Parasitic worms affect virus coinfection: a mechanistic overview

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Abstract

Helminths are parasitic worms that co-evolve with their host usually resulting in long term persistence through modulating host immunity. The multifarious mechanisms altering the immune system induced by helminths have significant implications on the control of coinfecting pathogens such as viruses. Here, we explore the recent literature to highlight the main immune alterations and mechanisms that affect the control of viral coinfection. Insights from these mechanisms are valuable in the understanding of clinical observations in helminth prevalent areas and in the design of new therapeutic and vaccination strategies to control viral diseases.

Helminths in a world of viruses: Friends or Foes?

Parasite worms or **helminths** (see **Glossary**) are parasites of almost all vertebrates, including humans as well as wild and domestic mammals. Globally, helminths affect nearly a quarter of the human population, essentially infecting individuals living under poor hygienic conditions in low- and middle-income countries [1]. Such epidemiologic situation explains the large geographic overlap between regions where helminth infections are endemic and areas where diseases caused by heterologous agents such as tuberculosis, malaria, or infection by the human immunodeficiency virus (HIV) are prevalent. Despite such overlap and epidemiological studies reporting association between parasitic worm infections and other pathogens, a gap of knowledge remains on the mechanisms through which helminth exposure could affect the host's response to coinfections with viruses.

Parasitic helminths of clinical significance in human essentially belong to the Phyla *Nematoda* and *Platyhelminthes* [2]. The Phyla *Nematoda* includes gastro-intestinal or filarial parasitic nematodes. *Platyhelminthes* are flatworms of the two classes *Trematoda* and *Cestoda* (**Table 1**). Most helminths cannot complete their life cycle within their **definitive hosts**. The parasite adult stages develop in the definitive hosts where eggs or larvae are produced. Then, the development of larval stages occur either in the environment or in **intermediate or paratenic hosts**. Only few helminths are able to complete a full lifecycle in their definitive host through auto-reinfection, e.g. *Taenia solium* or *Strongyloides stercoralis* [3,4]. Although helminth parasites are responsible for the induction of disease in their hosts, the induced pathology is in general due to a high burden of infection. The pathological effects of helminth infections can be induced by several mechanisms such as: tissue damage during parasite migration, attachment to the intestinal mucosa and blood feeding, nutrient deprivation, dysregulation of the metabolism, intestinal blockage, secretion/excretion of toxic compounds, and induction of immunopathology. However, helminths have been proven masters in modulating their host immune system and establishing, in many cases, subclinical or asymptomatic infections.

Experimental research using mouse models of helminth infection has largely contributed to better understand the diverse mechanisms of anti-helminth response, which mainly involves type 2 immunity [5]. Indeed, most parasitic worms induce type 2 immune responses in the infected host, except for flatworms such as *Schistosoma* sp. that induce mixed type 1 and type 2 immune responses depending on the lifecycle stage [6]. Besides its key role in the control of helminths, type 2 immunity is also involved in multiple aspects of homeostasis and disease [7], including lung homeostasis in neonates, thermogenesis, mammary gland development and wound healing. Thus, the elicited type 2 immune responses during helminth infection can act beyond a simple role of parasite clearance, and result in the modulation of host immune responses to unrelated pathogens such as viruses during coinfections [8]. Recent studies suggest that helminth infections can variably affect the outcome of a viral coinfection, in different contexts where helminth infection precedes or follows the virus infection [9]. The diverse outcomes during helminth-virus coinfections are the result of a finely balanced modulation of the immune response to helminth infection that is shaped based on several elements such as the helminth species, the portal of entry, the existence of migration stages within the host, and the tissue of residence. In this already intricate immune environment, a viral infection with its tissue tropism and specific antiviral immune responses adds more complexity and explains the variable outcomes, mostly depending on the tissue tropism of the parasite and virus, the viral species itself, and the nature of the protective antiviral response (**Box 1**) [9]. Data from experimental coinfection studies using helminth and viral infection models are still limited. However, they have identified in the last decade several key mechanisms induced by helminths that affect the outcome of viral coinfection, as summarized in **Figure 1** and **Table 2**. The present review will focus on the current knowledge on how helminth infection, resulting in type 2 immune responses can affect the response to bystander viral pathogens during coinfection (**Boxes 2 and 3**).

Local and distant helminth tissue sensing: consequences on virus coinfection

Early sensing of helminth infection by epithelial cells results in the local release of **damage-associated molecular pattern molecules (DAMPs)** that include alarmins, eicosanoids and neurotransmitters. The response to epithelial cell damage during skin penetration, migration, and extravasation of parasites into either the lung or intestine also includes the release of IL-33, a pleiotropic alarmin acting via the suppression of tumorigenicity 2 receptor (ST2). In concert with ATP released from damaged cells, the binding of IL-33 to its receptor ST2 (IL33R) results in the activation of type 2 immune cells, including ILC2s, basophils, and mast cells, leading to the release of the type 2 cytokines IL-4, -5, -9, and -13 [5]. On note, *H. polygyrus* secrete anti-IL33 and anti-IL-33 receptor products while promoting IL-1β production, leading to ILC2 suppression and worm persistence [10,11]. But IL-33 can also drive protective antiviral $CD8^+$ T cell responses [12], although the direct actions of IL-33 induced during helminth infection on antiviral $CD8^+$ T cell responses have not been addressed yet. IL-33 is not only induced in damaged tissues caused by the direct action of the parasite itself. Indeed, *N. brasiliensis* infection can induce epithelial stress and IL-33 release not only in the lung and intestine upon tissue damage, but also in the female genital tract (FGT), a tissue site distant from the tissues directly affected by the parasite [13]. Importantly, when *N. brasiliensis*-infected female mice were coinfected in the genital tract with human herpesvirus 2 (HHV-2 or **Herpesvirus simplex 2, HSV-2**), the induced lesions of genital herpes were severely exacerbated [13]. Strikingly, elevated IL-33 resulted in an **IL-4 receptor** α chain (IL-4Rα)-independent activation of ILC2s and production of type 2 cytokines including IL-5. IL-5 recruited eosinophils to the FGT, and these cells contributed to the exacerbated HSV-2-induced epithelial ulcerations, potentially through local release of major basic protein (MBP) and eosinophil extracellular traps. These observations offer a molecular explanation to the elevated type 2 cytokine profile in cervical fluid samples of patients infected by nematodes, their higher risk of viral infection and genital disease even though helminths do not typically populate the genital tract [14]. However, it remains unclear how epithelial stress and IL-33 release is triggered during *N. brasiliensis* infection.

