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REVIEWS

Science & Society

Neutralizing Antibodies against SARS-CoV-2 and Other Human Coronaviruses

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Coronavirus (CoV) disease 2019 (COVID-19) caused by severe acute respiratory syndrome (SARS)-CoV-2 (also known as 2019-nCoV) is threatening global public health, social stability, and economic development. To meet this challenge, this article discusses advances in the research and development of neutralizing antibodies (nAbs) for the prevention and treatment of infection by SARS-CoV-2 and other human CoVs.

Current Situation with SARS-CoV-2 and Other Human CoVs

Three emerging, highly pathogenic human CoVs are SARS-CoV, Middle East respiratory syndrome (MERS)-CoV, and COVID-19 virus, which was previously named 2019-nCoV by the World Health Organization (WHO), and is also known as hCoV-19 or SARS-CoV-2 [1]. Atypical pneumonia (SARS) was first reported from Guangdong Province, China in late 2002. SARS caused a global pandemic in 2003 with approximately 10% (774/8098) case fatality rate (CFR) [2]. SARS-CoV has not circulated in humans since 2004. MERS-CoV was first reported from Saudi Arabia in 2012 and has continued to infect humans with limited human-to-human transmission, leading to a CFR of approximately 34.4% (858/2494) in 27 countries, according to the most recent WHO report[i]. Both SARS-CoV and MERS-CoV are zoonotic viruses. They use bats as their natural reservoirs and transmit from bats to intermediate hosts (e.g., palm civets for SARS-CoV, dromedary camels for MERS-CoV), leading to infection in humans [2,3].

Different from SARS-CoV and MERS-CoV, SARS-CoV-2 was first reported in Wuhan, China in December 2019 and is characterized by its rapid spread and virulent human-to-human transmission [4], resulting in 125 048 confirmed cases including 4613 deaths (CFR 3.7%), particularly in Wuhan, China and in at least 117 other countries, territories, or areas as of March 12, 2020. With no vaccines or treatments on the horizon, researchers are exploring various medical interventions, including nAbs, to control the continuous spread of SARS-CoV-2 and the global COVID-19 pandemic [5]. SARS-CoV-2 is also a zoonotic virus with bats as its natural reservoir [4], but its intermediate hosts have not been identified.

Pathogenesis and Key Proteins of SARS-CoV-2 and Other Human CoVs

SARS-CoV-2 infection mainly results in pneumonia and upper/lower respiratory tract infection. Fever and cough are two major clinical symptoms, but others include shortness of breath, muscle pain (myalgias)/fatigue, confusion, headache, sore throat, and even acute respiratory distress syndrome, leading to respiratory or multiorgan failure [6]. For elderly people with underlying comorbidities such as diabetes, hypertension, or cardiovascular disease, SARS-CoV-2 infection may result in severe and fatal respiratory diseases. So far, its effects on children have been generally mild. The virus can be transmitted through respiratory droplets or close contact with infected surfaces or objects and is detectable in multiple samples, including saliva, stool, and blood [7]. To develop vaccines and therapeutics, we must understand the behavior of key proteins in SARS-CoV-2.

Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 is an enveloped, single-stranded, and positive (+)-sense RNA virus, belonging to the beta-CoV genera in the family *Coronaviridae* [4]. The genome of this and other emerging pathogenic human CoVs encodes four major structural proteins [spike (S), envelope (E), membrane (M), and nucleocapsid (N)], approximately 16 nonstructural proteins (nsp1–16), and five to eight accessory proteins. Among them, the S protein plays an essential role in viral attachment, fusion, entry, and transmission. It comprises an N-terminal S1 subunit responsible for virus–receptor binding and a C-terminal S2 subunit responsible for virus–cell membrane fusion [2,3]. S1 is further divided into an N-terminal domain (NTD) and a receptor-binding domain (RBD). SARS-CoV-2 and SARS-CoV bind angiotensin-converting enzyme 2 (ACE2) while MERS-CoV binds dipeptidyl peptidase 4 (DPP4), as receptors on the host cell expressing ACE2 (e.g., pneumocytes, enterocytes) or DPP4 (e.g., liver or lung cells including Huh-7, MRC-5, and Calu-3) [2,3,8]. Phylogenetically, SARS-CoV-2 is closely related to SARS-CoV, sharing approximately 79.6% genomic sequence identity [4]. During infection, CoV first binds the host cell through interaction between its S1-RBD and the cell membrane receptor, triggering conformational changes in the S2 subunit that result in virus fusion and entry into the target cell (see human CoV life cycle in Figure 1A) [2,3].

