IRL201104, a Novel Immunomodulatory Peptide, Prevents Inflammatory Infiltration and Proinflammatory Cytokine Release in a New Model of ARDS Associated to Influenza Infection

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Introduction

- Respiratory failure from Acute Respiratory Distress Syndrome or ARDS is a leading cause of mortality in patients with respiratory infections, in particular Influenza (1).
- IRL201104 (1104) is a novel clinical stage immunomodulatory peptide that has shown efficacy in a range of preclinical models of lung inflammation and ARDS/ALI without showing any signs of immunosuppression (2,3,4).
- Our aim was to establish a model that mimicked the dysregulated immune response associated with ARDS following influenza A virus (IAV) infection by using the viral mimetic polyinosinic-polycytidylic acid (poly I:C) to exacerbate Influenza-induced lung inflammation and, subsequently, to evaluate the efficacy of 1104 in this model.

Methods

Model set up

- Dose response study: In order to choose the optimal dose of poly I:C to exacerbate the Influenza induced lung inflammation, female Balb/c mice were infected intranasally with a H3N2 strain of influenza (A/X31;1x10^3 pfu). Three days after infection, at the time of peak viral load in the lung, animals received an intranasal dose of Poly I:C (10, 30 or 100µg/animal) or vehicle (PBS). A second group of non-infected animals also received an intranasal dose of Poly I:C. 24hrs after Poly I:C challenge, bronchoalveolar lavage fluid (BALF) was collected for differential cell counts.
- Time course study: After choosing the optimal Poly I:C concentration for exacerbation, female Balb/c mice were infected intranasally with IAV as described previously. Three days after infection animals received an intranasal dose of Poly I:C (30µg/animal) or vehicle (PBS). . As before, a second group of non-infected animals also received an intranasal dose of Poly I:C. BALF was collected for differential cell counts at 24, 48 and 72 hours after Poly I:C challenge.

Proof of concept study

- As described above, mice were infected intranasally with IAV and three days after infection animals received a dose of poly I:C (30µg) or vehicle (PBS). 1104 (80- 800 µg/Kg) or vehicle were administered q.d. intravenously 15min before Poly I:C and 24hrs later. Positive control Dexamethasone (Dex) was administered q.d. intraperitoneally (10mg/kg) 1hr before poly I:C challenge and 24hrs later. 48hrs after Poly I:C challenge, BALF was collected for differential cell counts and to measure cytokines using a multiplex immunoassay. Lung tissue was also collected to determine the viral titre.

BALF collection and differential cell counts

- Animals were overdosed with pentobarbitone and bronchoalveolar lavage was carried out using phosphate buffered saline. The isolated BALF was then centrifuged at 1500 rpm for 10mins at 4°C and the supernatant was aliquoted (400 µL) and stored at -80°C for cytokine analysis. Cell pellets were then re-suspended in 0.8mL of 0.2% w/v NaCl to induce haemolysis of any erythrocytes. After isotonization with the same volume of 1.6% w/v NaCl, the BALF cells were analysed for total and differential cell numbers using a XT-2000iV analyser (Sysmex). Results are expressed as cells/mL.

Cytokine/chemokine measurements

- A 12-Plex cytokine/chemokine panel (TNF-α, IFN-γ, IL-1β, IL-6, KC, IL-10, IL-17, MCP-1, MIP-1α, MIP-3α, G-CSF and GM-CSF) was run in BALF supernatant using a magnetic multiplex assay as per the manufacturer's instructions (Biotechne Ltd). Levels were measured using a Magpix system (Luminex Corp). Results are expressed as concentration in pg/mL.

Viral titre assay

- Serial dilutions of the lung samples were applied to plate wells containing an 80% confluency of MDCK cells. This was performed in triplicate. The inoculated cells were incubated at 37°C for 1 h, after which the inoculum was removed from the wells and the cells washed (twice) with phosphate buffered saline before applying an overlay of agar media (inc. growth media plus TPCK trypsin) to each of the wells. Once the agar media was set, the plates were placed in an incubator at 37°C with 5% CO_2 for 3 days after which the resulting plaques were counted. A second count was also conducted once the agar had been removed by fixing and staining the cells with crystal violet. Data are presented as mean ± sem pfu/g tissue for each group.

