# Complement receptor 3 plays a significant role in β-glucan induced ROS production by porcine neutrophils.

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## INTRODUCTION

Pro- and prebiotics are known to stimulate the intestinal immune system. One of the most commonly used immunostimulants in animal husbandry are β-glucans. β-glucans are conserved glucose polymers found in the cell walls of plants, fungi, yeasts and bacteria. Through binding to their receptors they can activate innate immunity, thereby enhancing defense barriers. Several studies in mice have established that dectin-1 is the most efficient receptor on macrophages and dendritic cells for phagocytosis of β-glucan-rich cell walls [1]. In humans, however, a recent study [2] showed an indispensable role for complement receptor 3 (CR3) in phagocytosis as well as reactive oxygen species (ROS) production by neutrophils in response to zymosan (β-glucan derived from *Saccharomyces cerevisiae*). The integrin CR3 is a heterodimer consisting of an α subunit (CD18) and a β subunit (CD11b). Dectin-1 and complement receptor 3 are both expressed on porcine neutrophils and monocytes [3,4], but which receptor contributes to the response of immune cells towards β-glucans in pigs is not known yet. Therefore, we studied the influence of β-glucan receptor inhibitors on the antimicrobial activity of porcine neutrophils.

## RESULTS AND DISCUSSION

β-glucan stimulation of porcine neutrophils results in the production of reactive oxygen species (ROS). Given the importance of dectin-1 in mice, we first investigated whether β-glucan induced stimulation of porcine neutrophils could be attributed to this receptor. Hereto, porcine neutrophils were isolated by Percoll gradient (n = 4, purity > 95%) centrifugation and pre-incubated with 1 mg/ml laminarin. Laminarin is a specific dectin-1 inhibitor. Afterwards, the cells were stimulated with different β-glucans (200 µg/ml), namely scleroglucan, curdlan, *Euglena gracilis*, macrogard, *Saccharomyces cerevisiae* and zymosan. ROS production was determined by chemiluminescence. The antimicrobial activity of porcine neutrophils to β-glucans was only slightly affected by blocking dectine-1 with laminarin (Fig 1). On the contrary, the addition of laminarin even increased the ROS production by neutrophils stimulated with zymosan. We conclude that dectin-1 is not involved in β-glucansignaling in porcine neutrophils. Hence, we investigated through which receptor this signalization occurs. Porcine neutrophils were pre-incubated with different β-glucan receptor inhibitors (anti-CD18, anti-CD11R1, anti-CD11R3 and laminarin). Antibodies against CD18 could inhibit the binding of β-glucans to the α subunit of the complement receptor 3 (CR3), while antibodies against CD11R1 and CD11R3 could inhibit binding to the β subunit of CR3, as both CD11R1 and CD11R3 have similarities with human CD11b (molecular weight or cellular distribution) [4]. The neutrophils were, subsequently, stimulated with the β-glucan zymosan. The antimicrobial activity of porcine neutrophils to β-glucans was only slightly affected by blocking CD18 or CD11R1. However, blocking CD11R3 resulted in the complete inhibition of ROS production by neutrophils (Fig 3, 4).

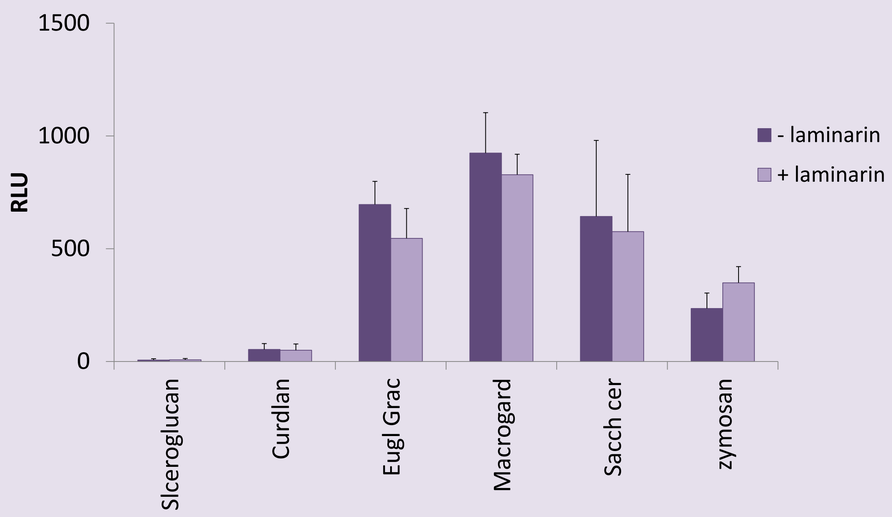


**Figure 1. Influence of laminarin on the reactive oxygen species (ROS) production by porcine neutrophils stimulated with different β-glucans.**
Data represents the average relative light units (RLU) ± SEM of four pigs. PMA (20 µg/ml) was used as a positive control and gave values of 520 RLU. Laminarin (1 mg/ml) gave a value of 7 RLU.

