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Immunomodulatory properties of heat-killed *Rhodococcus coprophilus* control the allergic reaction in a mouse model of food allergy

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Introduction & Aim

Actinomyces are soil bacteria with immunomodulatory properties that exert biological effects on intestinal epithelial cells in different inflammatory contexts. Our goal is to study the inhibitory effect of dead *Rhodococcus coprophilus*-Rc on activated epithelial cells exposed to pro-inflammatory stimuli, and in an experimental food allergy model.

Material & Methods

Colon cell lines (Caco-2 and Caco-luc) were cultured with flagellin (FliC) and the induction of cytokines (IL-1b, IL-6, TNFa) and chemokines (CCL20, IL-8 and MCP-1) were studied by qPCR, while Nf-κB was analyzed by immunoblotting. In addition, Balb/c mice were sensitized with cow´s milk proteins (CMP) plus cholera toxin by gavage, and orally challenged with CMP to induce intestinal inflammation and hypersensitivity symptoms. Activated cell lines were exposed to Rc before or during activation. On the other hand, mice received Rc by gavage during one week, and then they were sensitized.. The therapeutic effect of Rc was monitored in vivo (clinical score and cutaneous test) and *in vitro* (serum specific antibodies and cytokines by ELISA, and cell analysis by flow cytometry).

Results

In vitro assays

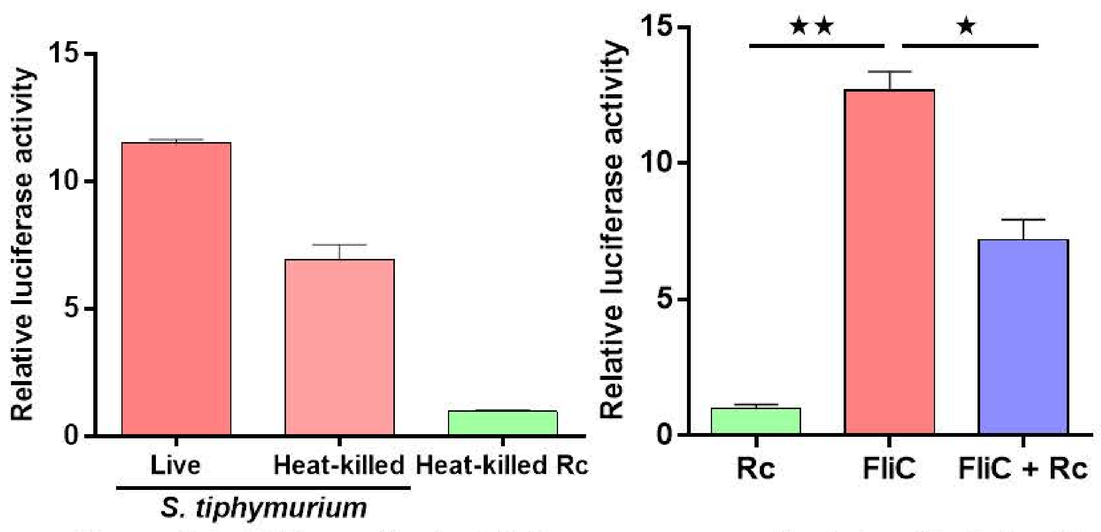

Figure 1. Inhibitory effect of Actinomyces on activated epithelial cells: FliC and *Salmonella*-induced cell activation with increased luciferase activity. We observed a statistically significant inhibition of cell activation with heat-killed *Rhodococcus coprophilus* (Rc).

