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## INTRODUCTION

Over 95% of the lung alveolar surface is covered by large flat Type I cells (AT1). These cells are generally felt to be terminally differentiated to perform their functions in gas exchange and ion/water flux and presumed to arise from Type II cells. The source of AT1 cells in fetal/developing lungs may be different. Recently, using AT1 cell specific antigens, pure populations of AT1 cells have been isolated and studied *in vitro*. In this system, AT1 cells have been shown to be active in the innate immune response<sup>1</sup>.

RAGE is a transmembrane receptor found exclusively in the lung on AT1 cells. RAGE activation by ligands such as HMGB1, and S100 calcium-binding proteins leads to NF-κB activation and production of inflammatory mediators.

We recently demonstrated that AT1 cells isolated from P1 mice had significantly higher levels of the chemokine CXCL2 (MIP-2) after 24 hours in culture compared to cells from adult mice. To examine whether RAGE might be involved in this response, we cultured newborn and adult AT1 cells with and without anti-RAGE antibodies and measured chemokine production.

## OBJECTIVES

- Determine the effect of RAGE blockade on CXCL2 production from AT1 cells isolated from newborn (P1) and adult mice
- Determine if AT1 cell chemokine production stimulation by anti-RAGE antibody is cell-type specific

## METHODS

### AT1 Isolation

- Newborn = < 24 hours old, adult = > 3 months old C57BL6 mice.
- FACS Sorting -Cells were incubated with hamster anti-mouse T1α followed by Alexa 488-labeled anti-hamster IgG. Only live (Propidium Iodide negative) collected.

### Cell culture

- Cells were cultured on fibronectin-coated Transwells (24 well size) in PBS/20% FBS and antibiotics in 10% CO<sub>2</sub>.
- 2 x 10<sup>3</sup> cells were incubated with anti-mouse RAGE (80 μg/ml), FPS-ZM1 (375 nM), or medium alone. FPS-ZM1 is a non-toxic RAGE inhibitor<sup>2</sup>.

### RAW 264.7 cells

- 4 x 10<sup>5</sup> cells were cultured in PBS/20% FBS with anti-mouse RAGE antibody (80 μg/ml), FPS-ZM1 (500 nM), LPS (10 μg/ml), or medium alone.

### CXCL2 Measurement

- ELISA

### CXCL2 RT-PCR

- After 24 hours in culture, mRNA isolated and analyzed by RT-PCR vs GAPDH housekeeping gene