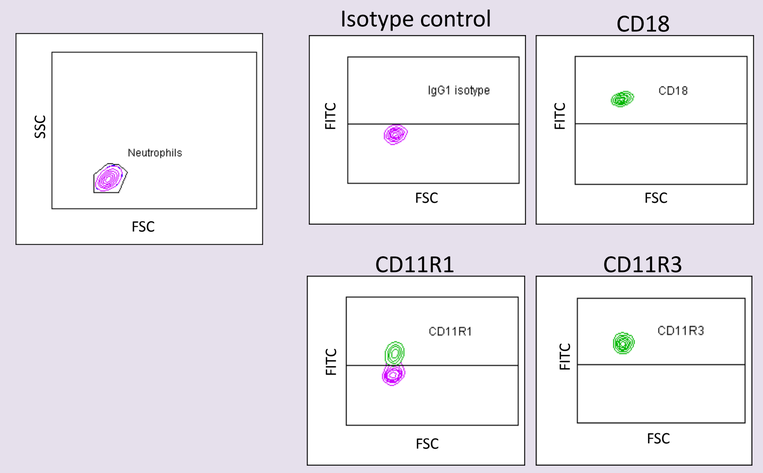


**Figure 2. Expression of complement receptor 3 on porcine neutrophils.**
Neutrophils were identified by forward and side scatter. An isotype-matched control antibody was used to measure the background fluorescence. This results show that all porcine neutrophils express the α subunit of CR3 (CD18) and CD11R3, however, only half of the cells express CD11R1.

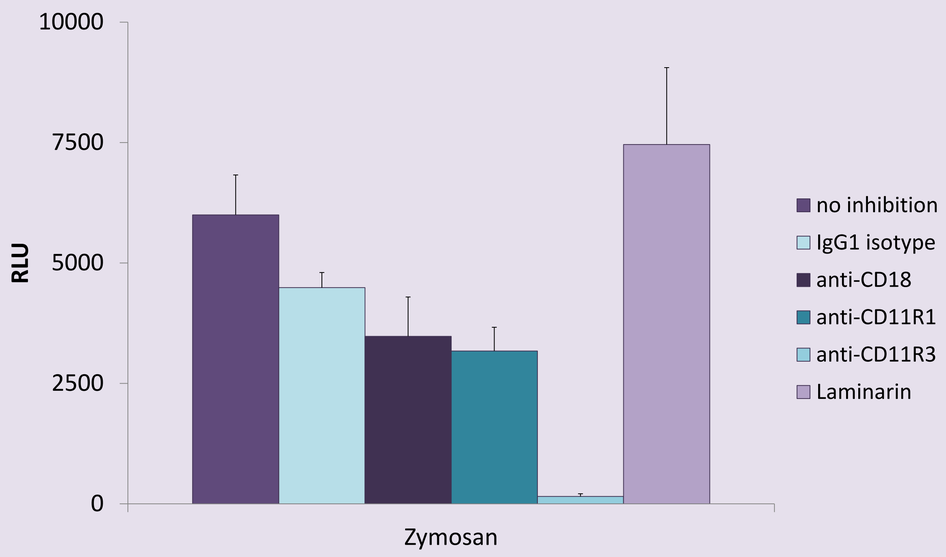


**Figure 3. Influence of laminarin, anti-CD18, anti-CD11R1 and anti-CD11R3 on the β-glucan induced ROS production by neutrophils.**
Data represents the average RLU ± SEM of four pigs. PMA (50 µg/ml) was used as positive control and gave values of 16 387 RLU.

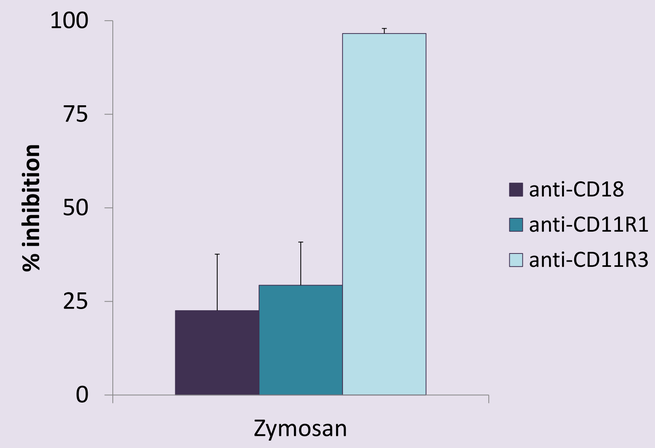


**Figure 4. Effect of anti-CD18, anti-CD11R1 and anti-CD11R3 on ROS production by neutrophils.**
The figure depicts the mean (± SEM) percentage inhibiton of ROS production by neutrophils.

## CONCLUSION

Although dectin-1 was described as the most important β-glucan receptor in mice, our experiments showed that dectin-1 is not involved in β-glucan induced ROS production by porcine neutrophils. Furthermore, we demonstrate the expression of CD18 and CD11R3 on all porcine neutrophils, where CD11R1 was only expressed on half of the neutrophils. Blocking CD18 and CD11R1 results in a reduced ROS production. However, phagocytosis of zymosan particles by porcine neutrophils is completely CD11R3 dependent. We conclude that as for human, CR3 also plays a cardinal role in β-glucan induced ROS production by porcine neutrophils. This observation can contribute to the optimalisation of the use of β-glucans as feed additive in pig industry.

## REFERENCES

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