Pentagrit
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HMPV
(Human Metapneumovirus)
Drug Screening Research Model

Immunogenic F Protein Fragment in B36 zebrafish strain.

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Background:

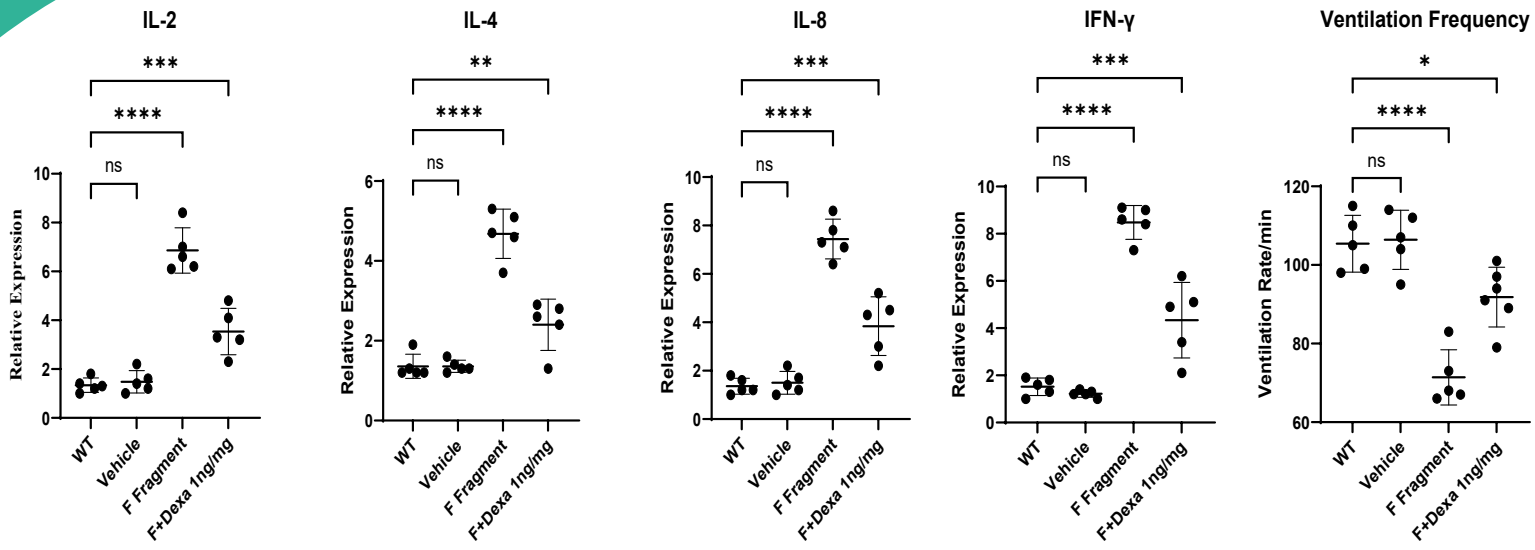
Global rising incidents of HMPV brings a dire need to be cautious of the virus. The Centre for Disease Control and Prevention, USA mentions commonly associated symptoms as cough, fever, nasal congestion, and shortness of breath. which may progress to bronchitis or pneumonia. While high risk groups include children under the age of 5, older people and those with weakened immune system; the spread of virus among all age groups and its ability to cause either mild flu like infection or severe respiratory failure is a subject of concern. Therefore, we developed a screening system that can identify candidates with therapeutic activity against HMPV pathology.

Research Model Strain Background:

Zebrafish B36 strain characterized at Pentagrit that carries a weakened innate immune system was employed to generate the model. B36 strain has low neutrophil count with a slow complement system and insufficient NK cells making the fish highly susceptible to infections such as mycobacteriosis, *Pseudocapillaria Tomentosa*, bacterial gill disease, microsporidia and tail rot infections. This strain was chosen since HPMV risk groups are those with poor innate immune system.

Induction Method:

To emulate HMPV immunogenicity, we employed a segment of the HMPV F protein which has proven immunogenic and antigenic property. During human infection, fusion of the HMPV virus with the cell membrane is mediated by the F protein which is highly conserved among strains of HMPV. Therefore, from the reference GeneID:37626966, a sequence was identified to be highly immunogenic and antigenic starting from the amino acid no 40 of F protein as follows RTGWYTNVFTLEVGDVENLT. To generate the model, 15ng of the F protein fragment was injected posterior laterally to the swim bladder region on day 8 post fertilization (dpf) with adjuvant. Post 1 day of induction dosing was carried for a subsequent 3 days and on 11th dpf assessments were made. Total inflammatory profile by RT PCR, ventilation rate, and alveolar smear for inflammation were measured and statistically analysed with GraphPad Prism10, with $n=6$ per group.


