Antiviral Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Short Communication

The use of plethysmography in determining the severity of lung pathology in a mouse model of minimally lethal influenza virus infection

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ARTICLE INFO

Article history:
Received 10 February 2014
Revised 29 April 2014
Accepted 1 May 2014
Available online xxxx

Keywords:
Influenza virus
Plethysmography
Lung function
Mice
H3N2 variant

ABSTRACT

To characterize the impact on lung function, we assessed plethysmography parameters in a course of infection with mouse-adapted A/Pennsylvania/14/2010 (H3N2) influenza virus. Several parameters, represented by enhanced pause (penh) and ratio of inspiratory/expiratory time (Ti/Te), were observed that had early (1–7 dpi) and robust changes regardless of virus challenge dose. Other parameters, characterized by tidal volume (TV), breathing frequency (freq) and end inspiratory pause (EIP), changed later (7–15 dpi) during the course of infection and had a virus challenge dose effect. A third category of lung function parameters, such as peak inspiratory flow, had early, virus challenge-independent changes followed by later changes that were challenge dependent. These parameters changed in a similar manner after infection with a non-mouse adapted virus, although the time-course of many parameters was delayed somewhat when compared with mouse-adapted virus. Histopathological assessment of lung samples corresponded with changes in lung function parameters. This study demonstrates the utility of plethysmography in assessing disease in a mouse model of mild influenza virus infection.

Influenza virus (IAV) causes significant morbidity and mortality worldwide, although the majority of infections are mild and infected individuals generally recover after a couple weeks. Lethal rodent models are commonly used to better understand influenza disease and to identify potential therapeutics (Barnard, 2009; Boltz et al., 2010; Smee et al., 2012). In order to develop a lethal mouse model after the emergence of an IAV strain, the virus must often be adapted to mice. This is accomplished through serial passage, which includes genetic changes to the virus. These changes, including enhanced receptor binding by the virus, result in increased pathogenesis (Ilyushina et al., 2010; de Jong et al., 2013). It is generally unknown how mouse adaptation influences the translation of discoveries in mice to the realm of clinical intervention in man. Therefore, it would be useful to develop a mouse model of mild influenza using non-adapted clinical isolates of IAV, but such a model would require a more sensitive method for the evaluation of disease, as morbidity and mortality would be reduced or absent.

Pulmonary tissue damage is often a consequence of influenza infection and is a key component of disease in man (Marsolais et al., 2009; Sanders et al., 2013). Lung impairment as a result of influenza infection in rodents may be a useful parameter for assessing therapies for the prevention or treatment of disease associated with influenza infection. Noninvasive techniques, including the use of a plethysmograph, may be used to longitudinally quantify lung impairment in rodents after virus infection, as opposed to more traditional methods, which include necropsy to assess lung damage (Julander et al., 2011). A recent study demonstrated significant loss of type I pneumocytes with associated impairment of lung function after severe influenza virus A/Puerto Rico/8/1934 (H1N1) infection or with a mild infection after A/Aichi/2/1968 x A/Puerto Rico/8/1934 (H3N2) influenza virus (x31) infection (Sanders et al., 2013). Based on our previous results (Julander et al., 2011), measures of lung function are also useful in antiviral studies.

The purpose of the present study is to characterize mild disease caused by influenza virus in mice using plethysmography, including a comparison of adapted and non-adapted (2 passages in MDCK cells) viruses. We obtained the A/Pennsylvania/14/2010 (H3N2) influenza virus in 2011 from the Centers for Disease Control and Prevention (CDC, Atlanta, GA). The virus was adapted to mice by passaging 7 times in female 13–15 g Swiss Webster mice obtained from Charles River (Wilmington, MA). For passage, inoculated mice were sacrificed 3 days after virus challenge and clarified lung homogenates were administered to a subsequent group of mice in a volume of 0.1 ml. After 7 passages followed by one passage in Madin–Darby canine kidney (MDCK) cells, the virus titrated at

