IRL201104, a Novel Immunomodulatory Peptide, Shows a Long Lasting, Dose Response and Wide Spectrum Effect Through Different Routes of Administration in a Model of Allergic Inflammation

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Introduction

IRL201104 is a novel peptide derived from M. tuberculosis (mTB) bacterial chaperonin 60.1 which has shown immunomodulatory and anti-inflammatory properties in a range of preclinical models of lung inflammation (Riffo-Vasquez et al, 2020; Man et al, 2021).

Despite a very short half-life (~10-15 min across species), IRL201104 shows a long-lasting effect in models of allergic inflammation of up 14 days (Riffo-Vasquez et al, 2019; Page et al, 2019).

The aim of this study was to compare, in an ovalbumin (OVA) driven re-challenge model of allergic inflammation, the impact of the molecule through two different routes of administration (subcutaneous and intravenous) on inflammatory cell lung infiltration and relevant cytokines both in serum and bronchoalveolar lavage fluid (BALF).

Methods

Model protocol

- Female Balb/C mice (~25g) were sensitised with ovalbumin (15 µg, s.c.) and Imject Alum as an adjuvant on days 1 and 7.
- In order to elicit a local inflammatory response in the lungs, mice were challenged on day 15, 16 and 17 with an aerosol of either 1% w/v ovalbumin in phosphate buffered saline (PBS) or just PBS, generated with an ultrasonic nebuliser (Aerogen) for 20 min.
- IRL201104 or vehicle were administered subcutaneously (s.c.; 0.2, 20 and 200 µg/Kg) or intravenously (i.v.; 0.2, 20 and 80 µg/Kg) 15 minutes prior to the OVA or PBS challenges on days 15, 16 and 17. 24 h after the final challenge to OVA or PBS (day 18), samples were collected.
- In a separate cohort of animals, a second series of challenges on days 27, 28 and 29 with an aerosol of 1% w/v ovalbumin or PBS were carried out without further drug treatment. Samples were collected from these animals 24 hrs after the final challenge (day 30).

BALF/serum collection and differential cell counts

- 24 hours after the last challenge (day 18 or 30) blood samples were taken by venepuncture (via the lateral tail vein) and placed into serum tubes. Each blood sample was kept at room temperature for 45 minutes to allow coagulation, before being centrifuged (2000g, 15 min at 4oC) and the resulting serum sample was stored at -80°C for cytokine analysis.
- Animals were then overdosed with pentobarbitone and bronchoalveolar lavage was carried out using phosphate buffered saline. The isolated bronchoalveolar lavage fluid (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 were expressed as cells/mL.

Cytokine/chemokine measurements

- A 12-Plex cytokine/chemokine panel (IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17, KC, Eotaxin, G-CSF, GM-CSF, MCP-1 and RANTES) was measured in BALF supernatant and serum using a magnetic multiplex assay as per the manufacturer's instructions. Levels were measured using a Magpix system (Luminex Corp). Results were expressed as concentration in pg/mL.

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. p< 0.05 was considered statistically significant.

Results (I)

OVA induced cell infiltration

24 hours post-challenge at day 18, exposure to OVA aerosol triggered inflammatory infiltration in the lung as measured by BALF differential cell counts. IRL201104 significantly reduced OVA-induced lung infiltration of eosinophils, neutrophils, lymphocytes and macrophages (Fig1A,B,C,D) in a dose response manner and through both routes of administration. The effect of IRL201104 was maintained upon OVA re-challenge (day 30), 13 days after the last dose of the compound (Fig1E,F,G,H).

