Assessment of viral load and time course of pulmonary inflammation in a murine model of H1N1 (PR8) influenza virus infection

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Introduction

Influenza virus infection in humans results in a respiratory disease that ranges in severity from sub-clinical infection to primary viral pneumonia. 40-60% of acute exacerbations in COPD are associated with respiratory virus infection; 85% of asthma attacks in children and 44% in adults are precipitated by upper respiratory tract infection.

We studied the effect of a single intranasal inoculation of amantadine sensitive A/Puerto Rico/8/1934 (H1N1) [PR8] (4, 40 or 400 PFU) in mice and evaluated the viral pathogenicity and pulmonary inflammation over a 7-day time course.

Methods

Female BALB/c mice were dosed intranasally with either vehicle (PBS) or H1N1 (PR8) influenza virus (4, 40 or 400 PFU/ animal) on one occasion (Day 0). Bodyweight, surface body temperature and clinical signs were recorded daily and penh recorded longitudinally using whole body plethysmography on Days 1, 3 and 5.

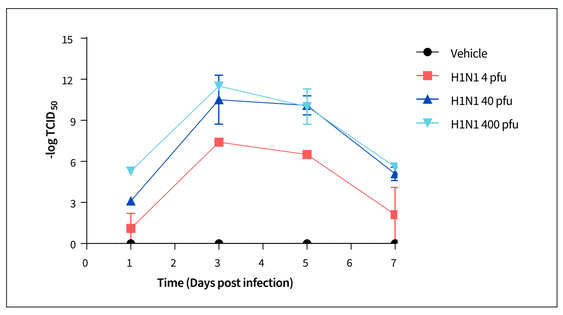
Cohorts of mice were euthanized on Days 1, 3, 5 and 7 after infection and recruitment of inflammatory cells and biomarker production was assessed in bronchoalveolar lavage (BAL) fluid. A total and differential cell count was performed using the Sysmex-XT-Vet and BAL supernatant analysed for inflammatory cytokines (IFNɣ, IL-1β, IL-6, IP-10, KC and TNF-α) using the Luminex 200. Lungs were insufflated with 10% neutral buffered formalin before wax embedding. 4-5 µm sections were cut and stained with H&E or periodic acid-Schiff for assessment of cell infiltrate and hyaline formation. The lungs were excised, weighed and lung tissue viral load determined using the cytopathic assay and a 50% Tissue Culture Infectious Dose ($TCID_{50}$) calculated.

Results

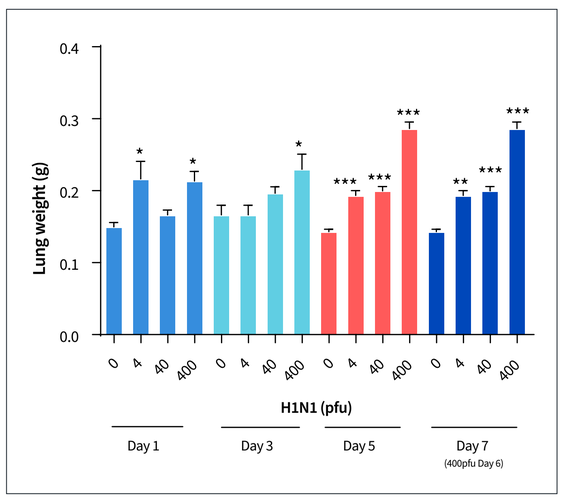
The peak lung tissue viral load was determined as either Day 3 or Day 5. Lung tissue titres increased from 1.1 to >7.4, 3.1 to >10.5 and 5.3 to >11.5 $TCID_{50}$ from Day 0 to Day 3 following 4, 40 and 400 PFU inoculation respectively. A subsequent decrease in lung viral titre was recorded on Day 7 in all inoculated groups.

