Optimization and applications of an in vivo bioluminescence imaging model of influenza A virus infections

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Dear Editor,

In vivo bioluminescence imaging (BLI) models of virus infection possess unique advantages over conventional assays. For instance, the BLI model enables rapid and real-time detection of viral load and dissemination in the same animal over time (Mehle, 2015; Wen et al., 2022). A major hurdle to establish an in vivo BLI model of viral infection is the requirement of recombinant viruses encoding luciferases, as the reporter viruses are usually either genetically unstable or attenuated due to limited tolerance of viral genomes. Recently, we had generated two stable and replication-competent recombinant influenza A viruses (IAVs) carrying the firefly luciferase (Fluc) gene, including PR8-Fluc (H1N1; originally designated as PR8-NSCE2-Fluc) and X31-Fluc (H3N2; originally designated as X31-NS2-Fluc) viruses. Further, robust in vivo BLI models of both subtype H1N1 and H3N2 IAV infections were established based on the PR8-Fluc and X31-Fluc viruses, respectively (Zhao et al., 2022). In the present study, we used these advanced animal models to address several critical issues in both basic and applied influenza virology that have been of concern.

Nasal inoculation is usually adopted to establish mouse models of influenza virus infection, while the documented protocols to perform nasal inoculation are slightly different; the volume of virus stocks used to challenge mice ranges from 25 to 100 μL (Belser et al., 2015; Govorkova et al., 2001; Kundasamy et al., 2020). We herein questioned whether the divergence affected the efficacy of infection. To answer this, female BLAB/c mice (4–6 weeks old) were inoculated intranasally with a sub-lethal dose (1000 TCID50) of the reporter PR8-Fluc virus in increasing volumes (10, 30, 50 and 100 μL) of sterile phosphate buffer saline (PBS). At day 1 post infection (p.i.), BLI was performed. It can be observed that the signals from the lung regions of mice became brighter as the inoculation volumes increased, indicating enhanced initial infection (Fig. 1A). In addition, the statistics of the bioluminescent signals clearly demonstrated that the efficacy of initial infection increased with increase in the nasal inoculation volumes (Fig. 1B). Notably, the bioluminescent signals from mice intranasally infected with 10 μL virus stocks generated an intragroup variation of as high as 0.68, signals from mice infected with 30 and 100 μL volumes generated comparable intragroup variations of 0.38 and 0.31 respectively, while signals from mice of 50 μL volume group showed the lowest intragroup variation of 0.15. Together, as intranasal inoculation of mice with IAVs in 50 μL stocks could guarantee a relatively high efficiency of initial infection and generate the lowest intragroup variation, we prefer using 50 μL over other volumes of virus stocks to infect mice intranasally in an optimized protocol.

Next, we adapted the BLI model as an advanced approach for rapid evaluation of influenza vaccines. Recently, our colleagues Si et al. (2022) had developed a novel live attenuated proteolysis-targeting chimeric (PROTAC) influenza vaccine (M1-PTD) based on the influenza A/WSN/33 (H1N1) virus. This vaccine can elicit robust and broad humoral, mucosal and cellular immunity in mice, promising protection against infections by both homologous and heterologous strains of sub-type H1N1 (Si et al., 2022). We wanted to assess whether this PROTAC vaccine has the potency to broadly protect mice from infections of other IAV subtypes. For this purpose, female BLAB/c mice (4–6 weeks old) were intranasally vaccinated with the PROTAC vaccine M1-PTD at a dose of 10,000 TCID50/mice. Mice inoculated with vehicles only were set as negative controls. At 28 days post vaccination, both control and vaccinated mice were challenged at a high dose (10,000 TCID50) with the reporter PR8-Fluc (H1N1) and X31-Fluc (H3N2) viruses, respectively. At day 2 p.i., BLI was carried out to monitor the virus infection. As a result, upon infection with both PR8-Fluc and X31-Fluc viruses, only

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bioluminescence signals at a level of background were collected from M1-PTD vaccinated mice, in contrast to the bright light observed from the control mice (Fig. 1C and D). These results indicate that M1-PTD confers complete protection against infection of both H1N1 and H3N2 subtype IAVs, implying its potential as a universal influenza vaccine (Du et al., 2021b).

To better evaluate the immunogenicity of M1-PTD, female BLAB/c mice (4–6 weeks old) were intranasally immunized with series doses of M1-PTD. At 28 days post vaccination, the mice were challenged with 10,000 TCID₅₀ of the reporter viruses PR8-Fluc (H1N1) and X31-Fluc (H3N2) separately. At 2 p.i., BLI was performed (E) and the bioluminescence signals from lungs were analyzed (F). Dose-dependent immunization of the attenuated influenza vaccine M1-PTD. Female BALB/c mice (4–6 weeks old) were mock treated or immunized with M1-PTD intranasally at different doses of 10, 100 and 1000 TCID₅₀/mice separately. At 28 days post vaccination, the mice were challenged with 10,000 TCID₅₀ of the reporter viruses PR8-Fluc (H1N1). At 2 p.i., BLI was performed (G) and the bioluminescence signals from lungs were analyzed (H). The kinetics of bioluminescence from the nasal tracts of each infected mouse. Dashed line indicates the background BLI signal. Error bars show the standard deviation (SD) of the mean from indicated replicates; *, *P* < 0.05; ***, *P* < 0.001; student’s t-test.
eliminated at day 6 or 7 p.i., bioluminescence can be detected in aged mice till day 10 p.i., suggesting markedly delayed viral clearance (Fig. 1G). The kinetics of viral residence in nasal tract of each young and aged mice was dissected in Fig. 1H, and it clearly demonstrated the slower and delayed viral clearance in the upper respiratory tract of aged mice, which is in accordance to the impaired innate mucosal immunity associated with aging (Aso et al., 2016; Bates et al., 2008). Based on these observations, we can state that the in vivo BLI-based aged mouse model can provide a powerful tool to develop novel therapeutics or adjuvants that restore impaired mucosal immune responses in aged individuals (Aso et al., 2016; Bates et al., 2008).

To summarize, in the present study we applied the in vivo BLI model of IAV infections in both basic and applied influenza virology. Compared to conventional assays, the BLI models possess many unique advances, such as the real-time and longitudinal measurements of viral load in living animals, as well as improved throughput of the assay. The advanced BLI model calls for further utilization and will provide more powerful tools in the future.

Footnotes

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References