nAbs against SARS-CoV, MERS-CoV, and SARS-CoV-2

Virus nAbs induced by vaccines or infected virus play crucial roles in controlling viral infection. Currently developed SARS-CoV- and MERS-CoV-specific nAbs include monoclonal antibodies (mAbs), their functional antigen-binding fragment (Fab), the single-chain variable region fragment (scFv), or single-domain antibodies [nanobodies (Nbs)] [8]. They target S1-RBD, S1-NTD, or the S2 region, blocking

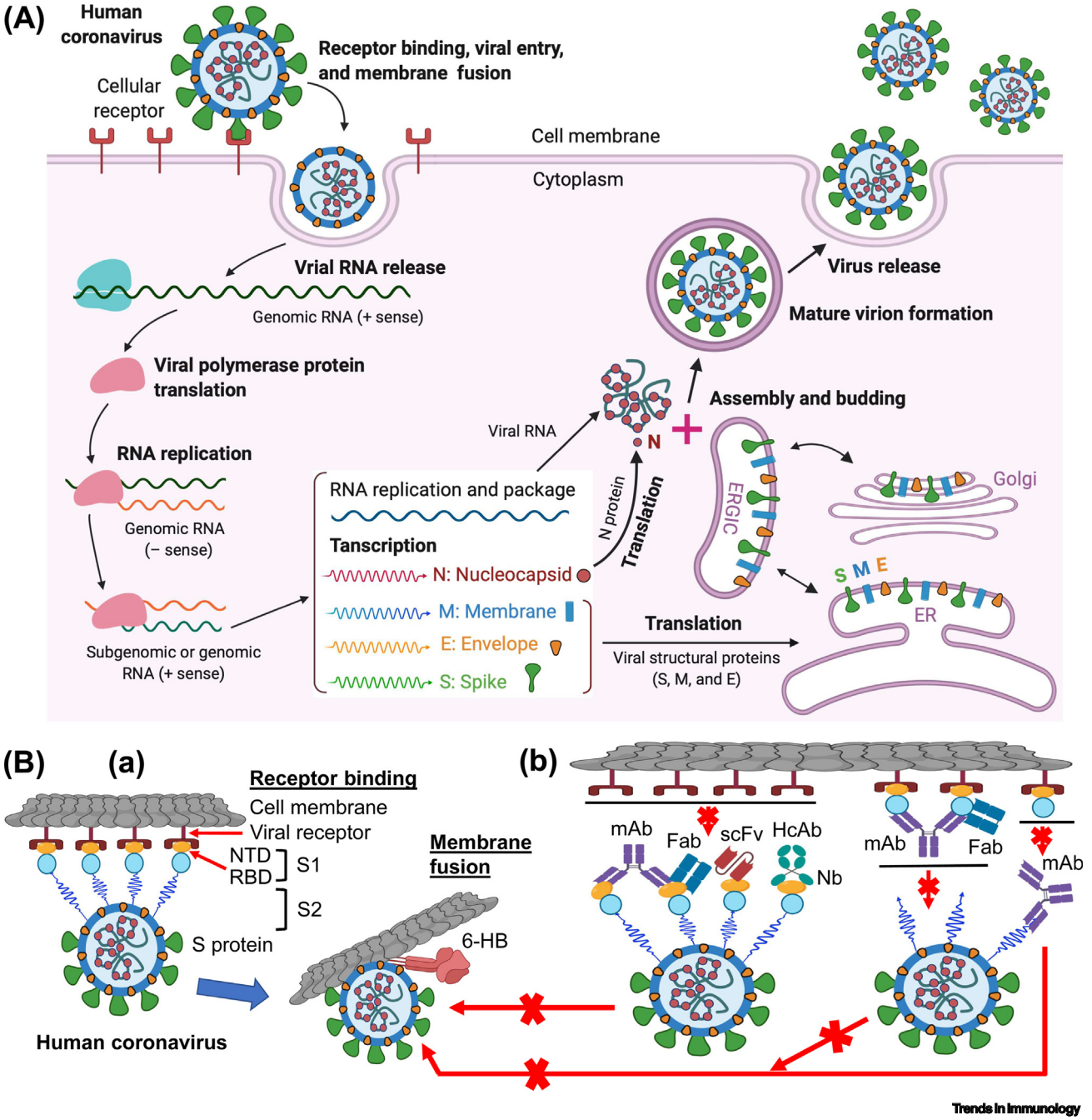

Figure 1. Life Cycle of Highly Pathogenic Human Coronaviruses (CoVs) and Specific Neutralizing Antibodies (nAbs) against These Coronaviruses. (A) Life cycle of highly pathogenic human CoVs. These CoVs enter host cells by first binding to their respective cellular receptors [angiotensin-converting enzyme 2 (ACE2) for severe acute respiratory syndrome (SARS)-CoV-2 or SARS-CoV and dipeptidyl peptidase 4 (DPP4) for Middle East respiratory syndrome (MERS)-CoV] on the membranes of host cells expressing ACE2 (e.g., pneumocytes, enterocytes) or DPP4 (e.g., liver or lung cells including Huh-7, MRC-5, and Calu-3) via the surface spike (S) protein, which mediates virus–cell membrane fusion and viral entry. Viral genomic RNA is released and translated into viral polymerase proteins. The negative (–)-sense genomic RNA is synthesized and used as a template to form subgenomic or genomic positive (+)-sense RNA. Viral RNA and nucleocapsid (N) structural protein are replicated, transcribed, or synthesized in the cytoplasm, whereas other viral structural proteins, including S, membrane (M), and envelope (E), are transcribed then translated in the endoplasmic reticulum (ER) and transported to the Golgi. The viral RNA–N complex and S, M, and E proteins are further assembled in the ER–Golgi intermediate compartment (ERGIC) to form a mature virion, then released from host cells. (B) Potential targets of nAbs against SARS-CoV-2 and other pathogenic human