An alternative macrophage response affects virus coinfection

Following parasitic helminth infection, the type 2 cytokines IL-4 and IL-13 signal through the shared IL-4Rα chain leading to the activation of the transcription factor signal transducer and activator of transcription (STAT)-6 [15]. Activated STAT-6 binds to the promotor regions of multiple genes and promotes their expression resulting in polarization and proliferation of **alternatively activated macrophages (AAMs)**. AAMs have been reported to mediate important roles in helminth infections, notably in modulating CD4^{+} Th2 responses and liver fibrosis during schistosomiasis [16–18], as well as promoting worm clearance [19]. Moreover, lung neutrophils were shown to interplay with AAMs in the lung and control *N. brasiliensis* larvae migration [20–22].

Compared to classically-activated macrophages by IFN-γ, IL-4/IL-13-polarized AAMs are less armed to control intracellular infections with bacteria or protozoa [8]. The role played by AAMs to control viruses in the context of helminth coinfection has also been investigated. IL-4Rα signaling in macrophages promoted the reactivation from latency of murid gammaherpesvirus 4 (MuHV-4) [23]. MuHV-4 is a rodent model of the two human oncogenic viruses Epstein Barr virus (EBV) and Kaposi's Sarcoma herpesvirus (KSHV). These viruses establish a persistent latent infection in myeloid cells and B lymphocytes, resulting in some cases in lymphomagenesis. Mechanistically, STAT-6 activation in latently infected AAMs during *H. polygyrus* infection or following administration of *S. mansoni* eggs or **IL-4 complex**, lead to the activation of the viral promoter N4/5 that in turn switched on the expression of the reactivation transactivator (Rta) gene encoded by viral ORF50 [23]. Importantly, combined IL-4 complex treatment and anti-IFN-γ antibody blockade potentiated viral reactivation induced by STAT-6, supporting the early finding that IFN-γ signaling can block gammaherpesvirus reactivation from latency [27]. IL-4 could also induce expression of viral reactivation transcripts and increased viral replication in KSHV infected cells, supporting that IL-4 could also reactivate gammaherpesvirus reactivation not only in mice, but also in human [23]. However, the IL-4Rα-mediated virus reactivation seemed to be cell dependent. Indeed, activated STAT6 could bind to the viral ORF50 N4/N5 promoter in macrophages but not in B lymphocytes [24], although the exact mechanism remains unclear. These observations suggest that cell-specific immunomodulatory mechanisms take place during helminth infections and could result in uncontrolled dissemination of gammaherpesvirus infections in-between hosts but also within the latently-infected host, therefore driving an increase in the pool of infected cells and the risk of developing lymphomas.

AAMs express characteristic signature genes that are involved in anti-helminth immunity and tissue repair [21,22]. Although the function of these effector molecules remains unclear, they seem to have a potential role in antiviral immunity. For example, AAMs expressing YM1 could impair the effective control of murine norovirus strain CR6 (MNV), an enterotropic virus. Mice infected with *T. spiralis* exhibited higher MNV titers in the ileum, an effect that was reproduced by the administration of IL-4 complex, whereas STAT-6- or IL-4Rα-deficient mice did not exhibit higher viral loads during coinfection [25]. The direct role of AAMs in being more permissive to MNV infection was investigated in bone marrow-derived macrophages and suggested that IL-4/IL-13 polarization of AAMs during *T. spiralis* infection could contribute to the observed increased MNV titers in coinfected mice. In addition, it was proposed that the effector chitinase-like protein YM1 produced by AAMs after *T. spiralis* infection contributed to limiting MNV-specific $CD8^+$ T cell expansion, resulting in increased MNV titers *in vivo* [25]. However, whether YM1 can directly alter antiviral cytotoxic T cell antigen priming and/or maintenance remains unclear. Besides macrophage polarization itself, IL-4 can also drive the proliferation of resident cavity macrophages during *L. sigmodontis* or *H. polygyrus* infection [26]. Other helminth species such as *S. mansoni* also trigger the recruitment of monocyte-derived macrophages, resulting in phenotypically distinct resident or monocyte-derived AAMs [27–29]. Thus, the nature of macrophage responses to helminth invasion either by local proliferation of AAMs in response to IL-4 or polarization of monocyte-derived macrophages are mechanisms that likely affect antiviral control and/or their intrinsic permissiveness to viral infection, which could result in a differential outcome in viral disease.

Tolerating helminths at the expense of antibody response to vaccines?

Helminths have coevolved with their hosts to achieve a finely regulated balance between immune control and tolerance of the parasite. To this end, helminths produce **excretory/secretory products (ES)** that contain a pharmacopeia of immunomodulatory molecules [30], some of them being able to expand $CD4^+Foxp3^+$ **regulatory T cells (Tregs)**. For example, filarial AvCystatin and *H. polygyrus*-produced TGF-β mimics expand Tregs *in vitro* [31,32]. In filarial infection, expansion of Tregs occurs and not only improves parasite survival but also contributes to avert parasite-induced pathology [33]. Similarly, Treg expansion induced by IL-2 administration *in vivo* contributes to *H. polygyrus* persistence [34], and transient depletion of Tregs during the first days of infection could promote expulsion of *Strongyloides ratti* [35]. However, these tolerance mechanisms could have an adverse effect when strong protective type 1 or type 2 immune responses are needed.