IL-2
IL-4
IL-8
IFN-γ
Ventilation Frequency
Relative Expression
Ventilation Rate/min
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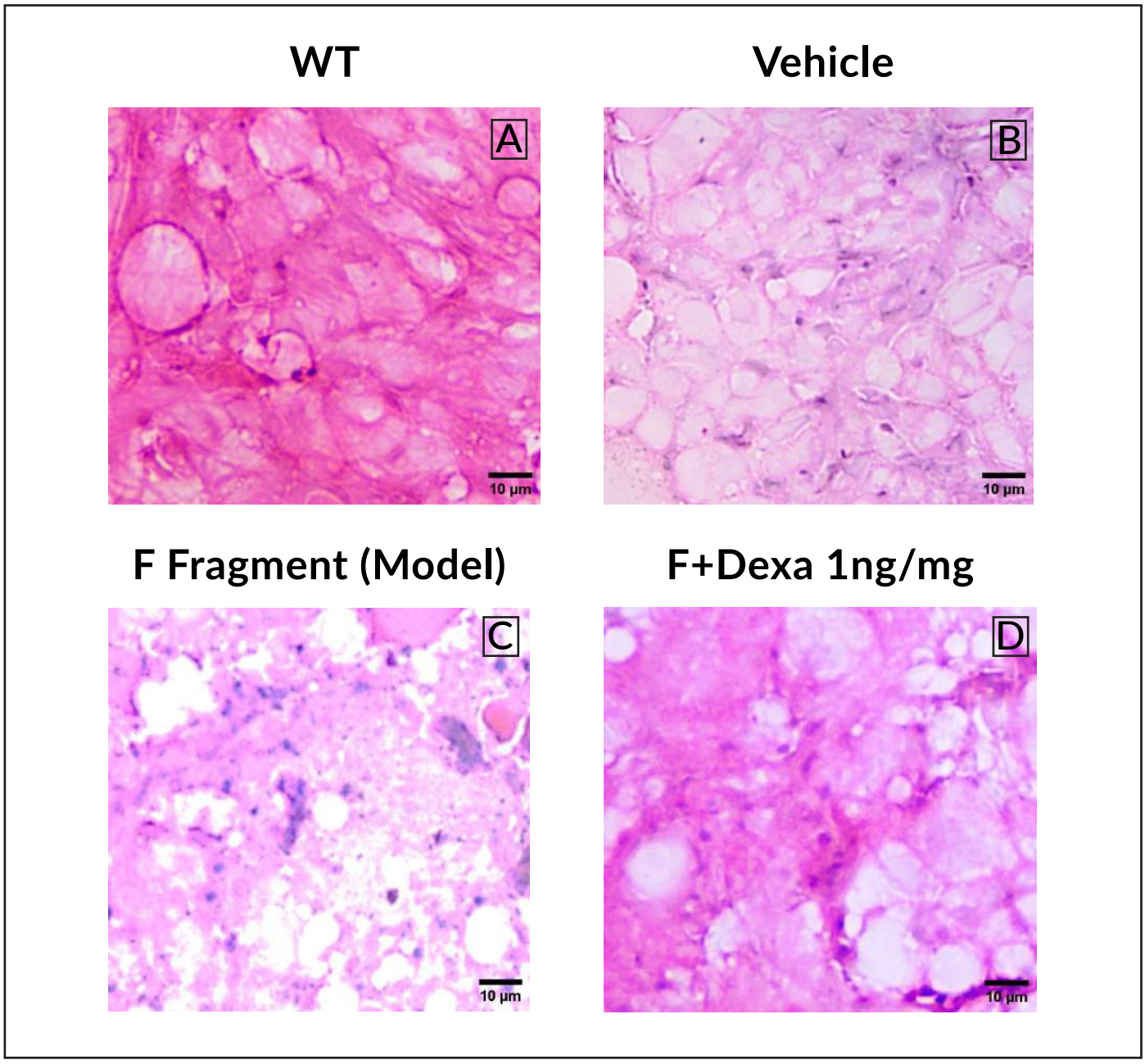
ns
WT
Vehicle
F Fragment
F+Dexa 1ng/mg