(Figure legend continued at the bottom of the next page.)

the binding of RBDs to their respective receptors and interfering with S2-mediated membrane fusion or entry into the host cell, thus inhibiting viral infections [2,5]. The putative targets and mechanisms of these SARS-CoV and MERS-CoV nAbs are shown in Figure 1B. Representative SARS-CoV and MERS-CoV RBD-specific nAbs are summarized in Table 1. No SARS-CoV-2-specific nAbs have been reported, but we herein introduce SARS-CoV- and MERS-CoV-specific nAbs in the context of their potential cross-neutralizing activity against SARS-CoV-2 infection.

SARS-CoV nAbs

All currently developed anti-SARS-CoV nAbs target the viral S protein. Most target the RBD, while a few target regions in the S2 subunit or the S1/S2 proteolytic cleavage site. For example, the human neutralizing mAbs S230.15 and m396 were isolated from SARS-CoV-infected individuals. They neutralize human and palm civet SARS-CoV infection by interacting with the RBD, thus blocking binding between the viral RBD and the cellular ACE2 receptor [9]. Other human mAbs, such as S109.8 and S227.14, have cross-neutralizing activity against multiple human, palm civet, and raccoon dog SARS-CoV infectious clones, protecting mice against four different homologous and heterologous SARS-CoV strains [10]. Human nAb 80R (scFv or mAb) neutralizes SARS-CoV infection by blocking the RBD–ACE2 interaction, although its protective efficacy has not yet been reported [11]. A variety of SARS-CoV RBD-specific mouse neutralizing mAbs are sufficiently potent to block RBD–ACE2 binding, thus neutralizing viral infection in ACE2-transfected HEK293T cells [12]. Despite their strong neutralizing activity and/or protection in cells or animal models, none of these SARS-CoV nAbs has ever been evaluated in clinical studies. Thus, to determine potential cross-neutralizing activity against SARS-CoV-2 infection, such studies should be vigorously undertaken.