Viral lung infection induced dose dependent significant increases in penh from Day 3 in those groups inoculated with 40 (308% increase v PBS control group) and 400 PFU (533% increase v PBS control group) and on Day 5 following 4 PFU inoculation (79% increase v PBS control group). Intranasal administration of H1N1 (PR8) induced a dose and time dependent decrease in bodyweight and surface body temperature. Significant bodyweight loss compared to pre-dose values were recorded from Day 3 at 400 PFU, (6.9%) peaking at Day 6 (22.7%); and Day 3 at 40 PFU (0.7%) peaking at Day 7 (20.8%); and only from Day 5 at 4 PFU peaking at 5.6% on Day 7. Significant surface body temperature decreases were only recorded in the 40 and 400 PFU dose groups from Day 5 and Day 3 respectively. Clinical signs, such as pilo-erection, hunched posture and partially shutting of the eyes increased in severity with increase viral lung titre. Wet lung weights were significantly increased in mice receiving 400 PFU on Days 1, 3, 5 and 7. Consistent significant increases in lung weights were recorded on Days 5 and 7 following 4 and 40 PFU inoculation when compared to the PBS treated animals.

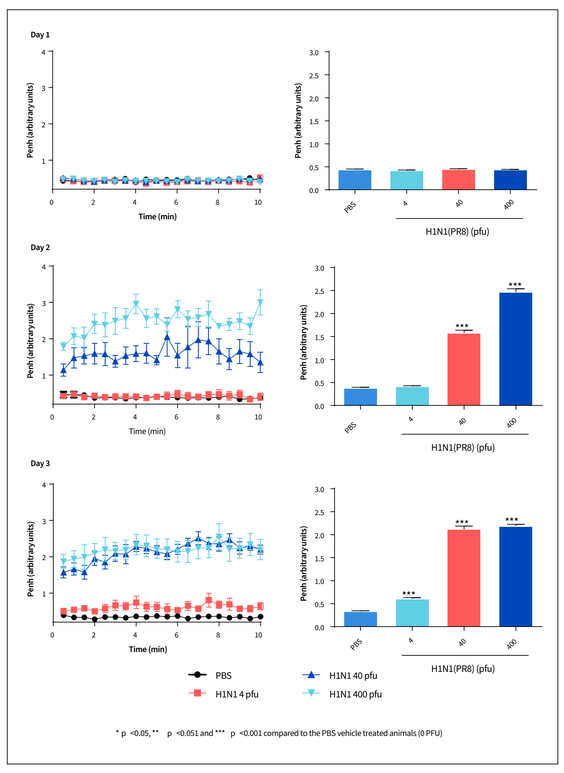
Administration of H1N1 (PR8) caused a statistically significant ($p<0.01$) recruitment of neutrophils, eosinophils and lymphocytes at all dose levels on Days 3, 5 and 7 post inoculation. Macrophages were significantly ($p<0.05$) increased at all dose levels as early as Day 1 but returned to PBS control levels by Day 7. Recruitment of cells into the lung was of a similar magnitude across all dose groups. Maximal recruitment of neutrophils (0.85 v 0.02 $x10^6$/animal in PBS control group), macrophages (0.55 v 0.15 $x10^6$/animal in PBS control group) and lymphocytes (0.14 v 0.003 $x10^6$/animal in PBS control group) was at Day 5 following either 4 or 40 PFU inoculation. Peak inflammatory cell infiltrate coincided with peak lung tissue viral titre. BAL cytokines IP-10 and KC were significantly ($p<0.05$) elevated in viral inoculated (400 PFU) animals as early as 24 h post infection (Day 1). IL-1β, (45 pg/mL; 40 PFU), KC (1865 pg/mL; 400 PFU) and TNF-α (146 pg/mL; 400 PFU) peaked at Day 3 and KC and TNF-α levels remained significantly ($p<0.001$) higher than the PBS control group out to Day 7. IFN-λ (12978 pg/mL; 40 PFU), IL-6 (12976 pg/mL; 40 PFU) and IP-10 (74391 pg/mL; 40 PFU) levels peaked at Day 7.