## RESULTS

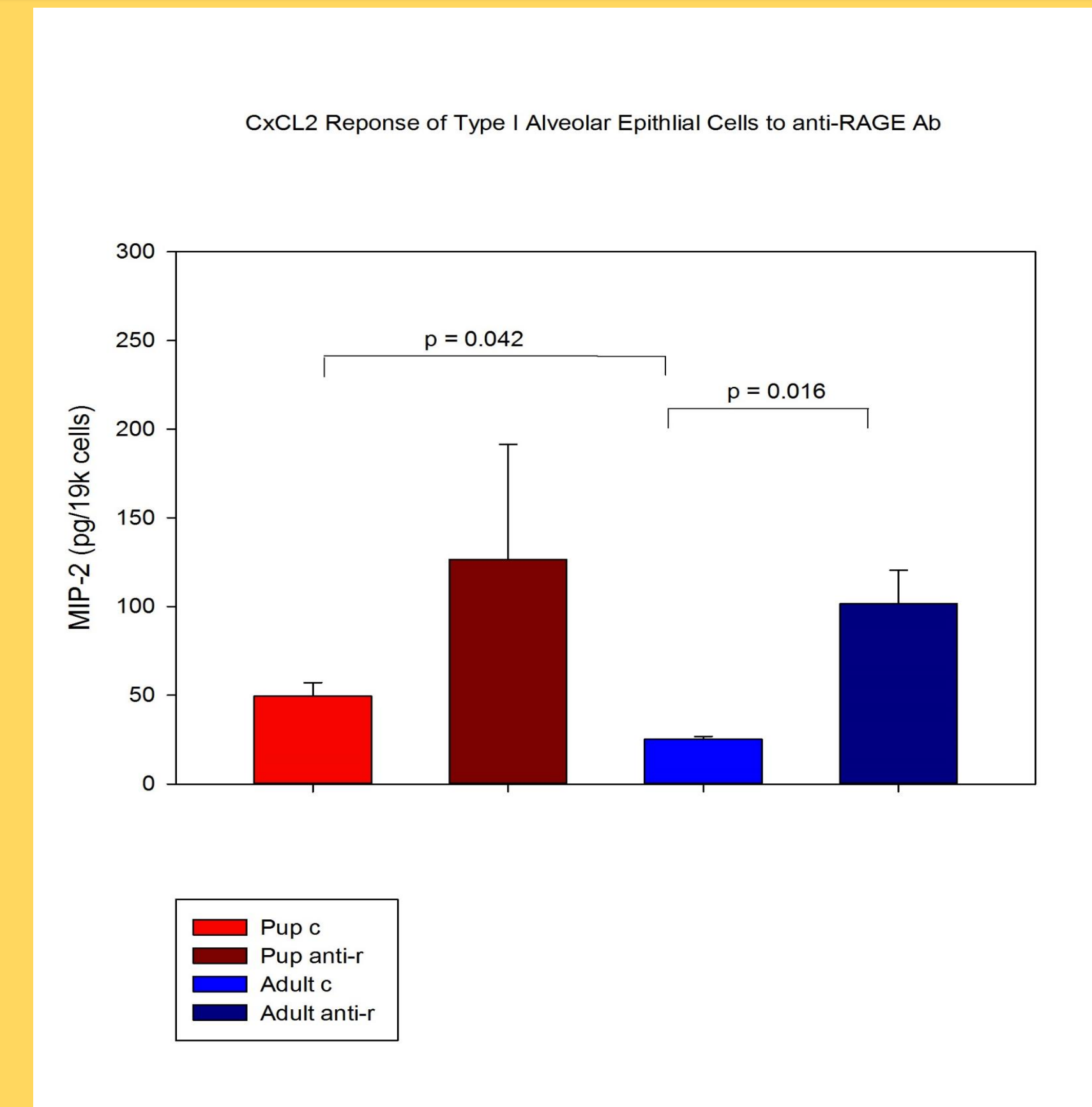


Figure 1 – Effect of Anti-RAGE antibody on medium CXCL2 levels after 24 hours in culture. Control newborn pup P1 mouse cells produced more chemokine than adult cells. Antibody exposure increased adult AT1 chemokine production. By ANOVA, antibody exposure increased chemokine production in combined P1 and adult AT1 cells ( $p = 0.027$ ). Mean  $\pm$  SEM