MERS-CoV nAbs

A number of MERS-CoV-specific nAbs have been reported, most of which target the RBD in the S protein [3,8]. A few recognize epitopes on the S1-NTD and regions of the S2 subunit [3]. Among these nAbs, human mAbs or Fabs (MERS-27, m336, MERS-GD27, or MCA1 isolated from humans), humanized mAbs (hMS-1, 4C2 h), mouse mAbs (Mersmab1, 4C2, or D12 isolated from mice), and Nbs (HCAb-83 or NbMS10-Fc isolated from dromedary camels or llamas) recognize epitopes on the RBD and have been demonstrated to neutralize pseudotyped and/or live MERS-CoVs [3,8]. Several human/humanized mAbs and Nbs can protect mice, rabbits, or common marmosets from MERS-CoV infection [3,8]. So far, only one MERS-CoV nAb isolated from transchromosomic cattle has been evaluated in Phase I trials (SAB-301)[ii] [8]. No other nAbs have gone to clinical trials, again suggesting the urgency of developing nAbs with potential cross-neutralizing activity against SARS-CoV-2 infection.

SARS-CoV-2 nAbs

Currently, polyclonal antibodies from recovered SARS-CoV-2-infected patients have been used to treat SARS-CoV-2 infection, but no SARS-CoV-2-specific neutralizing mAbs have been reported. Researchers are working hard to develop such mAbs and/or their functional fragments as putative prophylactic or therapeutic agents to prevent or treat COVID-19. Once such antibodies are produced, the next steps will involve *in vitro* testing for neutralizing and/or cross-neutralizing activity, *in vivo* evaluation in available COVID-19 animal models for protective efficacy, preclinical studies, and clinical trials testing the safety and efficacy before they are approved for clinical application. Therefore, it may take one to several years for such SARS-CoV-2 neutralizing mAbs or their fragments to be ready for human use.

However, since SARS-CoV-2 is closely related to SARS-CoV and since their S proteins have high sequence identity [4], researchers have attempted to discover SARS-CoV nAbs with potential cross-reactivity and/or cross-neutralizing activity against SARS-CoV-2 infection. Notably, a SARS-CoV RBD-specific human neutralizing mAb, CR3022, could bind SARS-CoV-2 RBD with high affinity and recognize an epitope on the RBD that does not overlap with the ACE2-binding site [13]. In addition, sera from convalescent SARS patients or from animals specific for SARS-CoV S1 may cross-neutralize SARS-CoV-2 infection by reducing S protein-mediated SARS-CoV-2 entry [14]. Moreover, SARS-CoV RBD-specific polyclonal antibodies have cross-reacted with the SARS-CoV-2 RBD protein and cross-neutralized SARS-CoV-2 infection in HEK293T cells stably expressing the human ACE2 receptor, opening avenues for the potential development of SARS-

CoVs. (a) Human CoV receptor binding and membrane fusion process. The CoV first binds a viral receptor (ACE2 or DPP4) through the receptor-binding domain (RBD) in the S protein, followed by fusion of the virus with cell membranes via the formation of a six-helix bundle (6-HB) fusion core. NTD, N-terminal domain. (b) Potential targets of nAbs on the S protein of human CoVs. Monoclonal antibody (mAb), antigen-binding fragment (Fab), single-chain variable region fragment (scFv), or single-domain antibody [nanobody (Nb) or VHH derived from camelid heavy chain antibody (HcAb)] binds to the RBD, S1 subunit (non-RBD, including NTD), or S2 of the viral S protein, blocking binding between the RBD and the respective receptor (for RBD-targeting nAbs), interfering with the conformational change of S (for S1-targeting nAbs), or hindering S2-mediated membrane fusion (for S2-targeting nAbs), leading to the inhibition of infection with pathogenic human CoVs in the host cells. This figure was created using BioRender (https://biorender.com/).