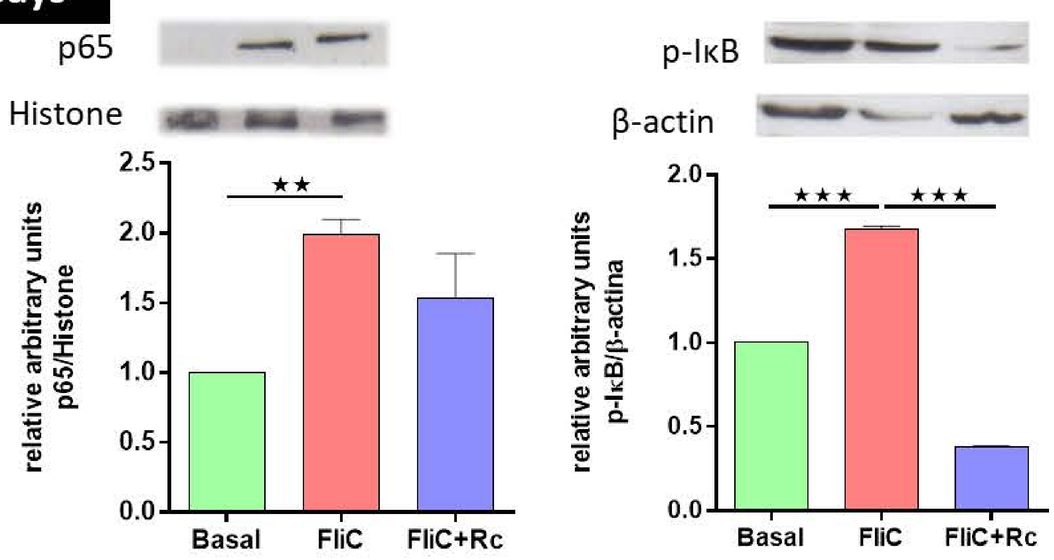

Figure 2. Modulatory effect of *Rhodococcus coprophilus* on NF-κB. Rc pre-treated Flic-activated cells modulated the translocation of the NF-kB component onto the nucleus.

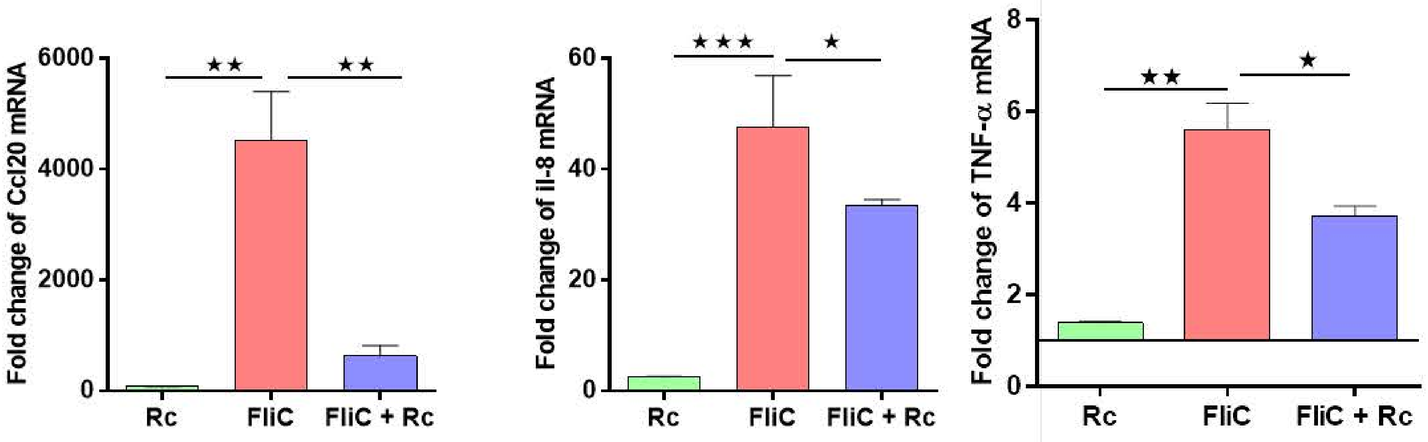

Figure 3. Chemokines and cytokines modulation by Rc: Treated cells with flagellin and *Rhodococcus coprophilus* expressed less amounts Ccl20, Il-8 and TNF-α than the cells treated only with Flic.

