenza A virus infection [21]. Microarray analysis of WT and AIMP1<sup>-/-</sup> BMDCs suggested its close correlation to the expression of antiviral genes. Challenge of AIMP1<sup>-/-</sup> mice with influenza A virus (H3N2) by aerosol spray resulted in 100% lethality, whereas WT mice showed only 20% lethality at a high infectious dose [a median tissue culture infective dose (TCID<sub>50</sub>) of 20] per mouse at 2 weeks post-infection. A sub-lethal dose of 7.50 TCID<sub>50</sub> per mouse still caused 60% mortality among AIMP1<sup>-/-</sup> mice, while all WT mice survived. Evaluation of survivors indicated that the levels of infiltrating neutrophils and macrophages in bronchoalveolar lavage fluid were significantly reduced in AIMP1<sup>-/-</sup> mice on day 7 post-infection. Moreover, IFN- $\gamma$ + T cells in the lung and levels of IgG2a (the T<sub>H</sub>1-specific antibody isotype) in the serum were highly reduced in AIMP1 knockout mice on day 15 post-infection. By varying the titers of infectious virus and investigating different time-points, this study revealed that AIMP1 deficiency can lead to insufficient innate and adaptive antiviral immune responses *in vivo* [21]. This was the first report on the role of MSC components in adaptive immunity during sequential phases of virus infection. However, further studies will be necessary to determine whether dissociation of AIMP1 from the MSC and its secretion by cells are required for its function against viral infection. Whether AIMP1 functions differently in intracellular and extracellular environments in infection should also be addressed by future studies. Such molecular details will strengthen the interrelated roles of AIMP1 in innate and adaptive immunity in infectious disease.

## 2.4. WRS as a primary responsive molecule to infection

In addition to its enzymatic activity, human WRS contains an N-terminal extension WHEP domain. WRS exists as two different forms; the major form is the full-length WRS (fWRS, aa 1–471), and the other form is a truncated version (mini-WRS, aa 48–471) in which the N-terminal WHEP domain is deleted by alternative splicing [51]. Both forms of WRS are secreted by various cells, and the fWRS is further cleaved into T1 (aa 71–471) or T2 (aa 94–471), which possess angiostatic activity, by leukocyte elastase or plasmin in the extracellular space [52]. Secreted WRS also plays an important role in high-affinity Trp uptake by human cells, which is linked with immune regulation [53].

Ahn et al. reported a critical role for human WRS as a primary responsive molecule to activate innate immune defense signaling against bacterial infection [22]. They showed that fWRS primes innate immunity upon bacterial infection, although infected cells secreted both fWRS and mini-WRS, suggesting that the N-terminal WHEP domain is responsible for control of infection [22].

These authors further showed that the secretion of WRS is significantly increased in sepsis patients infected with pathogens, including Gram-negative bacteria, Gram-positive bacteria, and fungi, but not in sepsis patients with sterile inflammation. WRS was secreted from

monocytes upon infection and directly bound to the TLR4-MD2 complex on macrophages to activate phagocytosis and chemokine production. WRS seems to function prior to the full activation of the immune response in infected cells. Its secretion occurred relatively early, at around 15 min post-infection, which is much earlier than TNF- $\alpha$  production, which could be first detected at 120 min [22]. These results suggest that WRS acts as a warning molecule to prime the first line of defensive signaling against invading microbes.

Interestingly, treatment of human peripheral blood mononuclear cells (PBMCs) with WRS induced their production of the chemokines CCL3 and CCL4, which recruit inflammatory cells, particularly neutrophils and macrophages. Moreover, secreted fWRS increased phagocytic cell numbers in mice and enhanced the phagocytic activity of mouse bone marrow-derived macrophages (BMDMs). Furthermore, administration of WRS to *Salmonella typhimurium*-infected mice increased neutrophil infiltration and decreased the bacterial load in the spleen and liver, which ultimately improved the survival of the infected mice [22].

It would be very interesting to see the molecular details underlying the rapid and infection-specific secretion of WRS. Further studies will also be required to identify the mechanisms through which WRS secretion is tightly controlled by different signals. Fig. 1 and Table 1 summarize the immunoregulatory roles of ARSs.