Vaccine responses have been reported to be poor in helminth-infected individuals [36,37]. Indeed, infection with helminths interferes with the response to different types of vaccines, such as Bacille Calmette-Guérin (BCG), tetanus toxoid (TT) and vaccines against measles or hepatitis B [37]. For example, the responsiveness to TT was decreased during lymphatic filariasis in human and associated with reduced IFN-γ production and increased TT-specific IL-10 production [38]. In addition, some studies have explored in animal models the role of filarial infections in viral coinfections and vaccine efficiency. Mice infected with the filarial nematode *Litomosoides sigmodontis* exhibited decreased levels of IgG2b and IgG2c antibodies against coinfecting retrovirus Friend Virus (FV), associated with increased viral titers [39]. Interestingly, *L. sigmodontis* infection has been shown to induce a systemic and sustained expansion of IL-10-producing CD49b$^+$LAG-3$^+$ type 1 regulatory T cells (Tr1) that persists even past the resolution of the infection [40]. In the context of coinfection with influenza virus (IAV), Tr1 cells were responsible for dampened antibody titers after a non-adjuvanted vaccination protocol against IAV that resulted in impaired viral control and lung disease upon viral challenge. Conversely, treatment with the filarial cystatin (AvCystatin/Av17) did not affect the antibody levels against subsequent infection with human respiratory syncytial virus (RSV) [32]. Nevertheless, AvCystatin administration could induce the expansion of IL-10$^+$ T cells which was associated with a milder inflammation after primary RSV infection, and vaccine-enhanced RSV lung inflammation. *L. sigmodontis* infection has also been associated with reduced antigen-specific B cells and antibody titers after vaccination with the model antigen DNP-KLH [41]. However, the involved mechanism was Treg-independent but rather due to reduced PD1$^+$CXCR5$^+$ **T follicular helper-like (T_{FH})** cells in germinal centers of the draining lymph nodes [42]. Interestingly, impaired vaccine efficacy during filarial infection also occurred using other model antigens as vaccine or a prime-boost influenza vaccine protocol, and was maintained even after parasite clearance [43,44]. Nevertheless, the efficacy of a prime-boost vaccine against IAV could be restored if applied in combination with *L. sigmodontis* deworming [45]. Thus, helminth persistent infection seems to be associated with peripheral immune tolerance with the induction of Tregs and IL-10-producing Tr1 cells, the latter being involved in dampening immunopathology induced by respiratory viruses coinfections but also affecting the response to vaccines, via impaired T_{FH} responses, at least during filarial infection.

A gut feeling for helminth affects antiviral responses

Most helminth species reside in the gut, where they interact with elements of the intestinal barrier such as the mucus and the epithelial cell layer itself but also with the microbiota and derived metabolites [46]. In response to helminths, the host’s gut mucosa has developed several mechanisms in an effort to manage worm infections, with potential consequences on concurrent viral infections. During intestinal worm infection, increased electrolyte production by the gut epithelium results in a “weep and sweep” response [47]. Although promoting parasite clearance, this mechanism also facilitates the caudal movement of parasite eggs and therefore, their dissemination. Helminths and their ES products can interact with epithelial cells, resulting in the induction of IL-25 release by intestinal **tuft cells** [48]. IL-25 in turn leads to the activation of ILC2s, and the initiation of a positive loop in which ILC2s produce IL-4 and IL-13 to expand tuft cells and goblet cells [47]. Importantly, tuft cell activation by helminths alters antiviral immunity during coinfection with West Nile virus (WNV), a neurotropic flavivirus that also targets enteric neurons [49]. Mechanistically, IL-25 released by tuft cells after sensing *H. polygyrus* worms resulted in the activation of IL-4Rα/STAT-6-dependent type 2 immunity and increased gut permeability and bacteriaemia when coinfected with WNV. Consequently, coinfected mice had impaired WNV-specific $CD8^+$ T cell responses resulting in higher viral burdens in the brain and intestine, and more severe viral disease [49]. Antibiotic treatment could rescue the control of WNV infection, suggesting that bacterial gut translocation could be involved in the disease exacerbation. *H. polygyrus* infection also exacerbated disease caused by coinfection with other flaviviruses, such as Zika and Powassan viruses [49]. During *H. polygyrus*/WNV coinfection the histologic splenic architecture was disrupted and increased apoptosis in WNV-specific CD8$^+$ T cells could be observed. Apoptosis of WNV-specific cytotoxic T cells could be possibly caused by an altered DC function, ultimately leading to impaired viral clearance and disease [49]. Importantly, another study investigating lymphocytic choriomeningitis virus (LCMV) and *Escherichia coli* coinfection confirmed that systemic bacterial dissemination can dampen CD8$^+$ T cell responses to viral infection [50].

A peak of type 2 inflammation occurs around 10-15 days after *H. polygyrus* infection when larvae have migrated from the submucosa to the intestinal lumen. This stage of the lifecycle can affect epithelial permeability, and constitutes an important stage of the lifecycle that could favor bacterial gut translocation, although most commensals reside in the colon rather than the small intestine where the adult worms reside [51]. Systemic treatment with IL-4-complex also resulted in disease exacerbation after WNV infection, as observed after *H. polygyrus* infection, suggesting IL-4 elicited responses are sufficient to induce bacterial translocation and impaired CD8$^+$ T cell responses. However, the gut tropism of the virus species is probably determinant as IL-4-complex treatment followed by LCMV infection only resulted in a mildly reduced CD8$^+$ T cell response with no bacterial translocation [49]. In addition to these observations during coinfection, helminth infections are also associated with changes in the **microbiome** composition [52], which could potentially interfere with antiviral immune responses although these effects have not been investigated yet.

Bacterial gut translocation is not the sole mechanism involved in impacting the cytotoxic T cell responses to coinfecting viruses during helminth infection. Indeed, MNV coinfection during the intestinal phase of *T. spiralis* infection resulted in increased MNV viral load in the intestine and impaired MNV-specific CD8$^+$ T cells [53]. However, although bacterial translocation was not directly addressed in that study, no link between gut microbiota and the observed impaired anti-MNV T cell response could be identified. Instead, antibody-mediated blockade of YM1 could restore effective antiviral T cell-mediated responses, suggesting that AAMs induced after helminth infection not only mitigate Th2 cell responses [21], but potentially also modulate CD8$^+$ T lymphocytes. Alternatively, MNV could also use an alternate strategy to invade helminth-infected hosts more efficiently. Indeed, MNV has been shown to specifically target tuft cells for host invasion via the receptor CD300lf, and IL-4 or systemic IL-25 treatment resulted in increased MNV replication in the intestine [54]. Thus, helminth-dependent activation and expansion of intestinal tuft cells could likely contribute to explain the increased permissiveness to MNV infection, although this has never been directly demonstrated. An additional proposed mechanism to explain the impaired adaptive immune response to viral coinfection is the systemic redistribution of naive lymphocytes to the mesenteric lymph nodes during intestinal persistent helminth infection [55,56]. As a consequence, immune cells are actively depleted from peripheral lymphoid organs, which results in impaired virus-specific T cell responses at peripheral sites during coinfection [55]. Such phenomenon could also provide an additional explanation to the poor efficacy of vaccine responses in areas where gastro-intestinal helminth infections are endemic [37].