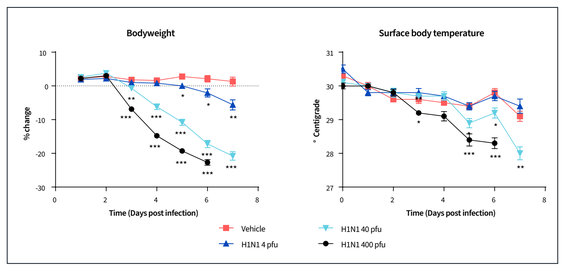
Lung tissue viral titre ($TCID_{50}$) post inoculation with H1N1 (PR8) influenza virus Mean ± sd (n=3/group).



Wet lung weights, Mean ± SEM (n=3/group) following H1N1 (PR8) influenza virus infection.



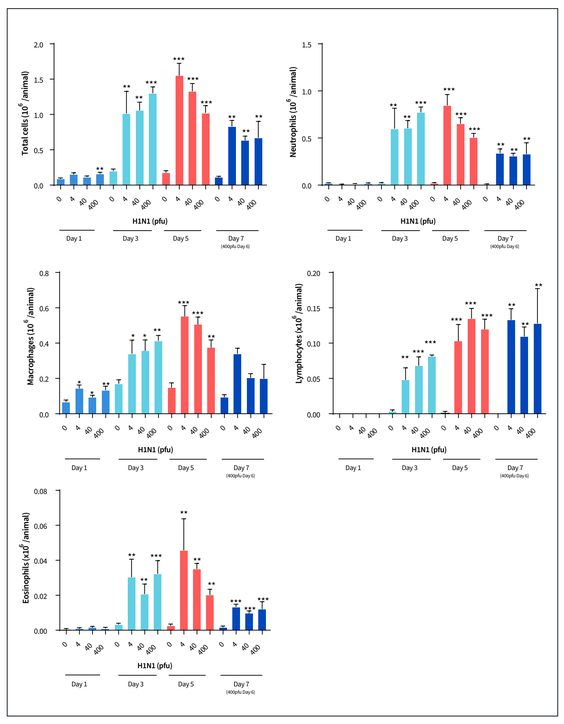
Effect of H1N1 (PR8) influenza virus infection on lung function (Penh) measured longitudinally by whole body plethysmography (a) Data recorded throughout 10-minute period (b) Mean ± SEM (n=6/group).



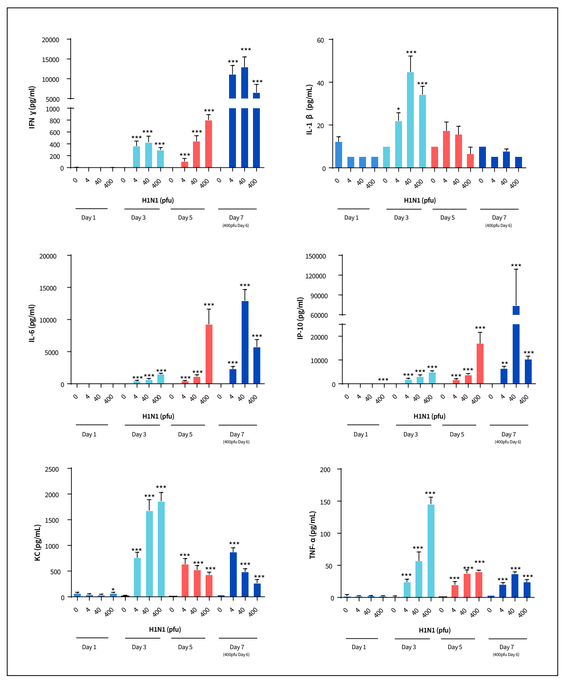
Bodyweight, surface body temperature and clinical observations following H1N1 (PR8) influenza virus infection.

Clinical Observations	4 PFU	40 PFU	400 PFU
Pilo-erection	Day 5	Day 2	Day 1
Rapid breathing	Day 5	Day 3	Day 2
Hunched posture	Day 6	Day 4	Day 3
Eyes partially closed	NAD	Day 3	Day 2
Dull eyes	NAD	Day 6	Day 3
Abnormal gate	NAD	NAD	Day 4

Table 1. Clinical observations.