Statistical analysis

- Data are shown as mean ± S.E.M. (standard error of the mean). Inter-group deviations were statistically analysed by a one-way analysis of variance (ANOVA) followed by a Dunnett's test or a Student's t-test when comparing infected vs uninfected Poly I:C groups. P< 0.05 was considered statistically significant.

Results (I)

Poly I:C dose selection and time course

Poly I:C administration at the peak of influenza infection elicited a synergistic inflammatory response in the lung as measured by neutrophilic infiltration which was maximal after a dose of 30µg/animal (Fig1A). The exacerbation of the lung neutrophilic infiltration peaked at 48hrs and was still significant 72hrs after Poly I:C administration (Fig1B).

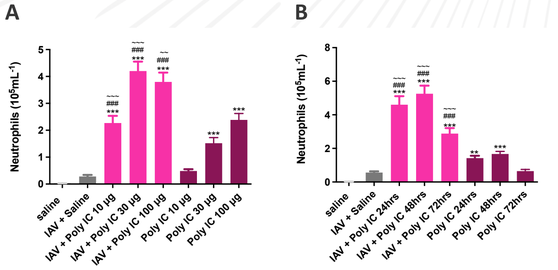

Figure 1 – Effect of Poly I:C on cellular infiltration. *(A) Effect of different Poly I:C doses (10-100µ) on exacerbating IAV associated neutrophilic lung infiltration 24 hrs after challenge. (B) Effect of Poly I:C (30µg) on exacerbating IAV associated neutrophilic lung infiltration at different time points after challenge (24, 48 and 72h). Data are expressed as cells per mL of BALF, mean ±SEM. Comparisons vs saline group were made using a one-way analysis of variance (ANOVA), followed by a Dunnett's test (**P<0.01;***P<0.001). Comparisons of IAV/saline vs IAV/Poly I:C groups were made using one-way analysis of variance (ANOVA), followed by a Dunnett's test (### P<0.001). Poly IC only groups were compared to IAV infection infected concentration/time matching groups using Student's unpaired t-test (~~ P<0.01; ~~~ P<0.001); n=8.*

Effect of 1104 on the IAV exacerbation model

1104 (80-800µg/kg) treatment significantly and dose dependently reduced the inflammatory infiltration in the lung associated to the IAV/Poly I:C combination. This was reflected on total white blood cells (Fig2A), neutrophils (Fig2B), macrophages (Fig2C) and lymphocytes (Fig2D). Unlike 1104, steroid dexamethasone was unable to have any significant impact on the lung infiltration.

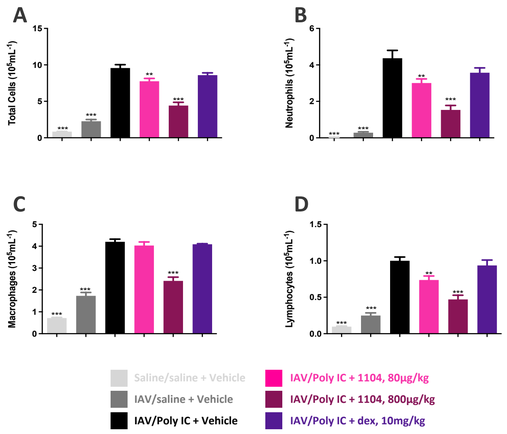

Figure 2 – Effect of IRL201104 on inflammatory infiltration after IAV/Poly I:C challenge. *Effect of IRL201104 on total cells (A), neutrophils (B), macrophages (C) and lymphocytes (D) in BALF. Data are expressed as cells per mL of BALF, mean ±SEM. Comparisons vs IAV/Poly I:C/vehicle group were made using a one-way analysis of variance (ANOVA), followed by a Dunnett's test,**P<0.01,***P<0.001; n=8.*

Results (II)

Effect of IRL201104 on cytokine/chemokine release in BALF

48 hours after Poly I:C challenge, IAV alone or in combination with Poly I:C elicited the release of TNF-α, IFN-γ, IL-1β, IL-6, KC, IL-10, IL-17, MCP-1, MIP-1α, MIP-3α, G-CSF and GM-CSF in BALF supernatant. As with the cellular infiltrate, 1104, unlike dexamethasone, showed significant dose dependent suppression of all these cytokines/chemokines (Fig3).

