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 reactive oxygen species (ROS) production by neutrophils in response to zymosan (a 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. Herein, porcine neutrophiles were isolated by Percoll gradient (n = 4, purity > 95%) centrifugation and pre-incubated with 1 mg/ml laminarin. Laminarin is a specific β-glucan inhibitor from the brown alga Laminaria. The neutrophils were, subsequently, 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 antimiocarial activity of porcine neutrophiles to β-glucans was only slightly affected by blocking dectin-1 with laminarin (Fig 3). 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-glucansingaling in porcine neutrophiles. Hence, we investigated through which receptor this signalisation occurs. Porcine neutrophiles 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 neutrophiles were, subsequently, stimulated with the β-glucan zymosan. The antimicrobial activity of porcine neutrophiles 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).

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 demonstrated the expression of CD18 and CD11R3 on all porcine neutrophiles, where CD11R1 was only expressed on half of the neutrophiles. Blocking CD18 and CD11R1 results in a reduced ROS production. However, phagocytosis of zymosan particles by porcine neutrophiles is completely CD11R3 dependent. We conclude that as for human, CR3 also plays a cardinal role in β-glucan induced ROS production by porcine neutrophiles. This observation can contribute to the optimisation of the use of β-glucans as feed additive in pig industry.

REFERENCES