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A long-distance relationship: the commensal gut microbiota and systemic viruses

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Recent advances defining the role of the commensal gut microbiota in the development, education, induction, function, and maintenance of the mammalian immune system inform our understanding of how immune responses govern the outcome of systemic virus infection. While characterization of the impact of the local oral, respiratory, dermal and genitourinary microbiota on host immune responses and systemic virus infection is in its infancy, the gut microbiota interacts with host immunity systemically and at distal non-gastrointestinal tract sites to modulate the pathogenesis of systemic viruses. Gut microbes, microbe-associated molecular patterns, and microbe-derived metabolites engage receptors expressed on the cell surface, in the endosome, or in the cytoplasm to orchestrate optimal innate and adaptive immune responses important for controlling systemic virus infection.

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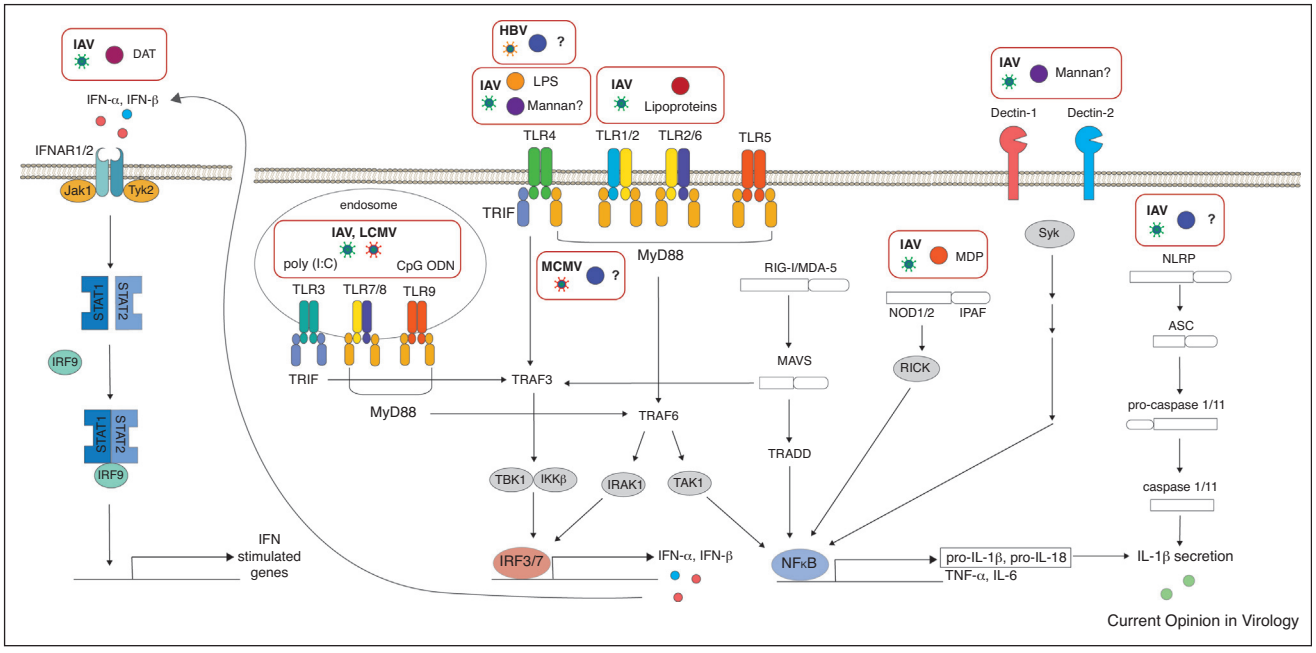
Introduction