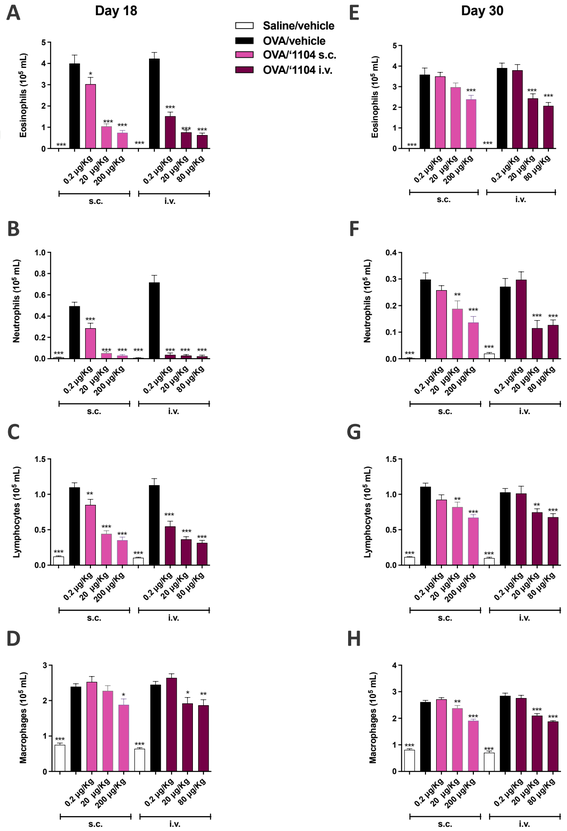

Figure 1 – Effect of IRL201104 on cellular infiltration. *Effect of IRL201104 s.c. and i.v at day 18 (A,B,C,D) and day 30 (E,F,G,H) on eosinophils, neutrophils, lymphocytes and macrophages lung infiltrate, respectively. Data are expressed as cells per mL of BALF, mean ±SEM. Comparisons to the vehicle treated animals exposed to OVA 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.

OVA induced cytokine/chemokine release in BALF and serum

At 24 hours post-challenge at day 18 and 30, exposure to OVA aerosol elicited the release of IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17, Eotaxin, GM-CSF, MCP-1 and RANTES in BALF supernatant and serum. IRL201104 significantly reduced the levels of all cytokines/chemokines at day 18 in a dose response manner and through both routes of administration. The effect of IRL201104 was maintained upon OVA re-rechallenge (day 30), 13 days after the last dose of the compound. Data on IL-4, IL-5 and IL-13 are shown in Figure 2 with a summary of the effect of the compound on the rest of the panel in Table 1.

Results (II)

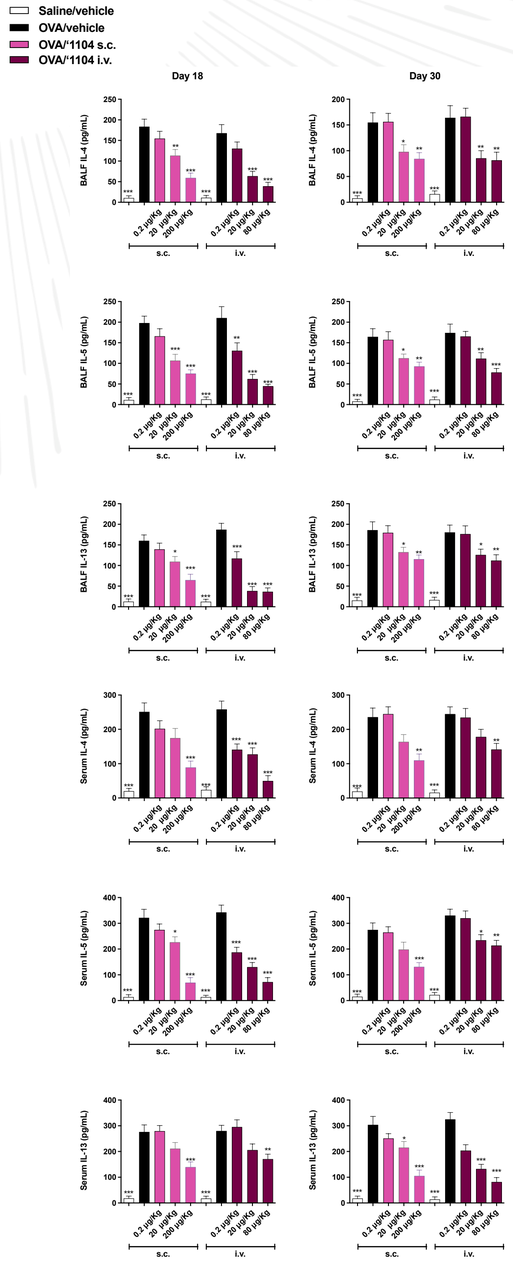

Figure 2 – Effect of IRL201104 on Th2 cytokines/chemokines in BALF and serum. *Effect of IRL201104 on IL-4, IL-5 and IL-13 in BALF and serum. Data are expressed as picograms per mL, mean ±SEM. Comparisons to the vehicle treated animals exposed to OVA were made using a one-way analysis of variance (ANOVA), followed by a Dunnett's test,*P<0.05, **P<0.01,***P<0.001; n=8.*