Despite exacerbation of disease in the context of WNV or MNV coinfection with intestinal helminths, infection with *H. polygyrus* rather protected against lower respiratory tract viral infection with RSV in mice [57]. Such duality in disease outcome could be dependent on the tissue targeted by viral infection, at least in part. *H. polygyrus*/RSV co-infected mice experienced less to moderate clinical signs and body weight loss, and reduced viral load. The protective effects of *H. polygyrus* against RSV were absent in mice lacking type I IFN signaling and in **germ-free mice**, but were unexpectedly retained in mice unable to respond to IL-4 or IL-13 (IL-4Rα$^{-/-}$) or devoid of T and B lymphocytes (RAG$^{-/-}$) [57]. Although bacterial gut translocation was not directly explored, *H. polygyrus* infection could be associated with an upregulation of IFN-stimulated genes in the lung, which would mediate clinical protection against RSV infection. Type I IFN response might be promoted by helminth-associated changes in gut microbiota and/or helminth-derived products, as previously reported [52,58]. Intriguingly, coinfection was associated with a reduced infiltration of cytotoxic CD8^{+} T cells, reflecting the bimodal role of cytotoxic T cells in killing RSV-infected cells but also contributing to lung pathology [59]. Thus, the impaired systemic CD8^{+} T cell responses during coinfection with intestinal helminths is not necessarily associated with deleterious outcomes but can also be beneficial for virus-induced lung pathology. Interestingly, *H. polygyrus* is not the only gastro-intestinal helminth species that protects against respiratory viral disease. Indeed, *T. spiralis* infection during the intestinal phase of its lifecycle also protected against respiratory IAV infection [60], and *S. mansoni* infection similarly protected mice from fatal respiratory distress upon infection with the pneumonia virus of mice (PVM), a mouse virus species phylogenetically related to RSV [61]. Thus, a general concept that emerges from these studies suggests that persistent intestinal helminth infections are associated with impaired induction and/or maintenance of virus-specific $CD8^+$ T cell responses to viral coinfection in peripheral lymphoid organs, which is associated either with exacerbation of systemic viral disease due to flavivirus infections, while conversely promote clinical protection against respiratory viral infection through type 2 immunity-independent and microbiota-dependent mechanisms. The exact mechanisms involved are however yet to be uncovered in their complexity.

Helminths go virtual to shape antiviral cytotoxic T cell responses

Besides being a major regulator of type 2 immunity, thymic IL-4 is also necessary to drive the differentiation of a specific subpopulation of memory-phenotype $CD8^+$ T cells independently of foreign antigen encounter. These cells are named “innate” or “virtual” memory T cells (T_{VM}) based on their respective location in the thymus or the periphery. In naive conditions, T_{VM} derive from low-affinity self-antigen recognition that leads to a memory phenotype characterized by upregulation of marker genes associated with memory, such as the marker CD44, the chemokine receptor CXCR3, and the shared IL-2/IL-15 chain IL-2 receptor β [62,63]. While IL-4 is required to provide the necessary signals for T_{VM} differentiation in the thymus, peripheral IL-4 produced in response to helminth infection can drive a significant expansion of T_{VM} in the lymphoid organs that are retained over time [64,65]. T_{VM} are described to have a conserved diversity of their T cell receptor repertoire and respond to their cognate antigen more rapidly than their naive equivalent [66], a characteristic that does not seem to be driven by IL-4 [65,67]. However, the IL-4-dependent expansion of T_{VM} during helminth exposure could directly be associated with a $CD8^+$ T cell-dependent enhanced control of respiratory MuHV-4 infection [65], as well as coinfection with bacteria [64]. In addition, T_{VM} maintenance relies on type I IFN signals [68], indicating that helminth-driven induction of IFN signature gene expression could contribute to T_{VM} function [58]. Thus, helminth infections are not always associated with impaired bystander CD8$^+$ T cell responses, but can also directly drive CD8$^+$ T_{VM} and efficient control of viral coinfections.

These observations can only add to the observation that the outcome of helminth/virus coinfections is tightly regulated based on the site of infection, the timing of coinfection, the virus species, and the nature of protection against viral disease [9]. IFN-γ and IL-4 are antagonistic cytokines at the core of the dichotomy of type 1 and type 2 immunity. Nevertheless, IL-4 induced during helminth infection can directly elicit CD8$^+$ T cells to express the transcription factor eomesodermin (EOMES), leading to upregulation of the chemokine receptors CCR2 and CCR5 and production of IFN-γ and granzyme A, independently of the microbiota as germ-free mice have similar T_{VM} proportions [9,62]. Such an intriguing connection between IL-4 and IFN-γ has real-world implications *in vivo* as IL-4 produced during helminth infection has beneficial bystander effects on IFN-γ-dependent antiviral effector responses by supporting T_{VM} development. Since anti-helminth immunity has a general negative impact on the establishment of effective antiviral responses, IL-4-promoted T_{VM} expansion could represent a safety mechanism developed by the immune system during evolution to produce highly responding CD8$^+$ T cells being able to quickly contribute to viral control.

Concluding remarks

Infections with parasite helminths and viral pathogens occur in largely overlapping geographic areas. Despite such epidemiological data abundance, only few studies address how helminths can affect the antiviral control and modulate viral disease during coinfection but also following deworming. This significant gap of knowledge showcases the need for more studies with high translational value. The role of specific cellular subsets involved or induced during the anti-helminth immune response, such as eosinophils, AAMs and/or T cell subsets can act as protective or exacerbating mediators in the outcome of a viral coinfection. What remains unclear, is how and when worm and virus coinfection would result in the engagement of immune mechanisms leading to beneficial or deleterious outcomes on viral disease development (**Figure 2**). The diverse nature of helminths with their complex lifecycles and the multifaceted anti-helminth immune response in association with viral coinfections could be seen as a Sisyphean task (see **Outstanding Questions**). Nevertheless, the existing studies have uncovered important immune mechanisms with real world implications and pursuing the investigations to unravel how these interactions are paramount in an era where emerging pathogens potentially causing pandemics arise, such as COVID-19 [69].