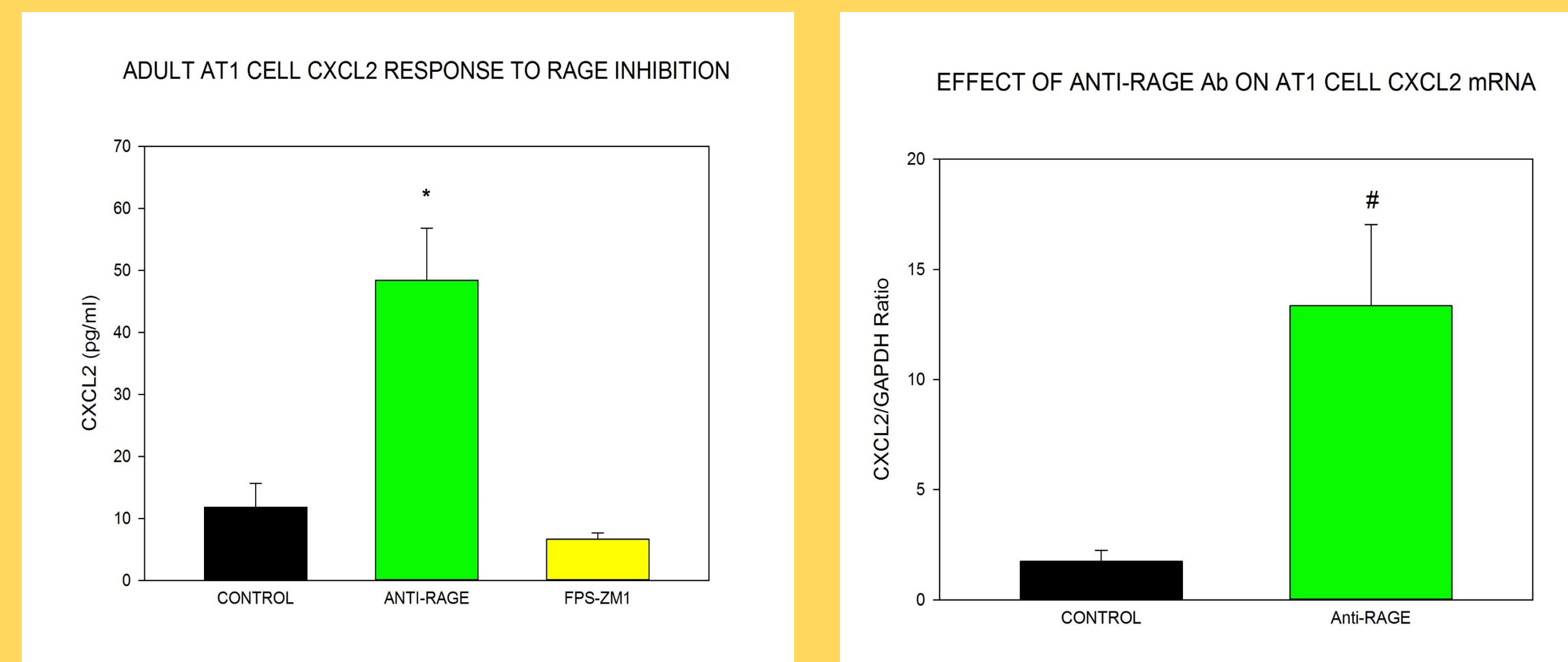


Figure 2. LEFT - Increased CXCL2 protein from adult AT1 cells after RAGE inhibition is specific for anti-RAGE antibody blockade. The multimodal chemical inhibitor FPS-ZM1 had no effect. \*  $p < 0.001$  vs Control and FPS-ZM1 groups. Mean  $\pm$  SEM  
RIGHT – Increased CXCL2 mRNA after exposure to anti-RAGE in adult AT1 cells. #  $p < 0.02$ . Mean  $\pm$  SEM