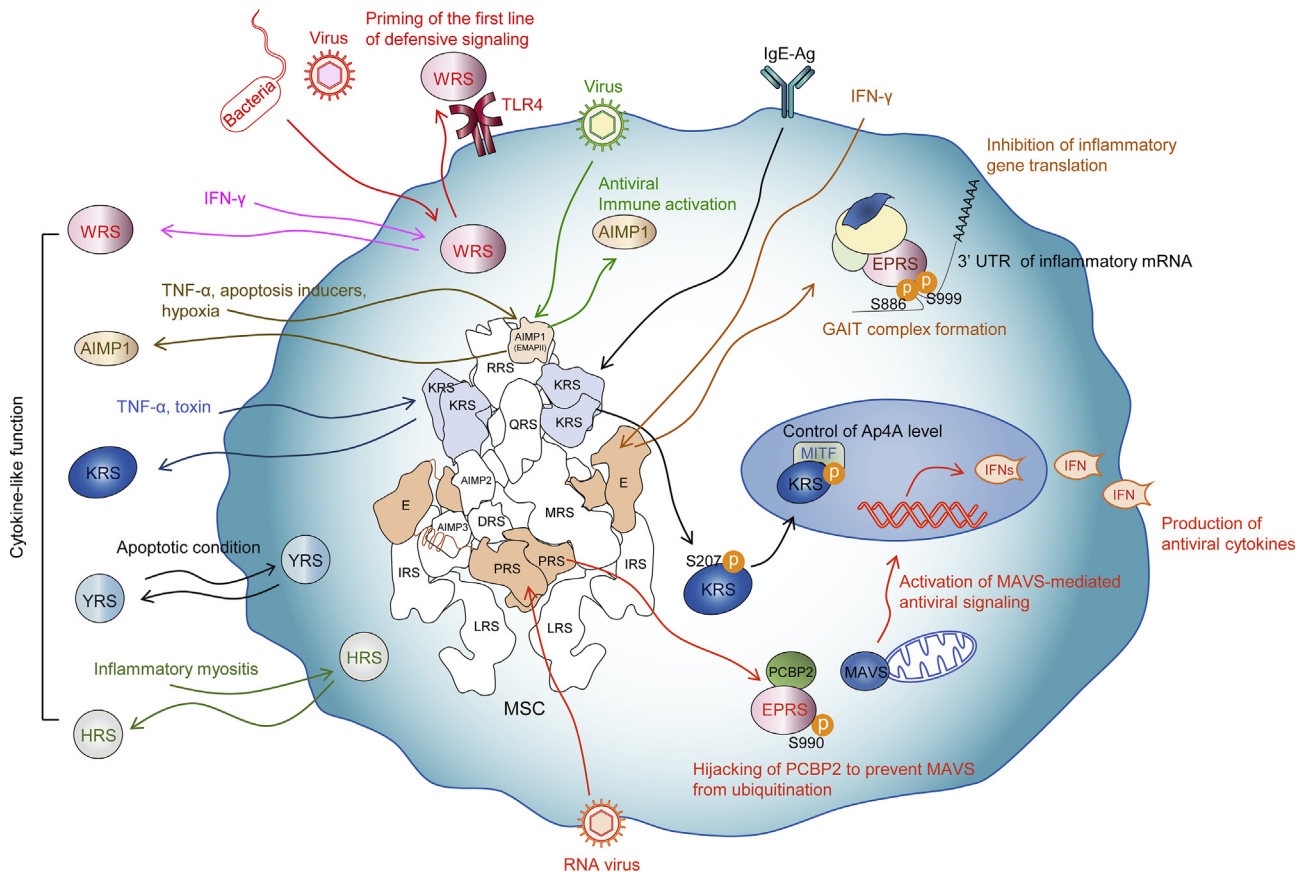
### 3. Communication between the mammalian ARS system and viruses

In general, RNA viruses have very small genomes, which limits their own protein synthesis [54]. Accordingly, viruses have to rely on the

host translational system for their survival and replication [55]. Unavoidably, the ARS system, including ARSs and tRNAs, is co-opted by viruses, which lies in contrast to its immunoregulatory role in defense against infection.

Viral hijacking of the host translational machinery, particularly the ribosomes and the ARS system, consequently reduces host translational fidelity [56]. Methionine misacylation was increased up to 10-fold in cells exposed to various viruses, including adenovirus and vaccinia virus [56]. In the case of vaccinia virus, its replication and progeny assembly take place exclusively in the cytoplasm of infected cells at distinct foci termed viral factories or viroosomes, which are enriched in viral DNA and proteins [57]. Interestingly, MSC was recruited to these active viral translation sites in vaccinia virus-infected cells [58]. Immunofluorescence analysis indicated that MSC proteins (KRS, MRS, and EPRS) and some free ARSs (SRS and YRS) are largely recruited to the viral factories [58]. Moreover, upregulation of diverse ARS genes after flavivirus infection in mice brain implies that host ARSs may be used by viruses for their propagation, based on the observation that flavivirus does not shut off host protein synthesis and therefore must compete with the cellular translational machinery for limiting factors [26].

Although the substrate specificity of ARS is sequence-dependent, several studies revealed that ARSs can recognize plant viral RNAs with different sequences from those of tRNAs [59]. Some viral RNAs have adopted structures that mimic those of tRNA, which increases the efficiency of their genomic RNA translation. tRNA-like structures (TLSs) have been found at the 3' end of RNAs from eight genera of plant viruses [60], including turnip yellow mosaic virus [61], bromo mosaic virus [62], and tobacco mosaic virus [63]. These TLSs can be aminoacylated specifically on valine, tyrosine, and histidine [64]. This mimetic



**Fig. 1.** Immune functions of ARSs. Viral infection releases EPRS from the MSC to exert antiviral activity by inhibiting MAVS degradation by PCBP2. AIMP1 also promotes antiviral immunity by potentiating the link between innate and adaptive immunity. WRS is secreted after bacteria or virus infection that subsequently primes innate immunity via binding to TLR4. Secreted cytokine-like ARSs are displayed with cognate stimuli. Each ARS function is represented by arrows in different colors. See text for details.

