Editorial: Unconventional Animal Models in Infectious Disease Research – Part I

Nadin Younes1 and Gheyath K. Nasrallah1,2*

1 Biomedical Research Center, Qatar University Health, Qatar University, Doha, Qatar, 2 Department of Biomedical Science, College of Health Sciences, Qatar University Health, Qatar University, Doha, Qatar

Keywords: vaccines, therapeutic agents, infectious disease, host-pathogen interactions, animal model

Editorial on the Research Topic

Unconventional Animal Models in Infectious Disease Research

The multiplicity of host-pathogen interactions associated with infectious diseases frequently necessitate the use of a complicated biological system. Therefore, animal models have contributed substantially to unraveling the physiopathology of infectious diseases. The feasibility to manipulate different animal models aids in (i) discovering the role of host or microbial factors in the infection pathogenesis, (ii) understanding different mechanisms of tissue invasion, host defense, pathogen dissemination. This Research Topic, which includes 8 high-quality research papers (7 original research articles, and 1 review), sheds the light on the use of unconventional model as a platform for advancement in infectious disease research and address novel ground-breaking findings in host-pathogen interaction studies, which paved the way for discovering new therapeutic targets and vaccine development. The most important findings are summarized in Table 1.

The first article focused on the role of autophagy during duck tembusu virus (DTMUV) infection using duck as a model (Hu et al.). The authors used two drugs as autophagy regulators, which are rapamycin (autophagy enhancer) and methyladenine and chloroquine (autophagy inhibitor). They discovered that DTMUV infection triggered autophagy in duck’s spleen and brain. They also demonstrated that the autophagy inhibitors suppressed DTMUV replication and reduced DTMUV-induced pathogenic symptoms. They concluded that autophagic regulation was linked to the expression of innate immunity genes such as the pattern recognition receptors, type I interferons, and cytokines.

The second article also utilized ducks to study the role of DEV ICP22 protein in duck enteritis virus (DEV) (Li et al.). The authors used two US1 genes duplicate US1 genes encode a DEV ICP22 protein. The authors reported that ICP22 protein, molecular mass of 57 kDa, can enter the nucleus by itself using conventional NLS motif. However, DEV ICP22 protein cannot enter the nucleus after mutating amino acid 309R, demonstrating that this amino acid is the crucial residue for ICP22 localization. Most importantly, they concluded that the DEV ICP22 protein is a non-essential immediate-early protein primarily found in the nucleus of infected duck-embryo-fibroblasts (DEF) cells and that US1 deletion can hamper DEV replication.

Johansen and Kremer were able to show that zebrafish embryos are susceptible to M. fortuitum infection in a dose-dependent manner. Interestingly, they showed that M. fortuitum was able to form granulomas in embryos as early as 2 days post-infection. In addition, they noticed that transient
TABLE 1 | Summary for the outcomes of all accepted articles in this special issue.

| Study type | Organism Name | Animal Model | Aim | Methods of assessment | Conclusion | Reference |
|------------|----------------|--------------|-----|------------------------|------------|-----------|
| Original article | DTMUV virus | Duck | To study the role of autophagy in facilitating DTMUV replication “in vivo” | • Western blot (WB) | DTMUV triggered autophagy, which facilitates its replication inside the cells and induces pathological symptoms | Hu et al. |
| Original article | DEV virus | Duck | To elucidate the roles of US1 and its NLS in DEV replication “in vivo” | • RT-PCR | The DEV US1 ORF is 990 bp | Li et al. |
| Original article | M. fortuitum | Zebrafish | To study the disease pathogenesis of M. fortuitum infections | • Creation of fluorescent M. fortuitum | Zebrafish embryos form granulomas as early as 2 days post-infection | Johansen and Kremer |
| Original article | G. mellonella larvae | S. aureus | To study the function of sRNAs during S. aureus infection “in vivo” | • Bacterial growth in the larvae | G. meltonella larvae is a suitable model to study sRNA-mediated pathogenesis in S. aureus | Ménard et al. |
| Original article | P. berghei | A. gambiae | Study the function of CLIPB10 in protease cascades “in vivo” using A. gambiae as a model | • RT-PCR | proPO plays an essential role in the cuticular melanization in insects | Zhang et al. |
| Original article | C. elegans | E. faecium | To analyze a panel of lab strains of E. faecium with deletions of targeted virulence factor “in vivo” using C. elegans as a model | • Colony forming unit assay | C. elegans is a high throughput infection model for studying the pathogenesis of E. faecium | Revtovich et al. |
| Original article | Mouse | Helicobacter, Clostridium, Lactobacillus, Klebsiella, Rodentbacter and Enterococcus | To study the effect of Autoinducer-2 on the process of necrotizing enterocolitis mouse model | | The AI-2 level was significantly decreased in the NEC group | Ji et al. |
| Review | Drosophila | Different pathogens | Gaining proper insight into host–pathogen interactions using drosophila as a model | | In vivo drosophila studies enabled the identification of humoral and cell-mediated host defense factors against a wide array of intracellular and extracellular pathogens | Younes et al. |
Me the decline in insect death rates is delayed. The CFTR conductance regulator (CFTR) in zebrafish by morpholino significantly increased the mortality rate, bacterial burden, and abscess formation. This is consistent with previous studies showing that CFTR expression on both innate and adaptive immune cells contributes to immunological dysfunction in cystic fibrosis (CF) (Hu et al.; Johansen and Kremer; Li et al.; Sermet-Gaudelus et al., 2003; Bruscia et al., 2009; Bonfield et al., 2012; Richards and Olivier, 2019).