Experiments demonstrating enhanced susceptibility of germ-free (GF) mice to virus infection in the 1960's first suggested a role for the microbiota, the commensal micro-organisms living on and in the host, in modulating virus infection (reviewed in Ref. [1]). In the past decade, our increased understanding of the impact of the various constituents that comprise the microbiota including bacteria, archaea, viruses, protozoa, and fungi on the development, education, induction, function, and maintenance of the mammalian immune system (reviewed in Refs. [2–6]) has important implications for how we think about virus infection. Indeed, using now more-readily available sequence-based and functional characterization of the microbiota as well as antibiotic (Abx) treatment to deplete the microbiota, culture-based techniques, and gnotobiotic mice, many groups have begun to dissect how the composition of microbial communities as well as specific microbial taxa modulate immune responses during virus infection (reviewed in Ref. [7]). While characterization of the impact of the local oral, respiratory, dermal, and genitourinary microbiota on host immune responses and virus infection is ongoing, it is now appreciated that the gut microbiota can impact immune responses both in the local gastrointestinal (GI) tract and at distal non-GI tract sites (reviewed in Refs. [8,9]). Accordingly, in this review, we discuss how the gut microbiota interacts with host immunity and distal non-GI tract sites to modulate the pathogenesis of systemic viruses using insights obtained from experimental mouse models.

Role of the gut microbiota in innate immune responses at non-GI sites

Although pattern recognition receptors (PRRs) were initially recognized for their role in sensing conserved molecular ligands called pathogen-associated molecular patterns (PAMPs) on pathogenic micro-organisms, it is now evident that signaling of commensal microbiota through PRRs via conserved ligands more generically termed microbe-associated molecular patterns (MAMPs) also shapes and modulates host immune responses (reviewed in Ref. [10]). Each PRR binds one or more conserved MAMPs such that sensing of viruses is achieved through binding of various forms of viral nucleic acids in the endosome or cytoplasm, while conserved components of bacteria, archaea, protozoa, and fungi are differentially sensed by an array of PRRs on the cell surface or in the cytoplasm (reviewed in Refs. [11–14])). Canonically, engagement of PAMPs from bacteria, protozoa, and fungi results in signaling cascades that drive production of certain chemokines and pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), Interleukin (IL)-6 and IL-1β while virus recognition by endosomal and cytoplasmic PRRs results in production of interferon α/β (Type I IFN) and interferon stimulated genes (ISGs) (Figure 1). However, an emerging appreciation of the cross-talk between what classically has been thought to be separate immune programs is changing our understanding of how pathogenic and commensal micro-organisms elicit differential immune responses even though they engage the same PRRs [15–17].

Figure 1



Receptor-mediated signaling pathways that modulate host immune responses during virus infection.
The interaction of microbe-associated molecular patterns and microbe-derived metabolites with host receptors such as pathogen-recognition receptors alters the type I interferon and/or pro-inflammatory cytokine response following viral infection.
IFN, interferon; IFNAR, IFN-α/β receptor; Jak, Janus Kinase; STAT, Signal transducer and activator of transcription; IRF, IFN-regulatory factor; poly (I:C), polyinosinic:polycytidylic acid; CpG ODN, CPG oligodeoxynucleotide; IAV, Influenza Virus; LCMV, Lymphocytic choriomeningitis virus; TLR, Toll-Like Recptor; TRIF, TIR-domain-containing adaptor protein inducing IFNβ; MyD88, myeloid differentiation primary-response gene 88; MCMV, Murine Cytomegalovirus; LPS, lipopolysaccharide; PG, peptidoglycan; HBV, Hepatitis B Virus; TNF, tumor necrosis factor; TRAF, TNF receptor-associated factor; IL, interleukin; IRAK1, Interleukin-1 receptor-associated kinase 1; TBK1, TANK-binding kinase 1; IKKβ, inhibitor of nuclear factor kappa-B kinase subunit beta; TAK1, TGF-β-activated Kinase 1; TRADD, Tumor necrosis factor receptor type 1-associated DEATH domain; NFκB, Nuclear Factor Kappa Beta; MDA5, melanoma-differentiation-associated gene 5; MAVS, mitochondrial antiviral signaling; RIG-I, retinoic-acid-inducible gene; NOD, nucleotide-binding oligomerization domain; RICK, receptor-interacting serine/threonine kinase; IPAF, ICE-protease-activating factor; ASC, apoptosis-associated speck-like protein containing a caspase-recruitment domain; SYK, spleen tyrosine kinase; NLRP, NOD-like receptor protein; MDP, muramyl dipeptide; DAT, desaminotyrosine; Tyk2, tyrosine kinase 2.