WT
Vehicle
F Fragment (Model)
F+Dexa 1ng/mg
A
B
C
D
10 μm

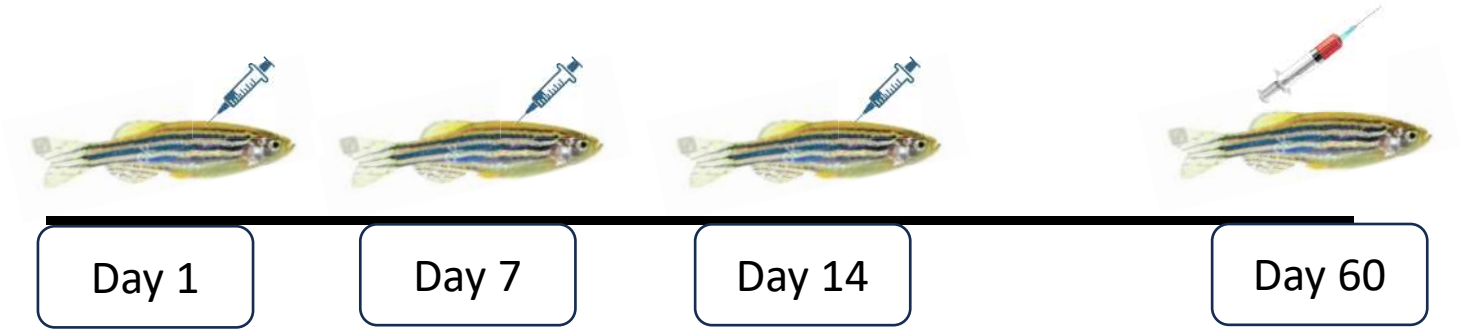
H&E of gill smear showing A - WT with healthy alveolar surface with intact bronchial architecture; this similar field is also observed in B – Vehicle.

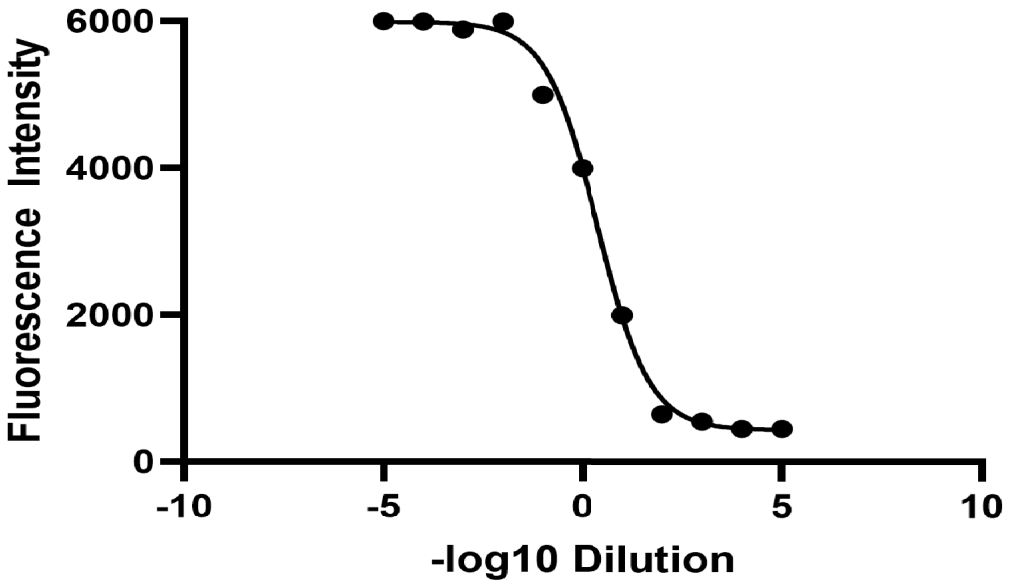
In C – F Fragment (Model) group shows marked inflammation with alveolitis and peribronchiolar and perivascular infiltrate observed with multiple hematoxylin stained cells. Loss of alveolar tissue architecture with initiation of intra-alveolar fibroblastic plugs indicating a pneumonia profile.

In D - F+Dexa 1ng/mg treated group, moderate rescue with lesser loss of tissue architecture and lesser number of infiltrates was observed.

Neutralizing Antibody Titration:

To measure antibody production, adult fish were exposed to repeated F fragment injections and on day 60, blood was collected through the dorsal aorta without sacrificing the fish. Serum dilutions were employed to titrate neutralizing effect on F fragment.





Results:

F Fragment was sufficient to induce an immuno-pathogenic response with an increase in proinflammatory and anti-inflammatory response that strongly corelated with breathing difficulties and alveolar pathology in the model. Dexamethasone showed a moderate rescue.

Neutralizing antibodies were identified at dilutions -5 to 5 fold indicating F fragment was sufficient to induce humoral immune elicitation.

Conclusion:

The current screening system offers a quick 3 days screening period to identify compounds for HMPV therapeutic development.

References

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