**Table 1**  
Immunoregulatory roles of mammalian ARSs.

ARS	Stimulus	PTM	Interactor	Function	Cell	Refs.
EPRS	- H1N1 influenza A virus - VSV - PolyI:C	Phosphorylation at Ser990	PCBP2	Activation of MAVS-mediated antiviral signaling	U937 Raw264.7	[20]
EPRS	IFN- $\gamma$	Phosphorylation at Ser886 and Ser999	NASP1 GAPDH L13a	Inhibition of inflammatory gene translation by heterotetrameric GAIT complex formation	U937	[32]
EPRS	IFN- $\gamma$	Phosphorylation at Ser999	GAPDH L13a	Inhibition of inflammatory gene translation by heterotrimeric GAIT complex formation	Raw264.7	[41]
WRS	- <i>S. typhimurium</i> - <i>E. coli</i> - <i>S. aureus</i> - RSV - H1N1 Influenza A virus	Not identified	TLR4	Priming the first line of defensive signaling	PBMC HEK293-PRRs	[22]
AIMP1	- LPS - H3N2 Influenza A virus	Not identified	Not identified	- MAPK signaling activation - T <sub>H</sub> 1 polarization - Adaptive immune activation	BMDC	[21]
KRS	IgE-Ag	Phosphorylation at Ser207	MITF	- Control of Ap4A level in nucleus - Immune activation by MAPK signaling cascade	RBL mast cell	[35]

PTM, post-translational modification; VSV, vesicular stomatitis virus; GAIT, IFN- $\gamma$ -activated inhibition of translation; RSV, respiratory syncytia virus; PBMC, peripheral blood mononuclear cell; PRR, pattern recognition receptor; MAPK, mitogen-activated protein kinase; T<sub>H</sub>1, T-helper type 1; BMDC, bone marrow-derived dendritic cell; IgE-Ag, immunoglobulin E-antigen; MITF, microphthalmia-associated transcription factor; Ap4A, diadenosine tetraphosphate.

activity is strategically valuable to viruses for evading the host's immune system as well as increasing their translational efficiency [65]. Structural analysis showed that the viral TLS can bind to ARS, eukaryotic elongation factor 1 alpha (eEF1A), and the ribosome, implying that the tRNA mimicry functions as a translation enhancer and a means of protecting the 3' end of viral genomic RNA [66]. Similarly, a 32-nucleotide RNA motif in the genome of the transmissible gastroenteritis coronavirus formed a similar structure with the GAIT element that can selectively interact with EPRS [67,68]. The sequence of this viral RNA motif showed a high identity with a known GAIT element that interacts with the first and second linker regions in the WHEP domains of EPRS [40]. Direct binding of EPRS to the viral GAIT-like RNA motif inhibited the translation of this viral RNA motif *in vitro* [68]. Interestingly, the viral GAIT-like RNA motif affected the host melanoma differentiation-associated protein 5 (MDA5)-mediated antiviral signaling pathway. Although disruption of this GAIT-like RNA motif did not affect viral growth, the innate immune response was exacerbated through MDA5 activation, indicating that this motif interferes with the host defense system by blocking the accessibility of EPRS to host sensors of viral RNA [68].

All retroviruses utilize host cellular tRNA isoacceptors as primers for reverse transcription [69]. Human immunodeficiency virus-1 (HIV-1) uses the specific tRNA<sup>Lys3</sup> to initiate reverse transcriptase-catalyzed synthesis of minus-strand DNA [70]. During viral assembly, human KRS was found to be selectively packaged into HIV-1 virions via direct interaction with the viral Gag protein, and a truncated KRS associated with the tRNA<sup>Lys</sup> packaging was found within the virion [70–72]. Gag codes for core structural proteins of the virus, which are the fundamental building blocks of the retrovirus particle [73]. The source of viral KRS has been a topic of significant investigation. One study showed that the incorporation of KRS into HIV-1 virions occurs rapidly and is sensitive to the inhibition of newly synthesized KRS [72]. Another study suggested that HIV-1 uses host mitochondrial KRS during the packaging process [74]. Because KRS is normally associated with the MSC, HIV-1 could exploit the dynamic nature of the MSC to redirect and co-opt cellular translation factors [75]. Indeed, HIV-1 infection triggers release of KRS from the MSC, possibly through phosphorylation at Ser207 [75]. This PTM is important for KRS packaging into virions and progeny virus infectivity [75]. The free pool of KRS translocates to the nucleus in a phosphorylation-dependent manner, although the relevance of nuclear KRS for viral infectivity is not yet entirely clear [75].

HCV is a major cause of chronic liver disease characterized by persistent infection and immune escape [76]. HCV E2 is an envelope protein that plays an important role in viral entry [77]. HCV E2 directly interacted with AIMP1 and led to its degradation [78]. Because AIMP1 is a negative regulator of tumor growth factor- $\beta$  (TGF- $\beta$ ) [79], the decreased cellular levels of AIMP1 in HCV infection caused liver fibrosis by increasing TGF- $\beta$  signaling. Examining whether this interaction is involved in the immune evasion strategy of HCV will be valuable, as AIMP1 was recently shown to exert antiviral activity by enhancing T<sub>H</sub>1 polarization [21].

