Automated Activity Monitors for Objective Monitoring of Influenza Infected Mice During Vaccine Studies

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Abstract

Influenza viruses continue to cause severe morbidity and remain a continuous pandemic threat. Virus challenge studies in animal models are performed during preclinical vaccine development. Improved methods are needed to improve data quality in animal models to reduce both animal stress and the animal numbers. By evaluating automated activity monitors as a tool for Influenza vaccine development, we demonstrate that activity in mice declines after viral challenge and that this reduction in activity can be prevented by vaccination. Notably, the decline in activity precedes weight loss and could thus provide an objective and early endpoint in Influenza virus challenge studies.

Results

To investigate the use of automated activity monitors in influenza vaccine studies, we performed an immunization study in the commonly used Influenza A/PR8 model. Mice were subcutaneously vaccinated with a single dose of HA (Influenza A/PR8) formulated in the CAF01 adjuvant, which has previously been evaluated in influenza virus animal models[3,4]. On day 18, the mice were identified with RFID transponders and observed for 6 days to set baseline activity before being challenged on day 24. Unvaccinated mice were used as controls. Subsequently, the mice underwent intranasal PR8 challenge (15 µl per nostril with 150 EID_{50}) on day 24 and were followed for 7 days. Daily weight monitoring was conducted, and virus titers were determined from nasal washes on days 4 and 7 post-challenge, as well as from lung homogenate on day 7 post-challenge. On day 7, a significant difference in weight loss was observed between the unvaccinated and the HA+CAF01 vaccinated groups (Figure 1A). Mice vaccinated with HA+CAF01 had significantly higher activity levels than the unvaccinated group from day 5 to day 7 (Figure 1B). Thus, influenza infection led to reduced activity, whilst vaccination prevented this reduction. Vaccination also led to a lower overall symptom score (Figure 1C). Weight loss correlated significantly with reduced activity (Figure 1D) and by following the decline in activity in individual mice in the unvaccinated group, it could be seen that the decline in activity directly preceded weight loss (sFig 1). Specifically, declined activity could already be observed at day 3 in two of three mice, whereas weight loss in these first occurred at day 5. By measuring nasal wash virus titers by RT-PCR on day 4 post-challenge, we detected virus in four of six HA+CAF01 vaccinated mice, although titers were significantly lower compared to the unvaccinated group on both days 4 and 7 ($p<0.01$) (Figure 1E). At day 7, virus was detected in all unvaccinated mice but not in the mice that had received HA+CAF01 (Figure 1E). Overall, these data demonstrate that vaccination using a single dose of HA+CAF01 vaccine effectively protected against influenza infection, reduced activity, and disease. In contrast unvaccinated mice had a decrease in activity, which preceded weight loss.