Type I IFN-mediated anti-viral immune response

The Type I IFN response and its induction of an innate antiviral effector program within infected and bystander cells is a central component of virus control (reviewed in Refs. [18]). While type I IFN production is canonically associated with virus infection, several studies using Abx-treatment or GF mice have demonstrated a role for the commensal gut microbiota in setting homeostatic type I IFN levels and calibrating the type I IFN response during systemic virus infection (Table 1). Abt *et al.* observed decreased expression of IFN-β and ISGs in peritoneal macrophages (MO) isolated from naïve Abx-treated mice [19•]. Moreover, peritoneal MO, dendritic cells (DCs) and splenocytes from Abx-treated and GF mice have an impaired ability to respond to stimuli such as IFN-β, IFN-γ, polyinosinic:polycytidylic acid (poly (I:C)), lipopolysaccharide (LPS), Sendai virus (SeV), Influenza virus (IAV), and Lymphocytic choriomeningitis virus (LCMV) *ex vivo* potentially due to decreased promoter binding of Interferon Regulatory Factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF_kB) or phosphorylation of Signal Transducer And Activator Of Transcription 1 (STAT1) [19•,20,21]. Using IFN-β reporter mice, Ganal *et al.* observed decreased IFN-β production in multiple organs of Abx-treated mice after *in vivo* poly (I:C) stimulation [21]. Decreased production of type I IFN and antiviral ISGs systemically and at distal non-GI sites following IAV, LCMV, Murine cytomegalovirus (MCMV), and SeV infection of Abx-treated and GF mice is associated with increased virus titers and/or enhanced susceptibility of mice to lethal infection (Table 1). In aggregate, these studies highlight a role for the commensal gut microbiota in priming type I IFN-dependent antiviral immune responses both systemically and at distal non-GI tract sites during systemic virus infection.

Pro-inflammatory cytokine-mediated immune response

In addition to the Type I IFN response, pro-inflammatory cytokines associated with NF_KB and inflammasome

Table 1

Interactions of the commensal gut microbiota with mammalian immunity during systemic virus infection