Influenza A virus encodes NS2, a nuclear export protein that plays an important role in overcoming host restriction [80]. The NS2 protein interacted directly with AIMP2 to protect it from ubiquitin-mediated degradation [81]. AIMP2 promotes virus replication by enhancing the stability of the virus M1 protein, facilitating the switch from ubiquitination to SUMOylation of M1 [81].

Collectively, these data demonstrate that viruses do not interact with the ARS system solely to co-opt the host translational machinery for their own protein synthesis. Instead, viruses also act through the ARS to regulate diverse cellular processes involved in viral propagation and replication.

#### 4. Therapeutic potential of mammalian ARSs

##### 4.1. Secreted ARSs acting as cytokines and chemokines

As key players in the innate and adaptive immune system, cytokines can be used as therapeutic agents for immune-related diseases [82]. The immune-modulatory activity of cytokines represents a promising feature of anti-infective therapeutics and immune adjuvants for vaccines directed against pathogens [83]. For example, interferon- $\alpha$  (IFN- $\alpha$ ) has been proven to be clinically useful in virus infection by inducing IFN-stimulated genes that limit virus replication and other genes involved in apoptosis of infected cells [84,85]. Several cytokines, such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), are produced as precursor proteins and cleaved into active mature forms by proteases, which are subsequently secreted into the extracellular space [86].

In the last few decades, it has been reported that several ARSs are secreted by intact cells and function like cytokines [51,87–90]. ARSs are secreted in response to extracellular stimuli and are also secreted continuously, suggesting they are constitutively and actively secreted

[43,89]. They are secreted as either full-length or truncated forms generated by alternative splicing or proteolytic cleavage and exhibit activity in cytokine signaling pathways [91,92]. For instance, under apoptotic conditions, YRS is secreted and cleaved into two distinct cytokines by an elastase, yielding an N-terminal catalytic domain (mini-YRS) and a C-terminal endothelial monocyte-activating polypeptide II (EMAPII)-like domain [87,93]. The latter showed potent chemotactic activity for leukocytes and monocytes and stimulated the production of myeloperoxidase, TNF- $\alpha$ , and tissue factor, whereas mini-YRS functioned as an IL-8-like cytokine via its Glu-Leu-Arg (ELR) motif, which is critical for polymorphonuclear leukocyte receptor binding [87].

WRS has strong structural homology to YRS [92]. As discussed above, WRS can be secreted in either a full-length or a truncated form. Based on a structural comparison, the ELR motif in YRS seems to be replaced with a Tyr-Gly-Tyr (YGY) motif in mini-WRS [94]. However, the YGY sequence of WRS was not essential for its angiostatic cytokine activity, but instead was important for maintaining structural stability. Alternatively, the Glu-His-Arg (EHR)-containing eight-residue motif (aa 382–389) in mini-WRS was directly involved in its angiostatic activity [94]. Whether the EHR motif of WRS affects binding to chemokine receptors and activates signal transduction should be investigated.

The extracellular signaling activity of KRS has been described [89]. KRS was secreted from intact human cells in its full-length form, which functioned as an active cytokine. The secreted KRS bound to monocytes and macrophages to induce immune responses through the ERK and p38 MAPK signaling pathways. TNF- $\alpha$  but not TGF- $\beta$  induced the release of endogenous KRS from cells [89]. Interestingly, Shiga toxins produced by *Shigella dysenteriae* and a subset of diarrheagenic *Escherichia coli* [95] triggered the dissociation of KRS from the MSC and its secretion by macrophages [96]. The secreted KRS contributed to Shiga toxin-mediated inflammatory signals by increasing the production of proinflammatory cytokines and chemokines from target cells. This study showed that a bacteria-derived virulence factor could use KRS as a cytokine to synergistically exacerbate inflammatory responses in the host. HRS secreted in its full-length form [97] induced lymphocyte migration and activated monocytes and immature dendritic cells through CCR5 binding [98].

AIMP1 is also secreted as a full-length form or as a 22 kDa C-terminal EMAPII domain [99]. Secreted AIMP1 displayed diverse biological functions, including upregulation of proinflammatory genes [43] and activation of immune cells [46,100]. The EMAPII domain activated host immune responses by acting as a proinflammatory cytokine or as a chemoattractant to induce endothelial/mononuclear cell migration [99,101,102].

Due to their specific cytokine-like and chemotactic activities, the secreted ARSs have great potential for boosting immunity against primary infections. Alternatively, blocking the release of ARSs from cells or targeting the secreted ARSs with specific antibodies or inhibitors could be used to alleviate severe inflammation during infections, similar to anti-TNF- $\alpha$  therapy in sepsis [103]. Although the mechanisms underlying ARS secretion have not been fully elucidated, recent studies showed that GRS and KRS are released via exosomes [104,105]. Application of an exosome pathway inhibitor (e.g., GW4869) or an ER-Golgi pathway inhibitor (e.g., brefeldin A) is a potential strategy to control ARS secretion and attenuate excessive inflammation induced by infection. Extracellular functions of ARSs in eliciting cytokine signaling responses are summarized in Fig. 1 and Table 2.