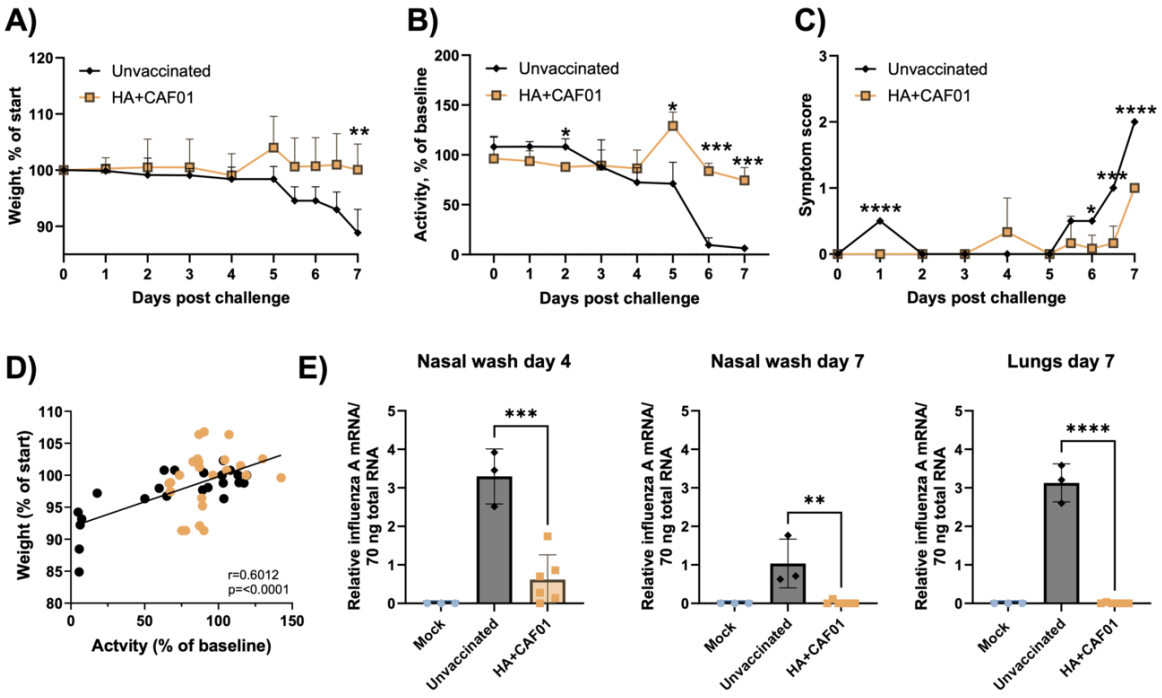

Figure 1: **Reduced activity in influenza vaccinated mice can be prevented by vaccination.** CB6F1 Mice (3-6 per group) were vaccinated with a single dose of hemagglutinin (PR8) formulated in CAF01. 21 days later, the mice were challenged with 150 EID_{50} of A/PR/8 virus. 6 days before challenge, the mice were tagged with RFID transponders. The average activity during the 6-day time period was used to set baseline activity for individual mice. Following challenge, the mice were followed for 7 days before termination. Weight loss and scoring was measured daily and activity was continuously monitored. Determination of viral titers in nasal washes (days 4 and 7) and lungs (day 7) was measured by RT-PCR. Error bars show standard deviation. Statistical analysis was performed by two-tailed unpaired t-test comparing unvaccinated and HA+CAF01 immunized mice for every time point (A-C). Correlation was analyzed by Pearson correlation (D) A two-tailed unpaired t-test was used to compare unvaccinated and HA+CAF01 immunized mice (E). * p-value [?] 0.05, ** p-value [?] 0.01, *** p-value [?] 0.001, **** p-value [?] 0.0001.

Discussion

Automated activity monitoring of laboratory animals may provide an objective measure to follow disease progression following infection with respiratory viruses. Here we tested a system using RFID transponders to follow the activity of individual mice challenged with mouse-adapted Influenza A/PR8 virus. We noted a decline in activity, which correlated with weight loss. In contrast, vaccination prevented this decline in activity. A similar decline in activity was observed in previous studies using computerized home cage monitoring (HCM) to measure average group activity of mice in the context of SARS-CoV-2 and influenza virus infection[5,6].

Automated activity monitoring presents a number of advantages over more subjective symptom scoring, which is typically performed by animal research technicians. By following the mice in their familiar environment and minimizing animal-human interactions, automated activity monitoring may cause less stress to the animals and has the potential to improve the data quality by offering an objective assessment. Furthermore, automated monitoring, could give less data variation, which may allow for reductions in the number

of animals used to test the protective efficacy of vaccines[7]. Notably, using automated monitoring of activity, we found that reduced activity preceded weight loss. An advantage of the RFID transponder system used in our studies is the ability to monitor mice at an individual level. Activity monitoring may therefore be used to inform animal caretakers to follow specific animals more closely and could potentially serve as endpoint in murine challenge studies. As an objective measure of animal health status, activity monitoring could thus be used to improve animal welfare by alerting animal caretakers upon a sudden decline in activity.

In summary, the use of automated activity monitors could present significant advantages for data accuracy and animal welfare and is increasingly used in preclinical research[8-10]. By providing continuous and objective monitoring, automated activity monitors may also be implemented for respiratory virus challenge models and vaccine testing. Here we tested their use in influenza vaccine studies and demonstrate that mice immunized with HA protein formulated in CAF01 retained their activity following challenge. The data demonstrate the promise of using automated activity monitors for challenge studies with influenza and possibly other respiratory viruses.