BAL total and differential cell counts post inoculation with H1N1 (PR8) influenza virus. Mean ± SEM (n=6/group).



BAL pro-inflammatory cytokine levels at 1, 3, 5 and 7 days post inoculation with H1N1 (PR8) influenza virus. Mean ± SEM (n=6/group).

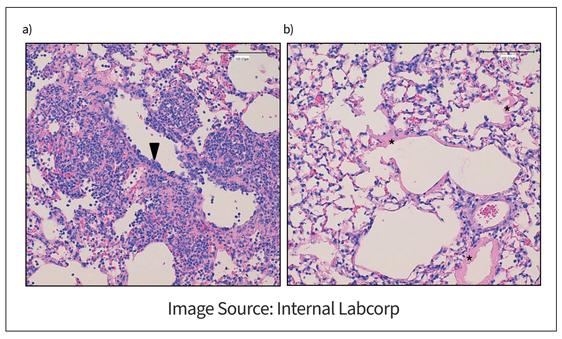


Image Source: Internal Labcorp

Photomicrographs of representative sections of lung from animals treated with 40 PFU of H1N1 virus on Day 7. (a) Destruction of alveoli and marked inflammation of lung interstitium & alveoli. The inflammatory infiltrate is predominantly composed of a mixture of granulocytes and lymphocytes. Degenerate bronchiolar epithelium indicated by an arrowhead. (b) Hyaline-like membrane formation (indicated by *). Photomicrographs of representative sections of lung from animals treated with 400 PFU of H1N1 virus on Days 1-7. Significant inflammation of lung interstitium & alveoli, extending to the peripheral regions of the lung (indicated by arrowheads) was seen at Day 7. Br = Bronchiole, BV = Blood vessel. Arrows indicate examples of areas with peri-bronchiolar or perivascular inflammation. Asterisk (*) indicates area of alveolar haemorrhage. All images taken at x40 magnification.

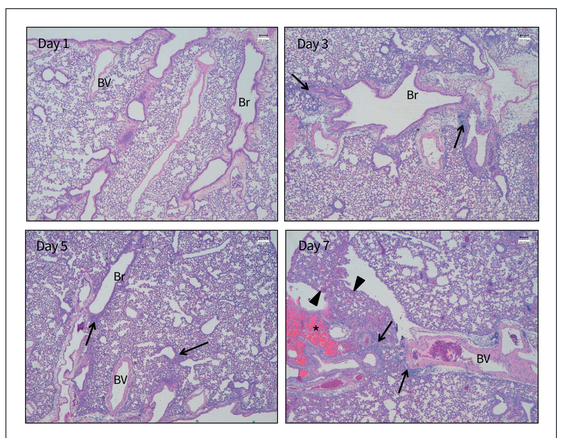


Image Source: Internal Labcorp

No significant lung histopathological lesions were recorded 24 h after H1N1 (PR8) influenza virus administration. An increase in severity of peri-bronchiolar/alveolar duct inflammation cell infiltrate and alveolar/interstitial/peri-vascular inflammation cell infiltrate was observed with increasing PFU concentration and time. Hyaline-like membrane formation was observed on Day 5 in animals receiving 400 PFU and on Day 7 in animals receiving 40 and 400 PFU.

Conclusion

We have demonstrated that inoculation with H1N1 (PR8) influenza virus at 4, 40 and 400 PFU in mice initiates viral replication within lung tissue resulting in the release of pro-inflammatory cytokines and airway cell infiltration. Histopathological, lung function changes and clinical signs were dose and time dependent with minimal effects observed following 4 PFU inoculation. We concluded that 40 PFU is the optimal H1N1 titre to study the efficacy of novel anti-inflammatory and anti-viral chemical entities in this BALB/c influenza pulmonary inflammation model.

Bibliography

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2. N.M Bouvier and A.C Lowen. Animal models for influenza virus pathogenesis and transmission. Viruses 2010. 1530-1563.

Animal experiments were conducted according to the Animals (Scientific Procedures) Act, 1986, and 2012 amendments following local ethical approval. Work was conducted in an AAALAC-accredited facility.