#### 4.2. ARS-derived peptides as potential anti-infective drugs

Development of human ARS-derived peptides is an attractive pharmacological approach in that they are relatively safe and well tolerated [106]. Currently, clinical approaches to treat acute infections depend mainly on the use of antibiotics or antiviral drugs. Host-directed therapies to augment anti-infective responses are greatly needed [107]. ARSs that have bioactive roles in anti-infectious signaling represent

potential candidates to enhance host defense. Our group showed that an EPRS-derived peptide has potential as a therapeutic agent against a broad range of RNA viruses [20]. A domain mapping study of EPRS showed that the EPRS L1 region (aa 168–196) was critical for protection of MAVS from PCBP2-mediated ubiquitination and activation of antiviral type I IFN signaling [20]. Based on this observation, an antiviral peptide called Tat-Epep was designed by fusing the EPRS L1 region to the cell-permeable HIV-1 Tat tag. Treatment of macrophages with Tat-Epep increased their production of antiviral cytokines and reduced RNA viral replication *in vitro*. Tat-Epep was shown to increase MAVS stability by inhibiting the negative regulation of PCBP2 in cells. To further examine the antiviral mechanism of Tat-Epep, structure-based modifications of the peptide should be introduced for optimization, and its activity should be functionally validated *in vivo*.

The ELR motif in human YRS is a representative leukocyte chemoattractant. The ELR peptide binds to host macrophages and is internalized, leading to enhanced secretion of the proinflammatory cytokines TNF- $\alpha$  and IL-6 [108]. Introduction of the YRS ELR motif into yeast cells, which lack this protein, led to a gain of cytokine function [109,110]. A structural analysis of the embedded ELR motif in YRS to investigate how it interacts with neighboring residues and binds with high affinity to the chemokine receptor CXCR1 only in the case of mini-YRS will support the development of this motif as a therapeutic agent to regulate inflammation as well as angiogenesis.

The WRS N-terminal region (aa 1–154), which acts as a danger signal early in infections, may also be a very promising target for an antimicrobial peptide [22]. Direct binding between the N-terminal region of WRS and TLRs stimulates anti-infective immune responses in the host. Identification of a functional motif required for this binding and annotation of specific residues by structural analysis of the WRS N-terminal domain will help us design potent drug candidates to treat infectious disease.

To overcome the general weakness of peptide drugs, including their short half-life, fast elimination, and low membrane permeability [106], small, synthetic compounds designed based on the identified ARS peptides may offer an effective solution for the development of anti-infectious drugs.

#### 4.3. ARSs as diagnostic biomarkers

Infectious diseases can be monitored by direct detection of pathogens. However, antigenic variation from a broad diversity of microbial pathogens and the presence of unknown antigens can make it difficult to diagnose the exact type of pathogen [111]. An approach that has the potential to address these challenges relies on monitoring the expression of host genes or proteins [112]. Analyzing gene expression profiles from virus-infected human blood showed that different pathogens elicited distinct host RNA signatures [113]. Host proteins involved in immune responses can also be an indicator of infection, and elevated levels of these factors can provide useful clinical information in the context of infectious disease. For example, detection of the concentrations of three proteins, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), interferon-inducible protein 10 (IP-10), and C-reactive protein (CRP), could be used to discriminate between bacterial and viral diseases and reduce antibiotic misuse [114]. High mobility group box 1 (HMGB1) protein is also an important mediator of inflammation, and elevated plasma levels are significantly associated with infection [115].

A close correlation between the secretion of ARSs and the presence of infection was observed in a previous study [22]. WRS was detected in supernatants from PBMC cultures 2 h after bacterial infection, while HMGB1 was not detected during this period [22]. This result suggested that, compared with HMGB1, which is secreted at a relatively late stage (at 12 h) after exposure to pathogens [116], WRS may be used as an early biomarker of infection [22]. Moreover, while HMGB1 is released in response to numerous inflammatory signals in addition to infection

**Table 2**  
Cytokine-like functions of ARSs.

ARS	Stimulus	Active form	Target cell	Receptor	Function	Refs.
YRS	Apoptotic condition	- Mini-YRS - C-terminal EMAPII	Leukocyte	CXCR1/2	- Chemo-attract leukocytes with ELR motif - Intercellular signal transducer - Production of cytokines by mononuclear phagocytes	[87]
WRS	IFN- $\gamma$	- Mini-WRS - Truncated T1, T2	Endothelial cell	VE-cadherin	Angiostatic activity with EHR motif	[52,94]
KRS	- TNF- $\alpha$ - Toxin	Full-length (exosome-associated)	- Macrophage - Monocyte	Not identified	- MAPK (ERK, p38) signaling activation - Macrophage migration - TNF production	[89,96,105]
HRS	Inflammatory myositis	- Full-length - Splice variants  (internal deletion of catalytic domain)	- Lymphocyte - Monocyte - Immature DC	CCR5	- Induce cell migration, chemokine activation - Initiation of adaptive immune response - Inflammatory function by WHEP	[97,98]
AIMP1	- Apoptosis inducers - Hypoxia - TNF- $\alpha$	- Full-length - EMAPII	- Macrophage - Monocyte - Endothelial cell - Epithelial cell	CD23	- Proinflammatory effect - Bi-phasic effect (anti-/pro-angiogenesis)	[99,101,102]

EMAPII, endothelial monocyte-activating polypeptide II; ELR, Glu-Leu-Arg; EHR, Glu-His-Arg; DC, dendritic cell.