	Mouse model		Influence of the gut microbiota on antiviral immunity			Associated PRRs, metabolites, or microbial constituents	
	Model of microbiota depletion	Outcome measured	Cytokine-mediated immune response	Innate cellular immune response	Adaptive immune response	MAMPs, PRRs, Metabolites	Beneficial gut microbes
Hepatitis B virus [38]	Oral Abx-treatment	Virus titers			**Virus-specific T cells:** IFN-γ production of splenic T cells **Virus-specific antibody production:** Generation of virus-specific titers in serum	TLR4	
Dengue virus [30•]	Oral Abx-treatment	Survival					
Influenza virus [19•, 23•,24••,29,39••, 42,43,45••,54]	Oral Abx-treatment [19•,23•,39••] GF mice [24••,54]	Lung pathology [19•] Weight-loss [19•,45••,54] Survival [19•,39••,45••] Virus titers [19•,23•,39••]	**In lung:** **1.** Expression of MO-associated antiviral genes [19•] **2.** Expression of inflammasome-associated genes [23•] **3.** Levels of pro-inflammatory cytokines [19•,23•]	**Function/ recruitment of CD103⁺ DCs:** **1.** Number of CD103⁺ DCs in mediastinal LN and lungs [23•] **2.** Expression of co-stimulatory molecules [23•] **3.** Presentation of viral antigen [23•]	**Virus-specific T cells:** **1.** Number of virus-specific CD8⁺ T cells in BAL, lungs, mediastinal LN, and spleen [19•,23•,39••] **2.** IFN-γ production by splenic virus-specific CD8⁺ and CD4⁺ T cells [23•] **3.** Expression of effector molecules on CD8⁺ T cells in lung [19•,39••] 4. Regulation of activation markers on virus-specific CD8⁺ cells in lung [19•] **Tregs:** Frequency of FoxP3⁺ CD4⁺ T cells in mediastinal LN and lung [23•] **Virus-specific antibody production:** Virus-specific IgM, IgG, IgG2b, and IgA titers in serum and nasal wash [19•,23•]	CpG DNA [23•] LPS [23•] Mannan [39••] NOD2 [42,55] Peptidoglycan [23•,29,42,55] Poly (I:C) [19•,23•] DAT [45••]	*L. paracasei* [29] *C. orbiscindens* [45••] *C. albicans* [39••] *S. cerevisiae* [39••] Wild mouse microbiota [24••] Non-SPF mouse microbiota [49]
Lymphocytic choriomeningitis virus [19•]	Oral Abx-treatment	Virus titers	**In spleen:** expression of antiviral genes **In serum:** levels of IFN-β and pro-inflammatory cytokines		**Virus-specific T cells:** **1.** Number of virus-specific CD8⁺ T cells in blood **2.** Expression of effector molecules on splenic CD8⁺ T cells **3.** Expression of inhibitory molecules on virus-specific splenic CD8⁺ T cells **Virus-specific antibody production:** virus-specific IgG titers in serum		

Table 1 (*Continued*)

	Mouse model		Influence of the gut microbiota on antiviral immunity			Associated PRRs, metabolites, or microbial constituents	
	Model of microbiota depletion	Outcome measured	Cytokine-mediated immune response	Innate cellular immune response	Adaptive immune response	MAMPs, PRRs, Metabolites	Beneficial gut microbes
Murine cytomegalovirus [21]	Oral Abx-treatment GF mice	Virus titers	**In spleen:** IFN-α/β responses in mononuclear phagocytes **In serum:** levels of IFN-α/β	**Function of splenic NK cells:** **1.** Priming **2.** IFN-γ production **3.** Cell-mediated toxicity		CpG DNA LPS Poly (I:C)	Altered Schaedler Flora
Sendai virus [20,48]	Oral Abx-treatment [48] GF mice [20]	Survival [48] Virus titers [48]	**In spleen:** levels of IFN-α/β **In lung BAL:** levels of pro-inflammatory cytokines		**Tregs:** number of FoxP3$^+$ CD4$^+$ T cells in lung and distal small intestine [48]		
West Nile virus [30•]	Oral Abx-treatment	Survival Virus titers			**Virus-specific T cells:** **1.** Number of virus-specific CD8$^+$ T cells in draining LN, spleen and brain **2.** Expression of effector molecules on virus-specific CD8+ T cells in draining LN, spleen and brain **Tregs:** numbers of FoxP3$^+$ CD4$^+$ T cells in draining LN and spleen		
Zika virus [30•]	Oral Abx-treatment	Survival Virus titers					

MAMPs, microbe-associated molecular patterns; PRRs, pathogen recognition receptors; Abx, antibiotic; GF, germ-free; TLR, toll-like receptor; MO, macrophage; BAL, bronchoalveolar lavage; DC, dendritic cell; LN, lymph node; Tregs, T regulatory cells; LPS, lipopolysaccharide; DAT, Desaminotyrosine; SPF, specific pathogen free; NK, natural killer; poly (I:C), polyinosinic:polycytidylic acid; IFN, interferon; CpG DNA, CPG deoxynucleic acid; NOD, nucleotide-binding oligomerization domain.