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Declaration of interests

The authors declare no competing interests.

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Glossary

Alternatively activated macrophages (AAMs): Alternative macrophages activated through the IL-4 receptor and involved in wound healing, resolution of inflammation, and tissue remodeling. AAMs upregulate signature genes like mannose receptor, arginase 1 (Arg1), chitinase-like (YM1/2), resistin-like molecule-α (RELM-α), or programmed cell death ligand 2 (PD-L2)

Damage-associated molecular pattern molecules (DAMPs): Released from damaged host cells and recognized by innate immune receptors

Definitive, intermediate and paratenic hosts: Definitive host in which sexual reproduction occurs; intermediate host in which the parasite passes obligatorily during its development and in which asexual multiplication may occur; paratenic hosts harbor larval parasite stages which do not develop further or multiply

Excretory/secretory products (ES): Released by helminth species in the extracellular space and composed of a mixture of proteins, peptides, nucleic acids, lipids, glycans, and small organic molecules that can have immunoregulatory functions

Germ-free mice: Mice that have no microorganisms living in or on them

Helminths: A general term referring to worms of four phyla: Nematoda (roundworms), Platyhelminthes (flatworms), Acanthocephala (spiny-headed worms), and Nematomorpha (hairworms). Only worms belonging to the first three are endoparasitic to vertebrates. The term “helminths” is in general restricted to parasitic worms of vertebrates, but many free-living species exists as well as nematodes infecting plants

Herpesvirus simplex 2 (HSV-2): Double-stranded DNA herpesvirus infecting human and responsible for genital herpes

IL-4 complex: A complex made of recombinant mouse IL-4 and anti-IL-4 monoclonal rat antibody to extend the half-life of the cytokine *in vivo*

IL-4 receptor: Type 1 and type 2 IL-4 receptors share the IL-4Rα chain. IL-4Rα interacts with the gamma common chain (type 1 IL-4 receptor) or with the IL-13-binding receptor alpha 1 (type 2 IL-4/IL-13 receptor)

Microbiome: The community of microorganisms residing in a particular environment, such as the intestine

Regulatory T cells (Tregs): Specialized $CD4^+$ T lymphocyte subset playing an important role in suppressing inflammation

T follicular helper cells: Specialized $CD4^+$ T lymphocyte subset that provides essential help to B lymphocytes for effective antibody responses

Tuft cells: Chemosensory epithelial cells having a characteristic ‘tuft-like’ brush of apical microvilli, functioning as a major constituent of the small intestinal response to parasitic helminths and protists

Virtual memory T cells (T_{VM}): Antigen-inexperienced $CD8^+$ T cells expressing of the main markers of immune memory independently of antigen encounter

“Weep-and-sweep” response: Intestinal response to intestinal helminth infections, characterized with increased luminal fluid and mucus (‘weep’) and increased smooth muscle contractility ‘sweep’ to clear out the intestinal helminths

Table 1. Mouse models of helminth infections

Phylum	Helminth species of importance	Mouse model	Natural host species	Infective stage and route of infection	Migratory tissue	Destination tissue	Reference
Nematoda	*Necator spp* and *Ancylostoma spp*	*N. brasiliensis*	Rat	L3 larvae (sc, id)	Skin, Lung	Small Intestine	[70]
		H. polygyrus	Mouse	L3 larvae (oral)	Intestinal Submucosa	Small Intestine	
	Ascaris spp	*A. suum*	Pig	Eggs (oral)	Blood, Peritoneum, Liver, Lung	Small Intestine	
	Trichuris spp	*T. muris*	Mouse	Eggs (oral)	-	Caecum and colon	
	Strongyloides spp	*S. ratti,* *S. venezuelensis*	Rat	L3 larvae (oral, sc, id)	Skin, Lung, Cerebro-spinal Fluid, Brain	Small intestine	
	Trichinella spp	*T. spiralis*	Mammals	Larvae (oral)	Blood, Lymphatics, Skeletal Tissue	Small Intestine (Adults), Skeletal muscles (Larvae)	[71]
	Filarial nematodes	*B. malayi*	Humans	L3 larvae (ip)	Blood, Lung, Liver, Spleen, Lymphatics, Skin	Lymphatics (Adults) Diverse Tissues (Microfilariae)	[72]
		L. sigmodontis	Cotton Rat	L3 larvae (sc, id)		Pleural Cavity (Adults) Diverse Tissues (Microfilariae)	
		A. vitae	Gerbil	L3 larvae (sc)		Subcutaneous Tissue (Adults)	

						Diverse Tissues (Microfilariae)	
Platy-helminthes	*Schistosoma spp*	*S. mansoni, S. japonicum*	Mammals	Cercariae (percutaneous)	Blood, Liver	Mesenteric veinules	[6]
		S. haematobium		Eggs (urogenital)	-	Bladder Vagina	
	Fasciola spp	*F. hepatica*	Ruminants, Pigs, Humans	Metacercariae (oral)	Gut	Liver	[73]
	Hymenolepis spp	*H. diminuta*	Rats	Eggs, Proglottids, Cysticercoids (oral)	-	Small Intestine	[74]
	Echinococcus spp	*E. multilocularis*	Canids	Protoscoleces (oral, ip, iv)	Blood, Liver, Lung	Liver, Lung, Brain	
	Taenia spp	*T. crassiceps ORF*	Humans and animals	Cysticercoids (oral, ip, sc)	Intestine	Muscles, Infected Tissue	
		Mesocestoides corti	Carnivores	Metacestodes (intercranial)	-	Brain	
	Spirometra spp	*S. mansonoides*	Humans, Cats And Dogs	Plerocercoids (oral, id)	Gut	Muscle, Connective Tissue	