Materials and methods

Mice

CB6F1 mice were ordered from Envigo (Horst, The Netherlands). Six–eight weeks-old female mice were used in all experiments. All mice were housed in the animal facilities at Statens Serum Institut, Denmark, and maintained in rooms with a controlled environment (20–23 ; relative humidity 55 ± 10 %; 12/12 h light/dark cycle). The mice were randomly allocated to conventional type III polycarbonate cages (820 cm^2). All animals were provided Aspen bedding and bricks (Tapvei), EnviroDri (LBS), and polycarbonate tunnels or houses or DesRes paper houses (LBS). Irradiated sunflower seeds, corn grains, and peanuts or bits of carrots were given to the animals once a week. A pelleted diet (Envigo Teclad 2916) and tap water were provided ad libitum. Mouse studies were conducted following the regulations set forth by the National Animal Protection Committee and following European Community Directive 86/609. The governmental Animal Experiments Inspectorate has approved the experiments performed under licenses 2020-15-0201-00525.

Immunizations and Influenza Virus Challenge

Mice were immunized subcutaneously (s.c.) at the base of the tail with 2 µg Influenza A H1N1 (A/Puerto Rico/8/1934) Hemagglutinin (HA) (Sino Biological) formulated in the cationic liposomal adjuvant CAF01, containing dimethyldioctadecylammonium (DDA) bromide (250 µg) and trehalose 6,6'-dibehenate (TDB) (50 µg), produced in-house (Statens Serum Institut, Copenhagen, Denmark) as described[11]. The vaccine was administered in a volume of 200 µl TRIS buffer (isotonic, pH 7.4, with 2% glycerol). Mice were challenged with 30 µL (15 µL/nostril) of the mouse-adapted influenza virus strain A/Puerto Rico/8/1934 (PR8) (150 EID_{50}). The virus was propagated in the allantoic cavity of 10-day-old embryonate hen's eggs. Allantoic fluid was harvested, clarified, and frozen at -80 until use.

Virus titer determination

Virus titers were determined in the lung homogenates and nasal washes. The lungs were removed into RPMI and homogenized by gentleMACS (Miltenyi Biotec). Nasal washes were collected by flushing with 150 µl PBS. The nasal washes and lung homogenates were stored at -80 °C, and the RNA was isolated by MagNA Pure 24 Total NA Isolation Kit on the MagNA Pure 24 instrument (Roche). The quality of the RNA was determined using NanoDrop 2000/2000c (Thermo Fisher Scientific). Virus titers were determined by the virotype Influenza A RT-PCR Kit (Indical Bioscience, Leipzig, Germany), using 70 ng of RNA. RT-PCR was performed on a LightCycler(r) 480 (Roche) using AbsQuant 2nd Derivative Max for obtaining the Ct value. PCR conditions were 10 min at 45 , 10 min at 95 , followed by 40 cycles of 15 s at 95 and 1 min at 60 . The relative mRNA amount was obtained by the $\Delta\Delta$Ct method[12], using β-actin as housekeeping gene.

Automated activity monitoring

Mice were anaesthetised with isoflurane, tagged subcutaneously with individual radio-frequency-

identification (RFID) transponders SID 102/A/2 (EURO I.D. Identifikationssysteme, GmbH, Germany) following analgesia with Caprofen. Mice were housed with 3 animals per cage. Cages were placed onto an RFID sensor plate consisting of 2 × 4 RFID sensors positioned in a grid-like formation (Activity Monitor, PhenoSys, Berlin, Germany). The unique RFID tag of each mouse was used to follow the position over time (sampling rate 3 Hz) and data were calculated as the mean daily distance traveled. The activity was calculated as the percentage reduction in activity compared to baseline (average distance traveled during 6 days before infection).

Statistical analysis

A two-tailed unpaired t-test analyzed differences between groups, and correlation was analyzed by Pearson correlation. Prism 7 software (GraphPad v10.0.2) was used for all statistical analyses.

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