activation, such as TNF-α, IL-6, IL-1β, and lL-18, play a critical role in limiting viral replication and modulating viral pathogenesis by orchestrating tissue-specific immune responses through their recruitment and activation of monocytes, MO, granulocytes, DCs, and natural killer (NK) cells (reviewed in Ref. [22]). Studies using Abx-treatment and GF mice uncovered a complex relationship between the gut microbiota, pro-inflammatory cytokines and systemic virus infection (Table 1). The decreased expression of *Nlrp3*, pro-*Il-1β*, and pro-*Il-18* mRNA as well as the reduced amount of IL-1β protein in the bronchoalveolar lavage (BAL) of IAV-infected Abx-treated mice suggests that the commensal gut microbiota may function to stimulate the inflammasome at distal sites [23•]. Moreover, the reduced amount of TNF-α and IL-6 detected in the BAL and spleen of Abx-treated mice during IAV and LCMV infection, respectively, suggests an additional role for the gut microbiota in stimulating pro-inflammatory cytokines at distal sites [19•,23•]. However, in a recent study, Rosshart *et al.* also observed decreased amounts of pro-inflammatory cytokines including TNF-α and IL-6 following IAV infection in the BAL of GF mice reconstituted with gut microbiota from wild mice compared to GF mice reconstituted with gut microbiota from specific pathogen free-mice [24••]. Since GF mice reconstituted with gut microbiota from wild mice are protected from IAV infection, these studies suggest a more nuanced role for the commensal gut microbiota in calibrating pro-inflammatory cytokine responses and regulating viral pathogenesis during systemic virus infection that may be dependent on how different constituents of the gut microbiota engage distinct components of the host immune response.

Innate cellular immune response

The gut microbiota plays a key role in hematopoiesis by regulating production of immune cell precursors in the bone marrow as well as controlling the turnover of myeloid cells such as neutrophils and inflammatory monocytes in peripheral circulation [25–28]. While a role for the gut microbiota in cell-mediated immune responses at non-GI sites during bacterial infection is well described (reviewed in Ref. [9]), studies using Abx-treatment and GF mice highlighted virus-specific roles of the commensal gut microbiota in promoting innate cell-mediated immune responses (Table 1). Belkacem *et al.* observed increased numbers of tissue resident and circulatory myeloid cells including alveolar and interstitial MO, DCs, and inflammatory and patrolling monocytes in the lungs of mice orally colonization with *Lactobacillus paracasei* at baseline, but not during IAV infection, which was associated with protection against lethal infection [29]. Moreover, fewer CD103$^+$ DCs were found in the lung and mediastinal lymph nodes (LNs) of naïve Abx-treated mice [23•]. While CD103$^+$ DC from Abx-treated mice retain the ability to cross-present exogenously added antigen *ex vivo* [23•,30•], Ichinohe *et al.* found decreased numbers and impaired antigen-presenting capacity of CD103$^+$ DC from the lungs of IAV-infected Abx-treated mice, as well as impaired migration of these cells into mediastinal LNs, a required site of antiviral CD8$^+$ T cell priming during IAV infection [23•]. While the development, differentiation, and maturation of splenic NK cells was maintained in naïve Abx-treated mice, Ganal *et al.* observed that NK cells from Abx-treated mice stimulated *in vivo* with poly (I:C), LPS, and CpG DNA were impaired in *ex vivo* cell-mediated cytotoxicity and IFN-γ production [21]. Moreover, the impaired NK cell priming by CD11c$^+$ DCs or other bystander cells in Abx-treated mice resulted in increased virus titers during MCMV infection [21]. Together, these studies demonstrate a role for the commensal gut microbiota in priming cell-mediated innate immune responses at distal non-GI sites during systemic virus infection.