id = intradermal, sc = subcutaneous, iv = intravenous

Table 2. Experimental models of helminth and virus co-infection

Outcome	Tissue localisation	Virus	Helminth	Main observations and mechanism insight	Reference
Positive outcome	Systemic	Murid gammaherpesvirus 4	*S. mansoni,* *N. brasiliensis,* *H. polygyrus*	IL-4-dependent T_{VM} expansion leads to increased antigen-specific $CD8^+$ T cell activation and enhanced control of lytic viral infection	[65]
		Hepatitis B virus	*S. mansoni*	Schistosome-induced IFN-γ had a prominent antiviral effect that outcompeted immunosuppressive effects of type 2 cytokines	[75]
	Lung	Pneumonia virus of mice	*S. mansoni*	IFN-I independent protection; likely due to TNFα- dependent goblet cell hyperplasia	[61]
		Respiratory Syncytial Virus	*H. polygyrus*	Protection of lung disease and viral replication dependent on intestinal microbiota, IFN-I-dependent but IL-4Rα-independent or adaptive immunity	[57]
			AvCystatin (filarial origin)	Protection correlated with an influx of $IL\text{-}10^+$ Tregs in the airway resulting in reduced neutrophilic inflammation and cytokine production in the airways	[32]
		Influenza A H3N2 (X31)	*T. spiralis*	TNF-α mediated but IL-10-independent reduction of neutrophil recruitment resulting in dampened CD4 and CD8 T cells responses	[60]
Negative outcome	Systemic	Friend virus	*L. sigmodontis*	Reduced production of virus-specific neutralizing antiviral antibodies	[39]
		Murid gammaherpesvirus 4	*S. mansoni eggs,* *H. polygyrus*	Latent viral infection is reactivated *in vivo* by helminth infection, which is characterized by the induction of the cytokine interleukin-4 (IL-4) and the activation of the transcription factor STAT-6. By causing STAT-6 to bind to the promoter of the viral reactivation transactivator gene, IL-4 promoted viral replication and counteracted the antiviral effects of IFN-γ	[23]

	Human T Lymphotrope Virus-1	*S. stercoralis*	IFN-γ, TNF-α, IL-12p40, IL-10 and IL-13 were elevated in humanized mice infected with HTLV-1, *S. stercoralis* or with the dual infection, as compared to background controls	[76]
	Recombinant Vaccinia virus (vPE16)[a]	*S. mansoni*	Impaired antigen-specific cytototoxic T cell response and delayed viral clearance	[77]
	Lymphocytic choriomeningitis virus	*S. mansoni*	Increased viral multiplication and morbidity caused by type 2 cytokines and IFN-I being suppressed	[78]
Lung	Vaccinia virus	*A. suum*	Increased inflammation in the early stages (neutrophils, eosinophils, macrophages) and impaired viral control	[79]
	Influenza A virus H1N1 Hamburg/05/09 and non-adjuvanted influenza vaccine (Begripal®)	*L. sigmodontis*	Reduced efficacy of influenza vaccination due to the expansion of IL-10^{+} Tr1 cells	[40]
			Impaired antibody response to seasonal influenza vaccine	[43]
			Restored response to prime-boost influenza vaccination after deworming	[45]
	Influenza virus (S15)	*A. suum*	Increased severity of swine influenza	[80]
	Influenza virus A2/Japan/170	*N. brasiliensis*	Exacerbation of pulmonary lesions when larvae are present in the lung	[81]
		H. polygyrus	Increased viral titers and reduced antiviral antibody response	[82]
Gut	Flavivirus (West-Nile, Powassan, Zika)	*H. polygyrus*	Fatal gut lesions resulting from impaired virus-specific cytotoxic T cell responses – IL-4Rα/STAT6-dependent and tuft cell-dependent induction of bacterial gut translocation	[49]
	Influenza A H1N1 (PR8)	*H. polygyrus*	Accumulation of naïve lymphocytes in the mesenteric lymph node associated with	[55]

				depletion from non-draining lymph nodes and impaired peripheral antiviral response	
		Murine astrovirus	*H. polygyrus*	Increased permissiveness of intestinal goblet cells	[83]
		Murine norovirus (strain CR6)	*T. spiralis*	Impaired polyfunctional antiviral cytotoxic T cell response caused by YM1-producing AAMs but independent of microbiota	[25]
	Female genital tract	Human herpesvirus 2 (Herpes simplex 2, HSV-2)	*N. brasiliensis*	Exacerbation of genital herpetic lesions caused by an IL-4Rα-independent activation of eosinophils, recruited by IL-33-activated ILC2s and IL-5	[13]
Depend on the stage of the infection	Systemic	Influenza A H1N1 (WSN/33)	*L. sigmodontis*	Beneficial at juvenile adult stage Detrimental at L4 larval stage	[84]

[a] recombinant vaccina virus vPE16 expresses HIV gp160

Figure 1. Mechanistic insights on how helminths shape the response to viral infections. Helminth sensing in the gut is followed by the release of the alarmins TSLP, IL-25 from tuft cells and IL-33 from stressed epithelial cells and the subsequent production of IL-4 and IL-13 from activated immune cells. The resulting type 2 immune response in the gut causes changes in the microbiome affecting antiviral immunity at distal sites. Microbiota-dependent upregulation of type I interferons (IFN-I) and interferon stimulated genes in the gut and lung results in a better control of respiratory syncytial virus (RSV) (1). Bacterial translocation increases the susceptibility to West Nile virus (WNV), a flavivirus that infects enteric neurons, due to the impaired activation of cytotoxic T lymphocytes (CTL) by dendritic cells (DCs) in the spleen (2). Type 2 cytokines IL-4 and IL-13 drive the alternative activation of macrophages (AAM). The production of chitinase-like protein YM1 by AAMs impairs the activation and proliferation of murine norovirus (MNV)-specific CTLs in the secondary lymphoid organs (3). Direct IL-4 signaling in AAMs latently infected with MuHV-4 promotes the viral reactivation (4). Simultaneously, IL-4 signaling in CD8$^+$ T cells causes the expansion of **virtual memory T cells (T_{VM})** in secondary lymphoid organs that contributes to the immunity against murid gammaherpesvirus 4 (MuHV-4), resulting in increased virus-specific CTLs producing granzyme B (GrzB) and interferon γ (IFNγ) (5). ILC2: group 2 innate lymphoid cells, Th2: CD4$^+$ T helper 2 cells, Eos: eosinophils. Created using Biorender.com

Figure 2. Helminth-virus coinfection situations and their implications on viral disease. Representation of the main positive and negative outcome prediction of helminth-virus coinfection in term of viral disease development based on studies in mice [23,25,39,49,55,57,60,61,65,77,79]. Although the outcome of a coinfection is a challenging task to predetermine given the multilayered factors to be considered, a few general pointers may assist in predicting disease outcome. In general, having a helminth infection before a viral infection was biased towards a positive outcome, especially if the viral infection coincided with the type 2 immune response, or was in a different tissue of tropism. The latter is suggested by a number of studies demonstrating helminth protection of respiratory viruses, but not for genital viral infections. However, helminth infection after viral infection is reported to either reactivate latent viral infection, or impair antiviral responses resulting in increased viral loads. Shared organ tropism may also indicate a bias towards a negative outcome of the viral infection. Created using Biorender.com

Box 1. Anti-helminth vs. antiviral immunity: how different?