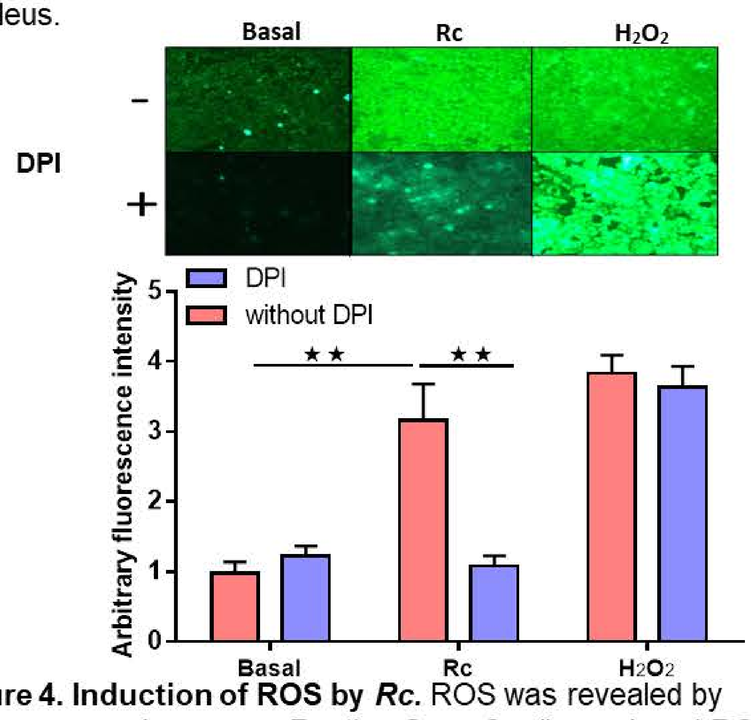

Figure 4. Induction of ROS by *Rc.* ROS was revealed by fluorescence microscopy. Resting Caco-2 cells produced ROS in the presence of Rc and it was inhibited in the presence of DPI (DPI: Diphenyleneiodonium).

In vivo assays

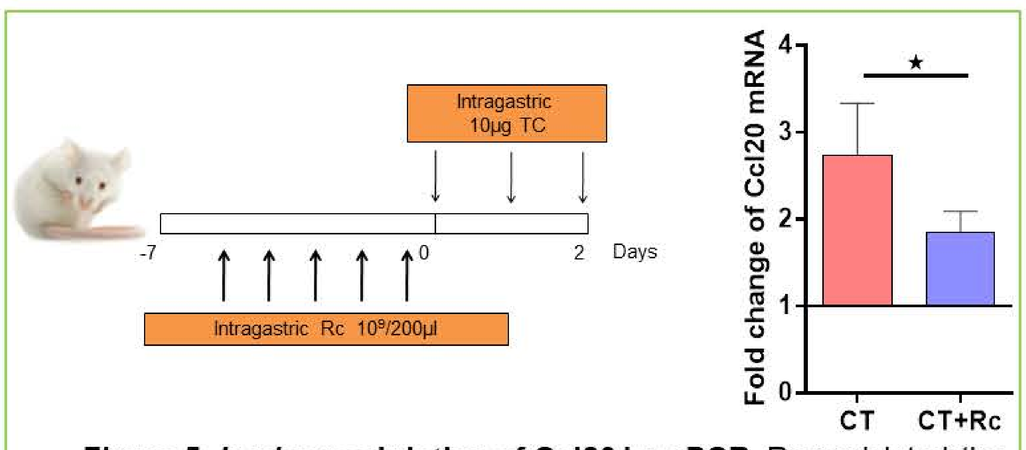

Figure 5. *In vivo* modulation of Ccl20 by qPCR. Rc modulated the expression of Ccl20 in jejunum

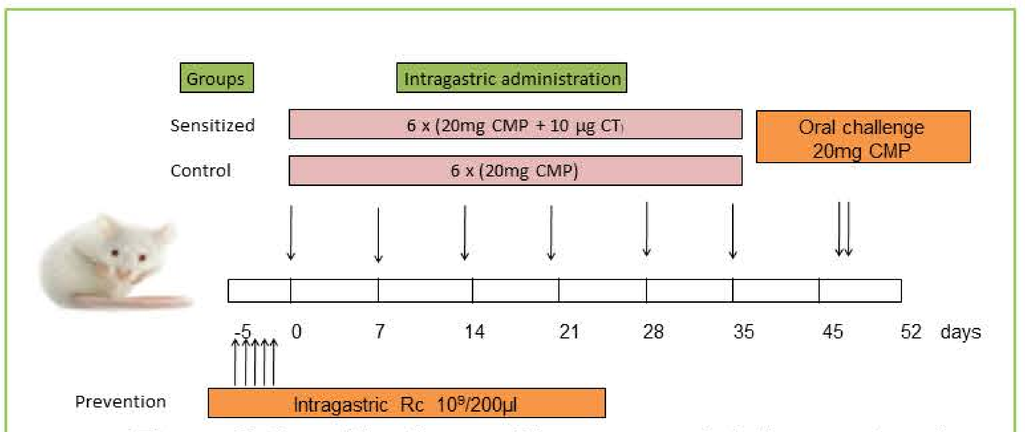

Figure 6. Sensitization and immunomodulation protocol.

Table 1. Clinical scores assigned to symptoms triggered following the oral challenge.