Role of the gut microbiota in adaptive immune responses at non-GI sites

A role for the commensal gut microbiota in shaping the adaptive immune response is now extensively documented (reviewed in Refs. [31,32]). The commensal gut microbiota is required for the development of secondary lymphoid tissues, as well as the differentiation, maturation, and function of T and B cells including virus-specific effector CD4$^+$ and CD8$^+$ T cells, FoxP3$^+$ CD4$^+$ T regulatory cells (Tregs) and Th17 cells, CD4$^+$ helper T cells, and B cells. Priming and activation of CD8$^+$ T cells by antigen-presenting cells (APCs) result in virus-specific effector T cells armed with the ability to kill virus-infected cells through the expression of effector molecules such as perforin and granzyme B, as well as the production of cytokines such as IFN-γ and TNF-α (reviewed in Ref. [33]). Additional interactions between APCs and CD4$^+$ T cells drive a cytokine-mediated Th1-polarized cellular program in other immune cells such as MO (reviewed in Ref. [34]). Moreover, CD4$^+$ T cells also have a crucial role following virus infection coordinating the generation of an optimal humoral immune response through their involvement in the germinal center reaction (reviewed in Ref. [35]). In short, an adaptive immune response requires complex coordination between innate immune cells and lymphocytes to initiate an optimal antiviral immune response capable of controlling systemic virus infection.

T cell-mediated adaptive immune response

While the commensal gut microbiota is known to regulate the differentiation and function of CD8$^+$ T cells and Tregs [36,37], its role in T cell-mediated antiviral responses during systemic virus infection is less well established. Studies using Abx-treatment and GF mice investigated the role of the commensal gut microbiota in the generation of virus-specific T cells and Tregs during systemic virus infection (Table 1). Fewer virus-specific effector CD8$^+$ T cells are found systemically in blood and

at distal sites including the lung, spleen, and brain of Abx-treated mice following Hepatitis B virus (HBV), IAV, LCMV, and West Nile Virus (WNV) infection [19•,23•,30•,38,39••]. Increased virus burden and/or increased susceptibility to lethal HBV, IAV, LCMV, and WNV infection of Abx-treated mice is likely in part due to decreased virus clearance by virus-specific effector $CD8^+$ T cells. While not examined in most studies, Ichinohe *et al.* also observed fewer virus-specific effector $CD4^+$ T cells in the spleens of IAV-infected Abx-treated mice suggesting a role for the commensal gut microbiota in priming both antiviral effector $CD4^+$ and $CD8^+$ T cells during systemic virus infection [23•]. In contrast, the role of the commensal gut microbiota in Treg induction appears to be more virus specific: fewer $FoxP3^+$ $CD4^+$ Tregs were found in the lung and spleen of Abx-treated mice following SeV and WNV infection, respectively, while a greater frequency of $FoxP3^+$ $CD4^+$ Tregs was found in the lung of Abx-treated mice following IAV infection [23•,30•,40]. In aggregate, these studies demonstrate a role for the commensal gut microbiota in generating an optimal effector and regulatory T cell response, the balance of which determines the outcome of systemic virus infection.

B cell-mediated adaptive immune response

While a role for the gut microbiota in shaping intestinal and systemic humoral immunity has been defined (reviewed in Ref. [41]), the mechanism by which the commensal gut microbiota regulates antibody responses during systemic virus infection is not known (Table 1). Virus-specific antibody responses are diminished systemically in Abx-treated mice following HBV, IAV, and LCMV, but not WNV, infection [19•,23•,30•,38,39••]. While these studies demonstrate that the commensal gut microbiota influences the humoral response during systemic virus infection, it remains to be determined if this effect is mediated directly through the influence of the gut microbiota on B cell function or indirectly through its influence on $CD4^+$ T helper cells that drive virus-specific antibody responses in the germinal center.