[115], WRS secretion is dependent on the presence of infection, which differentiates sepsis from systemic inflammatory response syndrome. Therefore, WRS may be a useful diagnostic tool for infectious diseases including sepsis without the necessity of detecting pathogenic antigens in patient serum.

KRS was also secreted by intact human cells in response to proinflammatory cytokines and stimulated TNF- $\alpha$  production by immune cells [89]. Bacterial toxins also induced KRS secretion, which in turn increased cytokine production [96]. This positive feedback loop augments the KRS signal during the infection, and may be utilized as a detection tool for infectious disease. Because the types and amounts of secreted ARSs might differ depending on the pathogen and the duration of the infection, the classification of detectable ARSs from human samples can provide valuable information about disease status [22,89].

Another strategy to detect infection is measuring ARS antibodies. Specific auto-antibodies are found in the sera of patients with autoimmune disease. Antisynthetase syndrome is a chronic autoimmune condition caused by the production of auto-antibodies against eight different ARSs, of which anti-HRS is the most prevalent [117,118]. Antisynthetase syndrome is a form of idiopathic inflammatory myopathy characterized by myositis, interstitial lung disease, and arthritis [119]. Although the exact underlying mechanism for this syndrome is unknown, it seems to occur after certain viral infections or exposure to certain drugs [119]. Indeed, infectious agents are thought to induce autoimmunity once self-tolerance is broken [120]. For example, infectious myositis, one of the symptoms of antisynthetase syndrome, is an acute, subacute, or chronic infection of skeletal muscle caused by viruses including HIV, bacteria, fungi, and parasites [121]. Therefore, ARS auto-antibodies may be clinically useful as biomarkers of infection.

Finally, a robust and specific ARS detection system is required for accurate prediction of infectious diseases. For purification of human ARSs as antigens, specific design is required to minimize cross-reactivity with pathogenic ARSs that contain conserved catalytic regions. Determination of antigenicity and specificity based on the host ARS sequence and structure should improve ARS detection [30]. Several ARS antibodies and sandwich ELISA systems are commercially available, but most utilize polyclonal antibodies and are quite expensive, and the sensitivities are not sufficient for use with human serum samples, which have high levels of background [122]. To overcome this low resolution, new diagnostic tools such as protein chips should be developed to identify multiple ARSs simultaneously [123]. PCR-based methods to detect host ARSs can also be used due to their high sensitivity and specificity [124]. The rational design of discriminative primers for human ARSs will promote the timely diagnosis of infections.

Moreover, in addition to secreted ARSs, examining the host ARS proteome or genome signature in cells isolated from patient peripheral blood or tissues will help correlate the abnormal expression of ARSs with infections [125–127].

Collectively, ARSs are novel biomarkers that may be used to identify early or secondary infections. The combined detection of pathogenic antigens and host ARSs that are specifically involved in infections will improve current diagnosis systems for infections.

## 5. Bacterial ARSs as targets for antimicrobial drug development

### 5.1. Representative compounds inhibiting essential functions of bacterial ARSs

There is a great deal of interest and concern about the rate at which new pathogens are emerging and causing significant human health problems. Among a wide variety of factors that predispose pathogens to invade host populations, the spread of antibiotic-resistant bacteria is a serious challenge in medical research.

Inhibition of protein synthesis by targeting tRNA aminoacylation has proven to be an effective strategy for anti-pathogenic drug development [128]. The catalytic domain of ARS has three distinct binding pockets responsible for recognition of amino acids, adenylate, and tRNA moieties [129]. Based on these substrate-binding sites, three classes of ARS inhibitors have been explored. While some inhibitors target a single specific pocket, many bind to one or more sites within the catalytic domain or the separate editing domain. Firstly, amino acid analogs can act as competitors for particular amino acids in ARS domains. Halofuginone binds to the proline- and tRNA-binding sites of PRS, and thereby inhibits its enzymatic activity and has been used as an herbal medicine to treat malaria [130–132]. Other biosynthetic products include indolmycin/chuangxinmycin (tryptophan analogs), cispentacin/icofungipen (an isoleucine analog), ochratoxin A (a phenylalanine analog), and SB219383 (a tyrosine analog), although they have not been used as therapeutic agents because of their poor antibacterial activity and low specificity [133]. Developing compounds that mimic aminoacyl-adenylate (AA-AMP) intermediate or bind to AA-AMP pockets may also be a powerful strategy [134]. Mupirocin is a mimetic of isoleucyl-AMP, which occupies the AA-AMP-binding site [135]. It is a very successful ARS inhibitor, exhibiting a high degree of specificity to several species of bacteria, including methicillin-resistant *Staphylococcus aureus*, *E. coli*, and *Bacillus subtilis* [135]. The clinical use of mupirocin validates the idea that ARSs can be effective antibiotic targets in human patients. Lastly, AN2690 inhibits fungal LRS by forming