Table 1. Representative SARS-CoV RBD- and MERS-CoV RBD-Targeting nAbs[a]

Ab name	Source	Neutralizing activity	Neutralizing mechanism	Protective efficacy	Refs[b]
S230.15 m396 mAbs	Human	Neutralize human (strains GD03, Urbani, Tor2) and palm civet (strains SZ3, SZ16) SARS-CoV infection	Recognize epitopes (residues 408, 442, 443, 460, 475) on SARS-CoV S1 protein, interfering with RBD–ACE2 receptor interaction	Protect mice against challenge of SARS-CoV (strains Urbani, rGD03, or rSZ16)	[9]
S109.8 S227.14 S230.15 mAbs	Human	Neutralize human (Urbani, GZ02, CUHK-W1), palm civet (HC/SZ/61/03), and raccoon dog (A031G) SARS-CoV infectious clones containing S variants	Inhibit the binding of SARS-CoV RBD–ACE2 receptor	Protect mice against challenge of SARS-CoV infectious clones (Urbani, GZ02, HC/SZ/61/03) or mouse-adapted strain (MA15)	[10]
80R scFv, mAb	Human	Neutralize live SARS-CoV (strain Urbani) infection	Recognize epitopes on SARS-CoV S1 (residues 261–672), blocking RBD–ACE2 binding and inhibiting syncytium formation	NA	[11]
CR3022 CR3014 scFv, mAb	Human	Neutralize live SARS-CoV (strain HKU-39849) infection; CR3022 could neutralize CR3014 escape variants	Recognize epitopes on SARS-CoV RBD (residues 318–510); CR3022 binds SARS-CoV-2 RBD with high affinity	CR3014 protects ferrets against SARS-CoV (strain HKU-39849) infection	[13]
33G4 35B5 30F9 mAbs	Mouse	Neutralize human (strains GD03, Tor2) and palm civet (SZ3) pseudotyped SARS-CoV infection	Recognize epitopes on SARS-CoV RBD, blocking RBD–ACE2 receptor binding	NA	[12]
MERS-27 m336 MERS-GD27 MCA1 mAbs, Fabs	Human	Neutralize divergent strains of pseudotyped and live (strain EMC2012) MERS-CoV infection	Recognize a number of key epitopes on MERS-CoV RBD protein, blocking RBD–DPP4 receptor binding	Prophylactically and therapeutically prevent and treat MERS-CoV (strain EMC2012) challenge in hDPP4-Tg mice, rabbits, or common marmosets	[3,8]
4C2 h hMS-1 mAbs	Humanized	Neutralize divergent strains of pseudotyped and live (strain EMC2012) MERS-CoV infection	Recognize epitopes (residues 510, 511, 553) on MERS-CoV RBD protein, blocking RBD–DPP4 receptor binding	Prevent MERS-CoV (strain EMC2012) challenge in Ad5/hDPP4-transduced or hDPP4-Tg mice	[3]
Mersmab1 4C2 D12 mAbs	Mouse	Neutralize pseudotyped and live (strain EMC2012) MERS-CoV infection	Recognize a number of key epitopes on MERS-CoV RBD protein, blocking RBD–DPP4 receptor binding	NA	[3]
HCAb-83 Nb	Dromedary camel	Neutralizes live MERS-CoV (strain EMC2012) infection	Recognizes epitope (residue 539) on MERS-CoV RBD protein	Prophylactically prevents MERS-CoV (strain EMC2012) challenge in hDPP4-Tg mice	[8]
NbMS10-Fc Nb	Llama	Neutralizes multiple strains of pseudotyped and live (strain EMC2012) MERS-CoV infection	Recognizes epitope (residue 539) on MERS-CoV RBD protein	Prophylactically and therapeutically prevents and treats MERS-CoV (strain EMC2012) challenge in hDPP4-Tg mice	[8]

[a]Abbreviations: Ab, antibody; Ad5/hDPP4-transduced mice, adenovirus serotype 5-hDPP4-transduced mice; hDPP4-Tg mice, human DPP4-transgenic mice; NA, not applicable; rGD03 or rSZ16, recombinant SARS-CoVs bearing the S protein of GD03 or SZ16; S, spike.
[b]Note: Due to space limitations, some review articles, rather than original research papers reporting the antibodies, are cited.