Puntaje	Signos clínicos observados
0	Sin síntomas
1	Rascado de nariz y cabeza y enrojecimiento alrededor de nariz y cabeza.
2	Hinchazón alrededor de ojos y boca, pelo erizado, baja actividad general y/o baja actividad con aumento de frecuencia respiratoria
3	Respiración dificultosa, cianosis alrededor de la boca y cola
4	Inactividad, convulsiones
5	Muerte

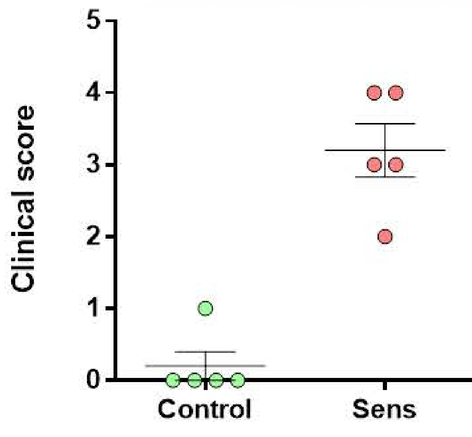

Figure 7. Assessment of the hypersensitivity response: clinical score was lower in Rc-treated animals compared with sensitized mice.

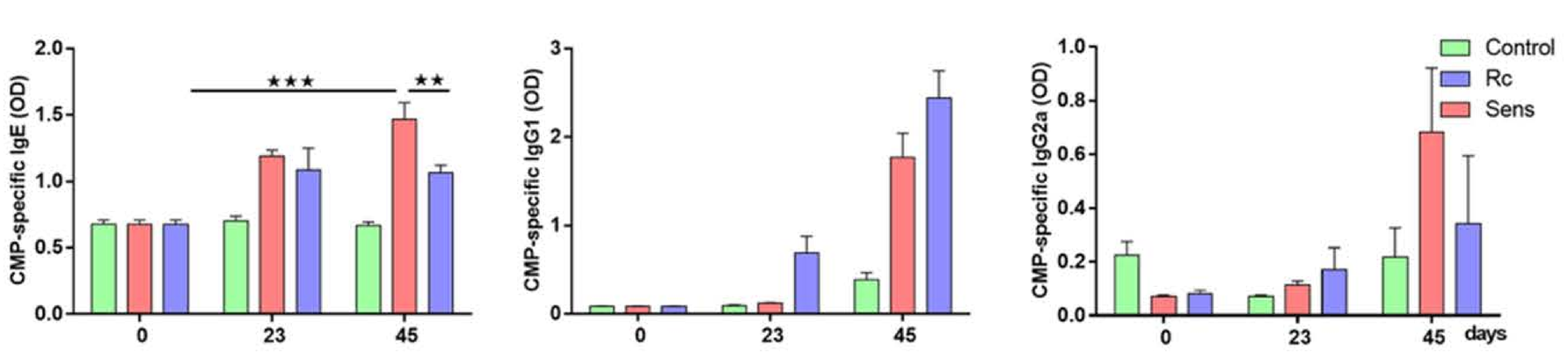

Figure 8. Serum specific immunoglobulin isotypes. We found that CMP-specific IgE and IgG2a were diminished at day 45.

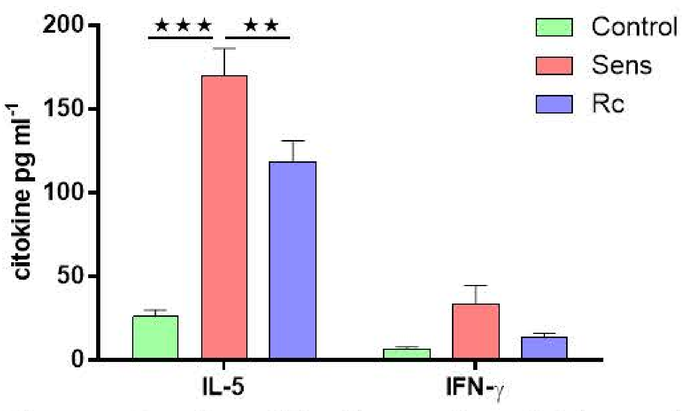

Figure 9. Quantification of cytokines in supernatants. Spleen cells from Rc-treated mice produced lower amounts of IL-5.

Conclusions

Rhodoccocus coprophilus modulated the NF-KB pathway, abrogated the production of pro-inflammatory cytokines and chemokines in intestinal epithelial cells and ameliorated hypersensitivity and the Th2-mediated immune response in the IgE-mediated food allergy mouse model.