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http://dx.doi.org/10.1016/j.antiviral.2014.05.002
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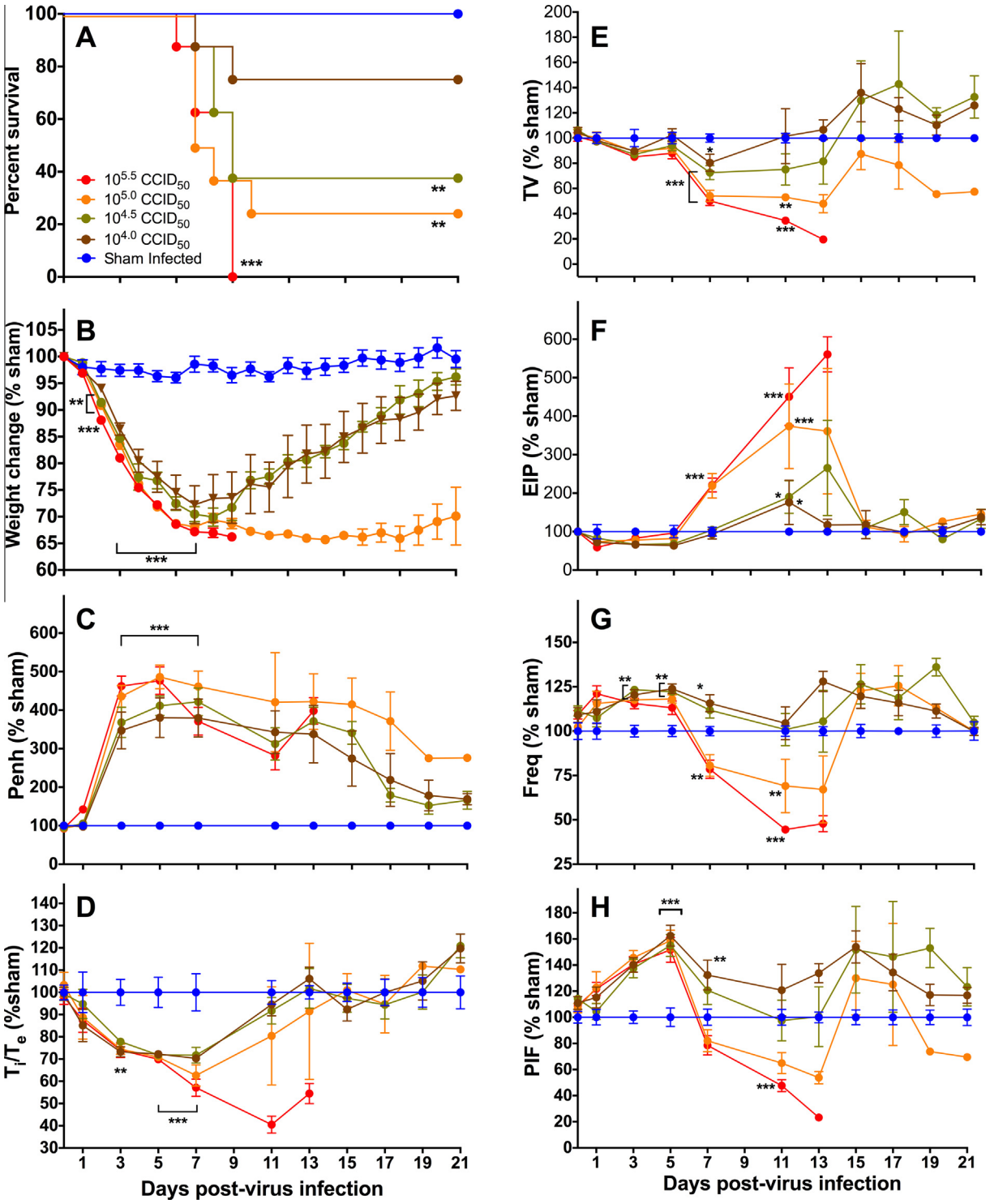

Fig. 1. Mortality (A) and mean weight change (B) of mice ($n = 8$) infected with various infectious doses of adapted A/Pennsylvania/14/2010 (H3N2) influenza virus are shown. The virus that was used had undergone 7 sequential passages through mouse lung. The representative lung function parameters of penh (C) and Ti/Te (D) show changes soon after virus challenge, while TV (E) and EIP (F) show dose-responsive changes later during the course of infection. The Freq and PIF measurements are representative of parameters that show early non-dose dependent changes followed by later virus challenge dose-dependent changes. Kaplan–Meier survival curves were analyzed by the log-rank test followed by a pairwise comparison using the Gehan–Breslow–Wilcoxon test. Mean weight change and plethysmography curves from each group were compared using a two-way ANOVA with a mean column effect analysis. Error bars represent the standard error of the mean (SEM) ($^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, as compared with mock-infected controls).

$10^{8.9}$ CCID$_{50}$/ml. A sample of the virus was submitted to CDC for genetic analysis where it was confirmed to be the A/Pennsylvania/14/2010 (H3N2) virus (data not shown). We titrated the passage 7-adapted virus in mice using survival, weight change, and longitudinal plethysmography measurement of lung function over the course of virus infection.

A commercially available plethysmograph, acquisition software, and mouse-sized plethymograph chambers (emka Technologies,

Please cite this article in press as: Julander, J.G., et al. The use of plethysmography in determining the severity of lung pathology in a mouse model of minimally lethal influenza virus infection. Antiviral Res. (2014), http://dx.doi.org/10.1016/j.antiviral.2014.05.002