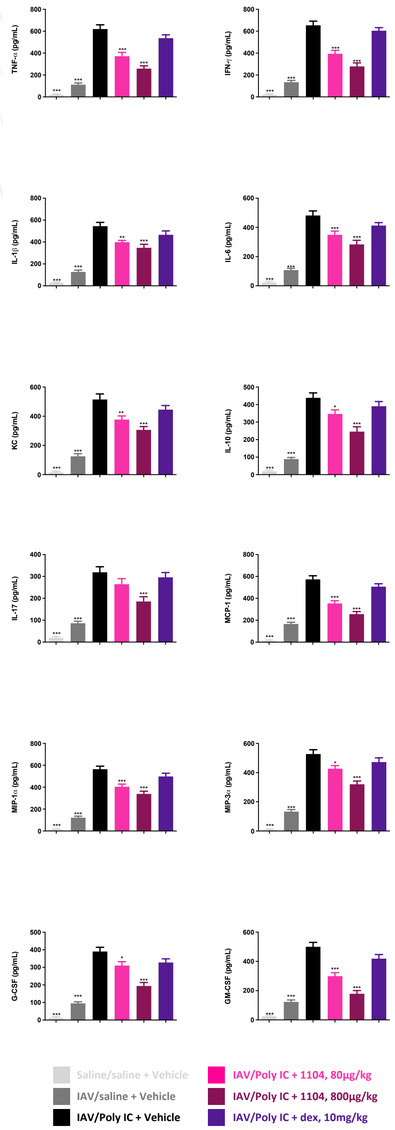

Figure 3 – Effect of IRL201104 on cytokines/chemokines in BALF after IAV/Poly I:C challenge. *Effect on TNF-α, IFN-γ, IL-1β, IL-6, KC, IL-10, IL-17, MCP-1, MIP-1α, MIP-3α, G-CSF and GM-CSF. Data are expressed as picograms per mL of BALF, mean ±SEM. Comparisons vs IAV/Poly I:C/vehicle group were made using a one-way analysis of variance (ANOVA), followed by a Dunnett's test,*P<0.05, **P<0.01,***P<0.001; n=8.*

Results (III)

Effect of IRL201104 on lung tissue viral titre

Viral titre in the lung 48 hours after Poly I:C challenge was similar in all IAV infected groups. Neither 1104 or Dexamethasone treatment seemed to have a significant effect on viral titre. This result highlights that 1104 can modulate the inflammatory response without causing immunosuppression (Fig4).

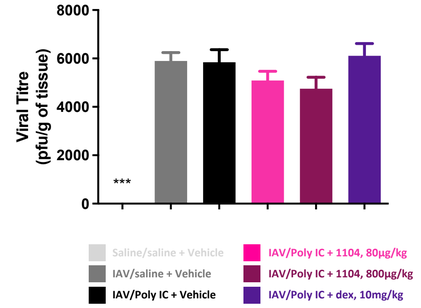

Figure 4. Effect of IRL201104 on viral titre in the lung after IAV/Poly I:C challenge. *Viral titre in the lungs from animals 48 hours after Poly I:C/saline challenge. Data are expressed as pfu/g of tissue, mean ±SEM. Comparisons vs IAV/saline treated animals were made using a one-way analysis of variance (ANOVA), followed by a Dunnett's test. *** P<0.001; n=8.*

Conclusions

- IRL201104 is an immunomodulatory peptide that recently completed two phase 2A clinical trials for Eosinophilic Esophagitis (NCT05084963) and Allergy (NCT05098522). The primary endpoint in the EoE trial was met with multiple secondary positive findings and the Allergy study data is pending.
- We have established a new model of influenza exacerbation that mimics certain aspects or ARDS associated to IAV infection, such us exacerbated inflammatory infiltration and associated release of relevant proinflammatory cytokines/chemokines, showing over activation of the immune system without an increase in the viral load.
- In this model, IRL201104, unlike dexamethasone, significantly inhibited both inflammatory infiltration and relevant pro-inflammatory cytokines usually elevated in ARDS associated to viral infection such us TNF-α, IL-1β, IL-6, IL-17, MCP-1 or MIP-1α (5). Furthermore, 1104 did not affect the viral titre, showing it can modulate the inflammatory response without causing immunosuppression.
- The results of this study highlight the potential for 1104 to be used in the treatment of ARDS associated to viral infections.

References

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Acknowledgements

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