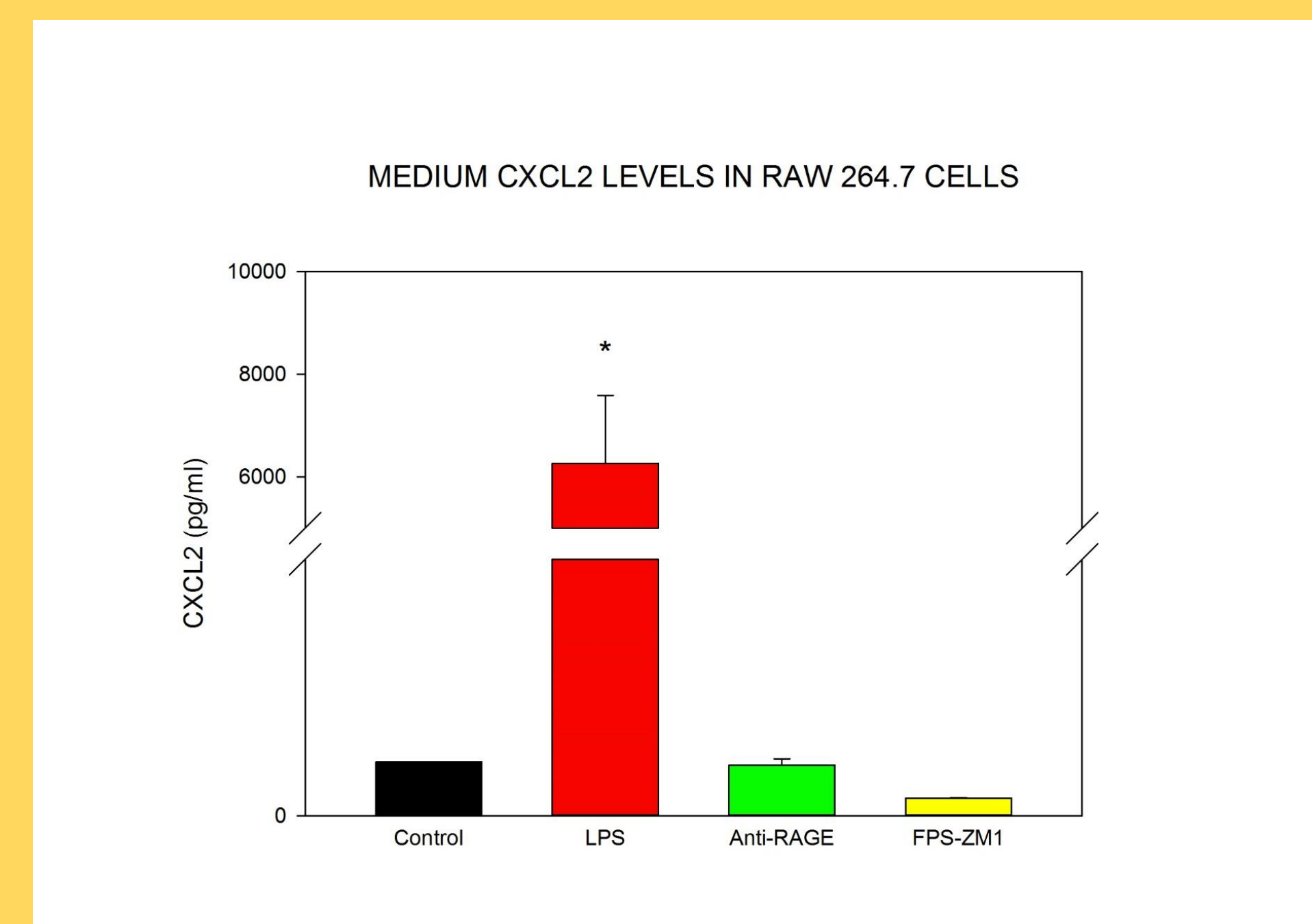


Figure 3. No effect of anti-RAGE antibody on mouse macrophage cell line RAW 264.7. LPS increased CXCL2 production vs all other groups (\*  $p < 0.001$ ). Mean  $\pm$  SEM

## CONCLUSIONS

- Anti-mouse RAGE antibody increased medium CXCL2 levels in freshly isolated AT1 cells. The rise was significant in adult cells. We speculate that increased spontaneous production in newborn P1 cells reduced the relative increase so that it did not reach significance –  $p = 0.200$  difference(Figure 1).
- The increase was due to the antibody and not seen with RAGE inhibition with the multimodal inhibitor FPS-ZM1 (Figure 2).
- The effect was specific for AT1 cells as mouse RAW 264.7 cells, which have RAGE receptors<sup>3</sup> showed no effect (Figure 3).

## SPECULATION

- Anti-mouse RAGE antibody may be a RAGE agonist. This would be specific for AT1 cells, since there was no agonist effect with the macrophage cell line RAW 264.7.
- The agonist effect is not likely due to LPS contamination because there was no similar effect with the RAW 264.7 cells.
- Anti-RAGE antibodies may interfere with the attachment of AT1 cells to the fibronectin Transwell. Unbound cells may then be primed to increase their inflammatory mediator production.
- Alternatively, the antibody could bind sRAGE and make it unavailable for binding to the extracellular matrix<sup>4</sup>. This could improve AT1 cell adherence and adherent cells may be the ones producing chemokine.

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