a tRNA<sup>Leu</sup>-AN2690 adduct in the editing site [136]. It traps the 3'-end A76 nucleotide of tRNA, thus inhibiting subsequent rounds of aminoacylation. Interestingly, borrelidin acts as a triple competitive inhibitor [137]. Co-crystal structures of borrelidin with TRS showed that borrelidin simultaneously occupied four distinct subsites, including substrate-binding sites for amino acids, ATP, and tRNA<sup>Thr</sup>, and an additional binding site [137].

### 5.2. Developing bacterial ARS inhibitors

The full set of 20 ARSs present in most bacterial pathogens offers great opportunities for the development of diverse antibacterial target-based drugs [133]. The considerable evolutionary divergence between prokaryotic and eukaryotic ARSs may reduce potential off-target effects of ARS inhibitors. Bacterial ARSs have many advantages as druggable targets. These enzymes are relatively easy to purify as recombinant proteins. Typical aminoacylation assays measuring the rate of AA-tRNA<sup>AA</sup> formation and kinetic assays to measure the rate of conversion of PPI into ATP using radioisotopes or specific organic compounds like malachite green are available for early characterization of ARS inhibitors [128,138].

The traditional approach to identify ARS-targeted drugs would include high-throughput screening with large libraries of chemicals or bacteria-derived natural products. Technological advances in automated high-throughput screening will enable the rapid, efficient, and inexpensive discovery of low molecular weight compounds that perturb bacterial ARS functions [139]. Potent inhibitors of *S. aureus* MRS were identified from a high-throughput screen by GlaxoSmithKline [140]. The compound was later characterized as a quinolinone derivative that acts by competition with a methionine substrate [141]. Anacor also used a combinatorial screening of a boron-containing small molecule library and discovered a novel synthetic inhibitor, AN2690, that inhibited fungal LRS [142]. However, a serendipitous discovery from a large chemical library would be like looking for a needle in a haystack, and such compounds may have a high risk of toxicity because they would work through unknown mechanisms.

Structure-based drug design is the most powerful approach for drug development. This technique could especially be applied to ARS inhibitors since the architecture of ARS catalytic domains is structurally conserved throughout evolution. The low amount of variation in the ARS active domains suggests that targeting bacterial ARSs may be a broad strategy for use against a range of pathogens, but selectivity between eukaryotic and prokaryotic ARSs might be difficult to achieve. As enzymes, the core structures of ARSs without additional domains (which are usually present in higher eukaryotes) are readily soluble, and many of their unique structures are publicly available in the Protein Data Bank. Atomic-level characterization with structure-guided drug design is critical for developing and modifying ARS-based drugs. Co-crystallization of ARSs and inhibitors clearly shows how small compounds can potentially control ARS function across a variety of species. The detailed structural and functional analysis of binding between the natural product borrelidin and TRS is a good example [137]. The authors determined the crystal structures of both human and *E. coli* TRS in complex with borrelidin and found that borrelidin has potent and redundant mechanisms to inhibit TRS during protein synthesis. This study revealed that borrelidin acted as a quadrivalent inhibitor by filling three substrate-binding sites and an extra space through its extended macrolide ring [137].

Growing reports on the crystal structures of ARSs showing the detailed catalytic sites and their availability provide valuable information for drug design and docking. Instead of resolving the co-crystal of an ARS and its inhibitor experimentally, new computational algorithms and approaches for modeling ARS drug interactions have become indispensable and time-saving tools [143]. Several bioinformatics tools are available to explore sequence and structure-derived enzyme active site residues to annotate drug inserts [143]. *In silico* methods including

virtual screening and structure-based drug design have been used to design ARS inhibitors targeting LRS, WRS, NRS, MRS, IRS, and TRS [144–146]. The inhibitory activities of these compounds must be confirmed. Moreover, ARS substrate-based modification such as exploiting the structure of tRNA can be considered. Trana Discovery has developed a modern bioinformatics tool to conduct detailed analyses of tRNAs and employed a high-throughput screening system with a fluorescent probe mimicking the T-loop region to identify novel drugs that bind at the anticodon stem loop region of tRNAs to inhibit pathogen growth [147]. In addition, despite the structural homology, specific sequence differences between prokaryotic and eukaryotic ARSs can be used to design discriminating and specific ARS-targeted drugs. For example, the successful application of mupirocin is derived from a mere two-amino-acid difference between the prokaryotic and eukaryotic ARS [148]. Mutational analysis of amino acid residues will further characterize the critical sites involved in the interactions between ARSs and their inhibitors.

Taken together, the integration of diverse methods will greatly enhance the rational development of more optimized and sophisticated ARS inhibitors that will meet the need for new antimicrobial, antifungal, and antiparasitic drugs.