- The nature of anti-helminth immunity consists in the induction of a coordinated type 2 immune response aiming for parasite killing, worm clearance from the intestine (“weep and sweep”), tissue repair, and immunoregulation [5]. The induction of these mechanisms will depend on the nature of the helminth species, the existence of a migratory phase in the parasite lifecycle, and the tissue in which the helminth resides.
    - Immune cells: the effector mechanisms against helminths are mediated by the crosstalk of innate and adaptive immune cells. Innate cells include group 2 innate lymphoid cells (ILC2), eosinophils, mast cells, macrophages, and dendritic cells (DC). Cells of the adaptive immune system include $CD4^+$ T helper 2 (Th2) cells and B lymphocytes [5].
    - Effector molecules and mechanisms: Alarmins (TSLP, IL-25, IL-33) are quickly released after cell damage and initiate the type 2 immune response [85]. Innate and adaptive cells produce type 2 cytokines (IL-4, IL-5 and IL-13), resulting in peripheral and tissue eosinophilia, goblet cell hyperplasia, mucus production, and alternative activation of macrophages (AAMs) in a coordinated response to control the parasite worm and repair the damaged tissue [22,47].

- Antiviral immunity aims to inhibit viral replication, neutralize viral particles, and kill infected cells, in a coordinated type 1 immune response, including antibody production and effector cytotoxic T lymphocytes [86].
    - Immune cells: the effector mechanisms against viruses are mediated by the crosstalk of innate and adaptive immune cells. Innate cells include group 1 lymphoid cells (ILC1) and natural killer cells, macrophages, and DCs. Cells of

the adaptive immune system include B lymphocytes, CD4$^+$ T helper 1 (Th1) cells, and cytotoxic CD8$^+$ T lymphocytes (CTLs) [86,87].

- Effector molecules and mechanisms: Innate molecules such as defensins, complement, IFNs and IFN-stimulated genes are important mediators of the innate antiviral response. In addition, IFN-γ and tumour necrosis factor (TNF) can activate macrophages to promote phagocytosis of infected cells. Virus-specific cytotoxic CD8$^+$ T cells migrate to the site(s) of infection, where they kill infected cells, notably by releasing perforin and granzymes. Upon antigen activation, B cells produce and release virus-specific immunoglobulin M (IgM) antibodies; while Th1 cells promote class-switching of germinal center B cells from IgM to IgG or IgA virus-specific antibody production. Effector functions of antibodies include viral neutralization, opsonization to promote phagocytosis, antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells, and complement activation [86,87].

Box 2. Main mouse models of helminth infection

Nippostrongylus brasiliensis: Rat parasite related to human hookworms. Direct cycle where eggs release larvae in the feces, and L3 penetrate the skin and migrate through the lung and airways to reach the small intestine. In mice, a strong type 2 immunity is induced, promoting lung tissue repair via AAMs and a **"weep-and-sweep" response** in the intestine, rapidly clearing the parasite from the gut [51].

Heligmosomoides polygyrus (bakeri): Natural mouse parasite, related to human hookworms and gastro-intestinal nematodes of ruminants. Direct cycle where eggs release release larvae in the feces, and L3 enter the host orally and invade the intestinal submucosa for ± 8 days before emerging in the lumen where adults persist weeks to months, depending on the mouse strain. Worms induce the expansion of Tregs and resistance depends on type 2 immunity, via AAMs and epithelial response [51].

Trichuris muris: Mouse nematode closely related to human whipworm *T. trichiuria.* Mice ingest infective eggs that hatch in the caecum and worms establish in the epithelial layer of the caecum and proximal colon. Resistance depends on effective type 2 immunity, involving mucus production. Variation in susceptibility exists depending on the mouse strain [88].

Trichinella spiralis: Encysted larvae are ingested by vertebrates, and larvae released in the stomach. Adult worms develop in the intestine for around one week, and the female worms release larvae that migrate in the host tissue to reach striated muscular cells in which they encyst. The intestinal phase induces a strong type 2 immunity, clearing the adult worms [71].

Litomosoides sigmodontis: Rodent filarial nematode use as a model of mosquito-borne human filariasis. L3 are transmitted during blood feeding of the mite intermediate host and migrate through lymphatics to the pleural cavity where adults develop and release circulating microfilariae. Worms persist during weeks to months, and induce type 2 immunity, expansion of Tregs and T cell anergy, depending on the mouse strain [89].

Schistosoma mansoni: Responsible for human schistosomiasis, fulfills its lifecycle in mice [6]. The intermediate host, a freshwater snail releases skin-penetrating cercariae which migrate to the lung and liver portal system. Adults live in mesenteric venules for months to years. Eggs are shed in the blood vessels and migrate through the intestinal layer, but many eggs are trapped in the liver and induce type 2 inflammatory granulomas, liver fibrosis, and portal hypertension. Eggs can be purified and injected parenterally to induce type 2 inflammation in mice.

Box 3. Main virus infection models in mice and tissue tropism

Virus species causing respiratory infections:

- *Influenza virus (IAV)*: negative single-stranded segmented RNA, enveloped virus of the family *Orthomyxoviridae*, responsible for seasonal flu in human. Laboratory strains have been adapted to laboratory mice (e.g. A/H1N1 PR8 and WSN, A/H3N2 X31) [90]. IAV induces acute, self-limited, upper respiratory viral infection.
- *Human Respiratory Syncytial Virus (RSV)*: Negative single-stranded non-segmented RNA, enveloped virus of the family *Pneumoviridae,* genus *Orthopneumovirus.* Responsible for lower tract respiratory viral infection. Poorly permissive in mice [91].
- *Pneumonia Virus of Mice (PVM)*: Negative single-stranded non-segmented RNA, enveloped virus of the family *Pneumoviridae,* genus *Orthopneumovirus.* Responsible for lower tract respiratory viral infection. Natural pathogen of mice (strains J3666 and 15) used as model for RSV infection [92].