Results (III)

A

Day 18	BALF (pg/ml)	OVA/vehicle s.c	OVA/1104 200µg/Kg s.c.	OVA/vehicle i.v.	OVA/1104 80µg/Kg i.v.
	IL-10	98±14	44±13**	112±12	348±8***
	IL-12p70	108±1	52±12***	113.5±12.5	23±7***
	IL-17	88±9	30±7***	94±7	25±8***
	Eotaxin	94±9	28.5±8.5***	100.5±10	47±7***
	GM-CSF	72±9	26±8.5**	91±11	27±8***
	MCP-1	92±13	38±10**	100±10	33±10***
	RANTES	81±11	38±9*	93±7	30±10***
Day 30	**BALF (pg/ml)**	**OVA/vehicle s.c**	**OVA/1104 200µg/Kg s.c.**	**OVA/vehicle i.v.**	**OVA/1104 80µg/Kg i.v.**
	IL-10	123.5±9	71±6***	135±12	62±8***
	IL-12p70	123±15	82±9*	154±12	79±15***
	IL-17	79±9	43±8**	85±8	38±9***
	Eotaxin	110±8	47±8***	98.5±13	39±10**
	GM-CSF	92±9.5	54±9*	98±8.5	36±9***
	MCP-1	102±8	64.5±12*	98±11.5	41±7***
	RANTES	103±11	81±9	105.5±16	61±9

B

Day 18	SERUM (pg/ml)	OVA/vehicle s.c	OVA/1104 200µg/Kg s.c.	OVA/vehicle i.v.	OVA/1104 80µg/Kg i.v.
	IL-10	177±25***	83±12	184±19	57±11***
	IL-12p70	113.5±11	54±14**	122±13	44±14***
	IL-17	143±15	67±10***	151±19	57±14***
	Eotaxin	134±17	48±18***	125.5±11	38±12***
	GM-CSF	110±10	58±14*	96±9	41±12***
	MCP-1	160±20	47±11.5***	168±19	37±11.5***
	RANTES	95±11	29±11***	108±10	37±15**
Day 30	**SERUM (pg/ml)**	**OVA/vehicle s.c**	**OVA/1104 200µg/Kg s.c.**	**OVA/vehicle i.v.**	**OVA/1104 80µg/Kg i.v.**
	IL-10	149±15	98±17	156±17	77±11***
	IL-12p70	130±16	90±10	124±13.5	81±10*
	IL-17	129±13	70±11.5**	148±15	57±10***
	Eotaxin	115±10	57±17**	101±9	40.5±13***
	GM-CSF	105±11	49±15**	91±9	26±13***
	MCP-1	178±16	82±13**	161±18	67±17***
	RANTES	112±10	80±9	127±13	74±11**

Table 1. Effect of IRL201104 on BALF (A) and serum (B) cytokines/chemokines. *Effect of IRL201104 on IL-10, IL-12p70, IL-17, Eotaxin, GM-CSF, MCP-1 and RANTES. Data are expressed as picograms per mL of BALF/serum, mean ±SEM. Comparisons to the vehicle treated animals exposed to OVA 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.*

Conclusions

- IRL201104 is an immunomodulatory peptide that is currently undergoing two phase 2A clinical trials for Eosinophilic Esophagitis (NCT05084963) and Allergy (NCT05098522).
- In the current study, IRL201104 shows a similar long lasting dose response immunomodulatory profile through two different routes of administration, intravenous and subcutaneous. IRL201104 has also shown similar effects when administered intranasally (Page et al, 2019) and sublingually (unpublished data).
- IRL201104 does not only affect eosinophils, but also neutrophils, macrophages and lymphocytes, indicating a broad spectrum of action on inflammatory immune cells.
- IRL201104 also exhibits a significant long lasting effect in a range of relevant cytokines and chemokines such us IL-4, IL-5, IL-13 and Eotaxin, both in BALF and serum.
- The present work shows the potential of IRL201104 to be used in the clinical setting through these routes of administration for the treatment of asthma and other allergic and inflammatory diseases.

References

1. Man et al. Am J Physiol Lung Cell Mol Physiol. 2021 Nov 1;321(5):L803-L813.
2. Page et al. Am J Respir Crit Care Med 2019;199:A2861
3. Riffo-Vasquez Y et al, Clin Exp Allergy. 2020 Apr;50(4):508-519.

Acknowledgements

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