### 5.3. Bacterial resistance against ARS-targeted antibiotics

Unfortunately, the use of antibiotics has inevitably led to bacterial resistance. ARS inhibitors are no exception. *S. aureus* is an extraordinarily adaptable Gram-positive pathogen with a proven ability to develop drug resistance [149]. Although mupirocin has been used to treat methicillin-resistant *S. aureus* clinically at a MIC of  $\leq 4 \mu\text{g/ml}$ , this strain appears to have evolved two types of resistance to mupirocin [150]. First, point mutations in the targeted *S. aureus* IRS led to the development of low-level (mupirocin MIC  $\leq 8$ – $64 \mu\text{g/ml}$ ) antibiotic resistance, while horizontal gene transfer of resistance genes (*mupA*, which encodes an alternate IRS, or *mupB*, which contains common IRS motifs) from either eukaryotes or other bacteria caused high-level resistance (mupirocin MIC  $\geq 512 \mu\text{g/ml}$ ) [151]. Additionally, although the MRS inhibitors were potent against a large number of pathogenic strains such as *S. aureus* and *Enterococcus*, *Streptococcus pneumoniae* exhibited a wide range of susceptibility to these compounds [140]. *S. pneumoniae* strains encoding a second, distantly related MRS enzyme called MRS2 are resistant to synthetically derived inhibitors, including REP8839 [141]. *S. pneumoniae* acquired the *mrs2* gene by horizontal transfer from Gram-positive pathogens or close relatives [141].

Many ARS inhibitors are naturally produced by several microbes. Because biosynthesized ARS inhibitors can be toxic to their sources by targeting their own ARSs, bacteria that produce ARS inhibitors require self-immunity. Here, self-immunity is defined as a resistance system against antimicrobial compounds that can kill related bacteria. Ironically, plasmids encoding these resistance genes (usually genes encoding secondary ARSs) are readily transmissible by conjugation and confer polymicrobial resistance. For example, *Pseudomonas fluorescens* produces mupirocin by quorum sensing to kill competitor bacteria in the local environment [152]. *P. fluorescens* protects itself by expressing the *mupM* gene, which encodes a second IRS. Transfer of *P. fluorescens* *mupM* to *E. coli* induced resistance to mupirocin [153]. Similarly, the *Agrobacterium radiobacter* strain K84 produces agrocin 84, which inhibits a plant pathogen, *A. tumefaciens* C58 [154]. Immunity to agrocin 84 is conferred by the *agnB2* gene, which encodes an active LRS. Introduction of this gene to pathogenic *A. tumefaciens* imparted antibiotic resistance, suggesting that the biocontrol organism carries a self-protected ARS to avoid cell death [155]. Thus, although ARSs are good drug targets due to their high selectivity and potency toward microbes, sharing of ARS resistance genes not only locally, among neighboring bacteria, but also across long distances by selective pressure is a major obstacle for ARS drug application [148]. Identification of ARS inhibitors that are not associated with self-immunity and that do not

transfer natural resistance genes should be identified. Finally, based on the different chemical properties of the 20 ARSs, targeting multiple ARSs simultaneously may be a safe way to overcome the emergence of resistance.

## 6. Conclusions and outlook

Infectious diseases remain an ever-growing risk to human health due to increasing antibiotic resistance, immune-compromised populations, and the emergence of new viruses that spread rapidly through the population due to globalization. Identification of novel drug targets to control these complex infective situations is inevitably of great importance.

Beyond their roles in protein synthesis, ubiquitously existed ARSs can sense and immediately respond to danger signals. MSC, a complex consisting of ARSs and non-enzymatic proteins, has been regarded as a hub for the cellular regulatory functions of ARSs [19]. The anti-infective functions of EPRS, which positively regulates antiviral signaling [20], and AIMP1, which increases the expression of antiviral genes [21], provide further evidence that the MSC serves as an immune regulatory system against infection. In addition, the non-MSC protein WRS is considered a primary defense molecule that helps cells of the immune system to respond rapidly to infectious signals [22]. Moreover, some secreted ARSs have evolved activities similar to those of classical proinflammatory cytokines [35,87]. All these functions demonstrate that ARSs are promising therapeutic targets that can boost defensive immune responses and control infectious pathogens. Additionally, because the duration of infection can range from days to years, screening for ARS expression and secretion could provide a potential prognostic maker for acute and chronic infections.

The emergence of multidrug-resistant microbes is a substantial threat to human health. Therapeutic applications of ARS-targeted inhibitors will offer the alternative drugs to overcome the inevitable bacterial resistance to currently used antibiotics. Although there are no known antiviral drugs targeting ARSs, identification of a GAIT-like RNA motif in the viral genome [68] or discovery of host ARS-RNA intermediates that are packaged into retroviruses may offer a new opportunities for the development of antiviral agents [148].

Still, limited studies are available on the efficacy of targeting ARSs against infections. Recent studies including our findings provide new insights on the roles of ARSs in infection and anti-infective immunity. We hope to stimulate further in-depth research in this field to advance both basic science and clinical applications of ARS.

## 7. Conflicts of interest

The authors declare no conflicts of interest related to this manuscript.

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