Insect models proved to be very valuable for infectious diseases studies. Ménard et al., developed a non-mammalian infection model using larval G. mellonella to investigate the function of small regulatory RNAs (sRNAs) during S. aureus infection. The authors collected total RNA from S. aureus at several time points during the infection. They concluded that the expression patterns of the examined sRNAs were distinct and fluctuated over time, with small pathogenicity island RNA D (sprD) and small pathogenicity island RNA C (sprC) expression increasing during infection and correlated with larval death. In addition, they found out that when either sprD or sprC is deleted, the decline in insect death rates is delayed. The findings of Ménard et al. shed lights on the usefulness of G. mellonella as an infection model to investigate all 50 bona fide sRNAs known to be expressed by S. aureus.

Zhang et al., also utilized insects as a model to study the function of CLIPB10 in protease cascades “in vivo”. Activation of prophenoloxidase (proPO), a form of immune system regulation, leads to the formation of eumelanin on foreign microorganisms. ProPO activation is tightly controlled by different mechanisms including clip domain serine proteases (CLIPs), proteolytically inactive homologs, and serpin inhibitors. The authors studied the function of the CLIPB10 in Anopheles gambiae, which is the most common malaria vector in Sub-Saharan Africa. They reported that CLIPB10 was required for the melanization of ookinete stages of Plasmodium berghei. In addition, recombinant serpin 2 protein, a key inhibitor of the proPO activation cascade, efficiently inhibited CLIPB10 activity in vitro. The authors concluded that CLIPB10 along with CLIPB9 plays a critical role as the second protease with prophenoloxidase-activating function in A. gambiae suggesting functional redundancy in the protease network that controls melanization.

Studying enterococcal pathogenesis generally requires vertebrates’ models, making them slow, expensive, and ethically problematic (Weiner et al., 2016). Therefore, Revtoovich et al., developed the first high-throughput Caenorhabditis elegans infection model. They successfully showed that E. faecium could effectively invade and colonize the intestine of C. elegans and elicit an immunological response. They used this model to examine a panel of lab strains with targeted virulence factor deletions. Although deletion of a single virulent factor such as Δmfs15 was sufficient to affect virulence, multiple deletions were generally necessary to alter the pathogenesis, implying that host-pathogen interactions are multifactorial.

Autoinducer-2 (AI-2) is thought to be a bacterial interspecies signaling molecule that plays an important role in the physiological behaviors of bacteria. Ji et al., studied the role of AI-2 in Necrotizing enterocolitis (NEC) mouse model. They randomly divided C57BL/6 mice into three groups: control, NEC, and NEC+AI-2 (NA). They reported that AI-2 significantly decreased in the NEC group. In addition, the intestinal injury scores, expression of TLR4, NF-κB, and proinflammatory factors were reduced in the NA group, whereas the expression of anti-inflammatory factors was increased in the NA group. They concluded that AI-2 partially reverses flora disorder and decreases inflammation in an NEC mouse model.

Younes et al., review discussed the use of drosophila in host–pathogen interaction research. Drosophila has recently gained a lot of attention due to the evolutionarily conserved features with higher vertebrates, such as cascades of the innate immune, pathways of signal transduction, and transcriptional regulators. The versatility, low cost, affordable maintenance, short life cycle, high fecundity, well-characterized genome made drosophila one of the most powerful model species (Younes et al.).

**AUTHOR CONTRIBUTIONS**

NY: drafted the editorial. GN: designed the work, revised the editorial critically for important intellectual content and provided final approval of the version to be published. All authors contributed to the article and approved the submitted version.

**REFERENCES**

Bonfield, T. L., Hodges, C. A., Cotton, C. U., and Drumm, M. L. (2012). Absence of the Cystic Fibrosis Transmembrane Regulator (CFTR) From Myeloid-Derived Cells Slows Resolution of Inflammation and Infection. *J. Leukoc. Biol.* 92, 1111–1122. doi: 10.1189/jlb.0412188

Bruscia, E. M., Zhang, P. X., Ferreira, E., Caputo, C., Emerson, J. W., Tuck, D., et al. (2009). Macrophages Directly Contribute to the Exaggerated Inflammatory Response in Cystic Fibrosis Transmembrane Conductance Regulator–/– Mice. *Am. J. Respir. Cell Mol. Biol.* 40, 295–304. doi: 10.1165/rcmb.2008-0170OC

Richards, C. J., and Olivier, K. N. (2019). Nontuberculous Mycobacteria in Cystic Fibrosis. *Semin. Respir. Crit. Care Med.* 40, 737–750. doi: 10.1055/s-0039-1693706

Sermet-Gaudelus, I., Le Bourgeois, M., Pierre-Audigier, C., Offredo, C., Guillemot, D., Halley, S., et al. (2003). Mycobacterium Abscessus and...
this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Younes and Nasrallah. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.