Potential mechanisms by which the commensal microbiota controls systemic virus infection

Although the molecular pathways through which the commensal gut microbial signals to generate optimal antiviral immune responses remain to be well defined, several groups have identified specific microbial constituents and signaling molecules that modulate host immunity and viral pathogenesis during systemic virus infection (Table 1). Consistent with the idea that the commensal gut microbiota modulates antiviral immune responses through the engagement of PRRs by MAMPs (Figure 1), the intrarectal administration of multiple toll-like receptor (TLR) agonists (LPS, peptidoglycan, poly (I:C), and CpG DNA) can restore inflammasome-dependent cytokines, $CD103^+$ DC priming and migration, and effector $CD8^+$ T responses in IAV-infected Abx-treated mice resulting in protection from lethal infection [19•,23•]. In addition, intravenous administration of a specific peptidoglycan component, Muramyl dipeptide (MDP), induces a NOD2-dependent type I IFN response which is protective against lethal IAV infection [42,43]. Recently, Jiang *et al.* found that oral administration of *Candida albicans* and *Saccharomyces cerevisiae* or the intrarectal administration of fungal-derived Mannan could also restore antiviral effector $CD8^+$ T cell responses resulting in protection of IAV-infected Abx-treated mice [39••]. This observation is consistent with the idea that the commensal gut microbiota acts to prime host immunity through the engagement of PRRs, since mannan can engage TLRs and C-type lectins (reviewed in Ref. [13]). Signaling through TLR4 may be required for the commensal gut microbiota to clear HBV antigen, while TRIF and MyD88-dependent signaling is required for microbiota-mediated priming of NK cells and control of MCMV infection [21,38]. In summary, these studies suggest that commensal gut microbiota-derived MAMPs engage PRRs to orchestrate innate immune responses that in turn generate optimal adaptive immune responses during systemic virus infection.

Commensal gut microbial-derived metabolites play an important role in the regulation of host immunity (reviewed in Ref. [44]), however; the role of microbiota-derived metabolites in systemic virus infection is not well defined (Table 1). Recently, Steed *et al.* identified a microbial-derived metabolite, desaminotyrosine (DAT), that is produced by the human-associated commensal gut bacteria *Clostridium orbiscendens* [45••]. Oral administration of *C. orbiscendens* or DAT protected mice from lethal IAV infection through reduced immunopathology in the lung in a phagocyte-dependent process potentially through augmentation of the Type I IFN amplification loop (Figure 1). When coupled with the major role of commensal gut microbial-derived metabolites in the regulation of host immune responses, this study suggests that many other metabolites will modulate the outcome of systemic virus infection.

Conclusions and future questions

Although many investigations using Abx-treated, GF and gnotobiotic mouse models over the past 50 years demonstrate a role for the commensal gut microbiota in priming antiviral innate and adaptive immune responses at non-GI tract sites during systemic virus infection (Table 1), many details about this long-distance interaction remain to be answered. While past studies have established a role for commensal bacteria and fungi in modulating host immunity during infection with several systemic viruses (Table 1), the role of commensal viruses and protozoa is

not well understood (reviewed in Refs. [46,47]). Moreover, because of the pervasive influence of the commensal gut microbiota on host immunity, additional systemic viruses may also be impacted. More recent studies have identified a role for specific bacteria and fungi, as well as MAMPs, metabolites, PRRs and downstream signaling molecules in the regulation of host immunity during systemic virus infection (Table 1). However, a more thorough understanding of the molecular mechanisms by which specific constituents of the commensal gut microbiota modulate host immunity is needed. Furthermore, despite studies which suggest that both the gut and local microbiota can independently influence host immunity in the lung during infection with several respiratory viruses [48–50], the relative contributions of the distal gut microbiota and local oral, respiratory, dermal, and genitourinary microbiota during systemic virus infection are not well established. Lastly, while this review has focused on regulation of systemic and distal non-GI tract host immune responses by the commensal gut microbiota, studies showing that systemic virus infection alters the commensal gut microbiota [51–53] suggest an additional layer of complexity in the interaction between the commensal gut microbiota and systemic virus infection that should be explored.

Conflict of interest statement

Nothing declared.

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