CoV RBD-based vaccines that might eventually prevent SARS-CoV-2 and SARS-CoV infection [15]. It is also possible that SARS-CoV RBD-targeting nAbs might be applied for prophylaxis and treatment of SARS-CoV-2 infection in the current absence of SARS-CoV-2-specific vaccines and antibodies. However, robust testing lies ahead.

Concluding Remarks and Future Perspectives

SARS-CoV-2 continues to infect people globally with the concomitant urgency to develop effective nAbs as prophylactic and therapeutic agents to prevent and treat its infection and control its spread. Studies from SARS-CoV and MERS-CoV have demonstrated that many fragments (S1-NTD, RBD, S2) in S proteins can be used as targets to develop nAbs. Still,

RBD-specific antibodies have greater potency to neutralize infection with divergent virus strains, suggesting that the RBD of SARS-CoV-2 can also serve as an important target for the development of potent and specific nAbs. Cocktails comprising antibodies specific for RBD and other regions in the S protein may further improve the breadth and potency of nAbs against SARS-CoV-2 and its escape-mutant strains. Human sera from convalescent patients have been used to treat COVID-19, but lessons learned from SARS show that some non-nAbs targeting the non-RBD regions in the S protein may cause an antibody-dependent enhancement (ADE) effect on viral infectivity and disease, as well as other harmful immune responses [2]. On a positive note, some anti-SARS-CoV nAbs have shown cross-reactivity or cross-neutralizing activity against SARS-CoV-2 infection *in vitro*. Thus, overall, research on SARS-CoV- and MERS-CoV-specific nAbs should provide important guidelines for the rapid design and development of SARS-CoV-2-specific nAbs.

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Resources

[i]www.who.int/emergencies/mers-cov/en/

[ii]https://clinicaltrials.gov/ct2/show/NCT02788188

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Spotlight

'Nervous' Immunity: Walking the Tightrope

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There is a major gap in our understanding of how the intestinal immune and nervous systems are integrated to regulate protective adaptations to enteric infections while maintaining tissue homeostasis. Three recent complementary reports published in *Cell* (2020) provide new mechanistic insights into how this enteric neuro-immune crosstalk may occur.

The gastrointestinal (GI) tract is a portal through which toxins and pathogens, along with nutrients, can gain entrance into the body. The intestine acts as a guard to sift through ingested material so that beneficial nutrients are absorbed, while toxins and pathogens are neutralized and expelled. Some of these protective functions are achieved through primary physiologic responses, such as vomiting and diarrhea, which are regulated by the neuro-epithelial sensory system and underlying neural circuits. A second line of response to danger requires the immune system, whose diverse cell types can provide both sensory signals and effector responses. Analysis of evolution, embryonic development, and functional interactions between the nervous and immune systems suggests that the two systems are integrated in gut protection. In mammals, this integration occurs through crosstalk between immune cells and gut-innervating neurons that are either extrinsic to the gut wall or reside inside the gut as a part of the enteric nervous system (ENS) [1,2]. Three recent complementary reports provide new mechanistic insights into how this enteric neuro-immune crosstalk maintains GI health and how it is impacted in response to enteric infection [3–5] (see Figure 1).

Infections by entero-invasive bacteria, including *Salmonella enterica* serovars, pose a major threat to human health, especially in light of rising antibiotic resistance. Despite our understanding of early immunological events in response to *Salmonella* evasion, the neuro-immune circuits that regulate resistance to