Virus species causing enteric infections:

- *Mouse norovirus***:** Positive single-stranded non-segmented RNA, non-enveloped virus of the family *Caliciviridae.* Natural pathogen of mice (strains CW3 and CR6). A model for human norovirus (Norwalk), responsible for viral gastro-enteritis in human [93].

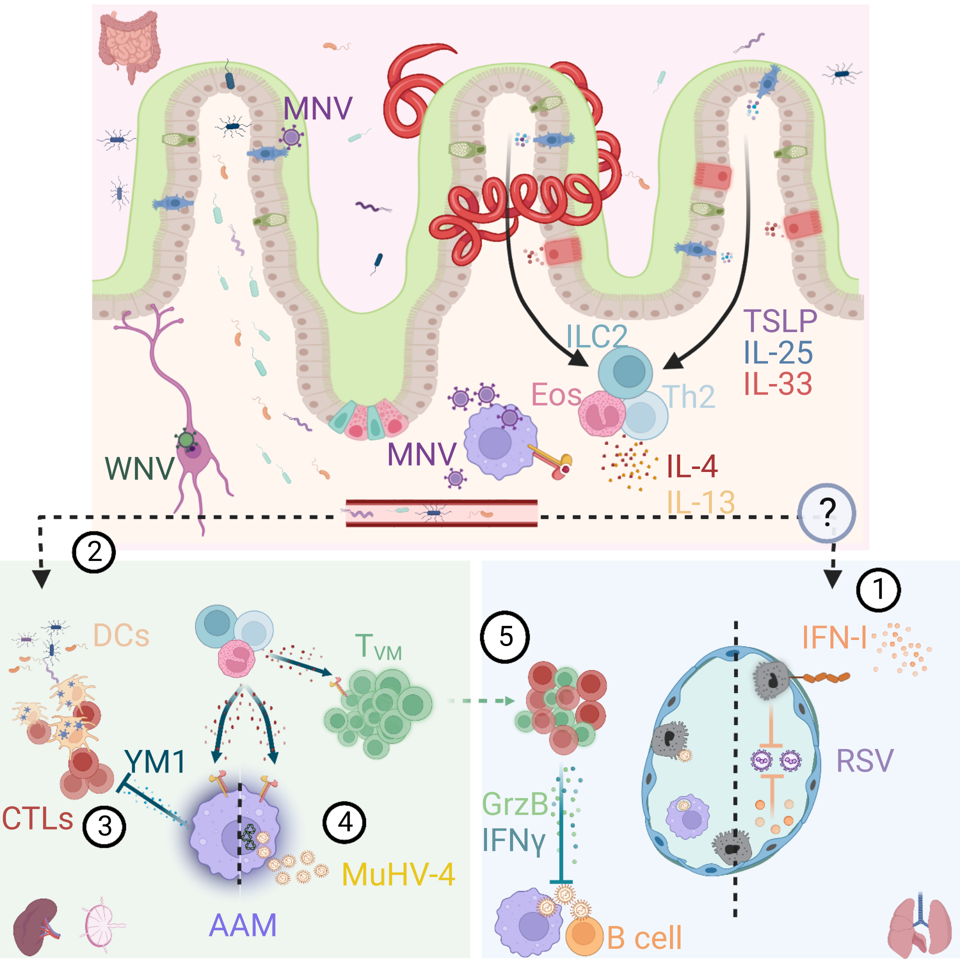
Virus species causing systemic infections:

- *Flaviviruses:* Arthropod-borne positive single-stranded non-segmented RNA, enveloped virus species, of the family *Flaviviridae*. Flaviviruses are responsible for West Nile Fever, Yellow Fever, Zika, Dengue, Japanese encephalitis, tick-borne encephalitis, Murray Valley encephalitis, Powassan encephalitis, and others. Several strains have been adapted to mice and display various disease severities depending on the mouse strain [94]. Flaviviruses are responsible for (meningo)encephalitis and hemorrhagic fevers.

- *Murid gammaherpesvirus 4 (MuHV-4):* Double-stranded DNA, enveloped virus of the family *Herpesviridae*. MuHV-4 infects mice through respiratory or genital routes and establishes a latent persistent infection in myeloid cells and B lymphocytes [95]. MuHV-4 is used as a model of oncogenic Epstein-Barr virus (EBV) and Kaposi Sarcoma-associated herpesvirus (KSHV), responsible for lymphomas in human.
- *Lymphocytic choriomeningitis virus (LCMV):* Negative single-stranded segmented RNA, enveloped virus of the family *Arenaviridae,* infecting the nervous system in rodents. Strain Armstrong is neurotropic and rapidly cleared in immunocompetent mice. Clone 13 is a strain that was derived from Armstrong and has a tropic for visceral organs and induces a chronic infection in mice [96]. LCMV is zoonotic and can induce meningoencephalitis in humans.

Virus species causing genital infections:

- *Human herpesvirus 2* (herpes simplex virus 2, HSV-2): Double-stranded DNA, enveloped virus of the family *Herpesviridae*. HSV-2 is a sexually transmitted pathogen that infects the genital tract and is responsible for human genital herpes [97]. Permissiveness in mice is improved after progesterone treatment [97].


MNV
TSLP
IL-25
IL-33
ILC2
Eos
Th2
MNV
IL-4
IL-13
WNV
?
②
①
DCs
T_{VM}
⑤
IFN-I
YM1
CTLs
③
④
MuHV-4
AAM
GrzB
IFNγ
RSV
B cell

Positive Outcome

- Helminth precedes viral infection
- Virus infection at peak type 2 immune response
- IL-4-induced T_{VM}, potentiating antiviral cytotoxic CD8$^+$ T cells
- Viral disease = immunopathology
- Coinfection in distant tissues
- Gut-lung immune axis

Negative Outcome

- Virus precedes helminth infection
- Virus infection before or after peak type 2 immune response
- Helminth impairment of antiviral responses (e.g dampening of IFN-γ)
- Viral disease = virus replication
- Coinfection in the same tissue
- Gut-genital tract axis