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Cell type-specific differences in β-glucan recognition and signalling in porcine innate immune cells

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ABSTRACT

β-glucans exert receptor-mediated immunomodulating activities, including oxidative burst activity and cytokine secretion. The role of the β-glucan receptors dectin-1 and complement receptor 3 (CR3) in the response of immune cells towards β-glucans is still unresolved. Dectin-1 is considered as the main β-glucan receptor in mice, while recent studies in man show that CR3 is more important in β-glucan-mediated responses. This incited us to elucidate which receptor contributes to the response of innate immune cells towards particulate β-glucans in pigs as the latter might serve as a better model for man. Our results show an important role of CR3 in β-glucan recognition, as blocking this receptor strongly reduced the phagocytosis of β-glucans and the β-glucan-induced ROS production by porcine neutrophils. Conversely, dectin-1 does not seem to play a major role in β-glucan recognition in neutrophils. However, recognition of β-glucans appeared cell type-specific as both dectin-1 and CR3 are involved in the β-glucan-mediated responses in pig macrophages. Moreover, CR3 signalling through focal adhesion kinase (FAK) was indispensable for β-glucan-mediated ROS production and cytokine production in neutrophils and macrophages, while the Syk-dependent pathway was only partly involved in these responses. We may conclude that CR3 plays a cardinal role in β-glucan signalling in porcine neutrophils, while macrophages use a more diverse receptor array to detect and respond towards β-glucans. Nonetheless, FAK acts as a master switch that regulates β-glucan-mediated responses in neutrophils as well as macrophages.

1. Introduction

β-glucans exert immunomodulatory effects upon oral and i.v. administration (Lehne et al., 2006; Ramberg et al., 2010). These glucose polymers can be derived from several sources, such as fungi, yeast, some bacteria, seaweeds and cereals (Du et al., 2013). In seaweeds, they serve as storage carbohydrates, while in bacteria, fungi, yeast and cereals, such as oat and barley, they play a role as structural frame and define cellular shape and rigidity (Miyanishi et al., 2003). The oat and barley cell walls contain unbranched β-glucans with 1,3- and 1,4-β-linked glucopyranosyl residues, whereas β-glucans from bacterial origin and some algae are unbranched 1,3-β-linked glucopyranosyl residues (Brown and Gordon, 2003; Estrada et al., 1997). In contrast, cell wall β-glucans of yeast, fungi and β-glucans produced by some algae consist of 1,3-β-linked glucopyranosyl residues with small numbers of 1,6-β-linked branches. Both the frequency and length of these branches differ depending on the source.

β-glucans are recognised as microorganism-associated molecular patterns (MAMPs) by immune cells via several pattern recognition receptors, including dectin-1, complement receptor 3 (CR3), lactosylceramide and scavenger receptors (Janeway, 1992). Receptor activation results in an enhanced production of pro-inflammatory cytokines, chemokines and reactive oxygen species by innate immune cells in several species (Adachi et al., 1994; Brown et al., 2002; Rice et al., 2002; Ross et al., 1985; Williams, 1997; Zimmerman et al., 1998). Dectin-1 has been recognised as the most important receptor for β-glucans in mice and consequently most studies have focused on the function of dectin-1 in the immunomodulating effect of β-glucans (Brown et al., 2002). This receptor is expressed by dendritic cells (DCs), neutrophils, monocytes

Abbreviations: CR3, complement receptor 3; ROS, reactive oxygen species; FAK, focal adhesion kinase; Syk, spleen tyrosine kinase; MAMPs, microorganism-associated molecular patterns; DCs, dendritic cells; LAL, limulus amebocyte lysate; mAbs, monoclonal antibodies; MDM, monocyte-derived macrophages; RLU, relative light units; PI, propidium iodide; siRNA, small interfering RNA; Cq, quantification cycle.

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and macrophages (Olynych et al., 2006; Reid et al., 2004; Taylor et al., 2002; Willment et al., 2001; Yokota et al., 2001). The intracellular domain of this receptor contains a hemi-immunoreceptor tyrosine-based activation motif (hemITAM), probably indicating the need for receptor dimerisation to properly activate signalling pathways. Dually phosphorylated ITAMs serve as docking site for spleen tyrosine kinase (Syk), a key player in the activation of immune cells (Kingeter and Lin, 2012; Mocsai et al., 2010; Underhill et al., 2005). Recently, several studies have questioned the role of dectin-1 in β-glucan recognition (Marakalala et al., 2013; Qi et al., 2011). Indeed, dectin-1 was not involved in triggering inflammatory responses to zymosan in mice (Marakalala et al., 2013). Likewise, although neutrophils in man express dectin-1, phagocytosis of β-glucans and β-glucan-mediated ROS production is here completely dependent on CR3 (van Bruggen et al., 2009). Interestingly, Bose et al. (2014) demonstrated that human monocytes use distinct receptors (dectin-1 and CR3) for oxidative burst in response to different physical forms of β-glucans. CR3, a β2 integrin, is usually associated with complement function, but recognises an array of various ligands. The ability of CR3 to bind diverse ligands is mainly attributed to a consensus binding site within its CD11b subunit (Yakubenko et al., 2002). The carbohydrate binding domain is however spatially separated from this consensus binding domain (Ross, 2002; Thornton et al., 1996; Vetvicka et al., 1996; Xia and Ross, 1999). Upon ligand recognition, the cytoplasmic domain of CR3 interacts with kinases, such as focal adhesion kinase (FAK), regulating the interaction between cells and the extracellular matrix and controlling several integrin-dependent processes (Kasorn et al., 2009; Parsons, 2003; Yuan et al., 2012). In pigs, dectin-1 and CR3 are expressed on immune cells, including neutrophils, monocytes, macrophages and DCs (Sonck et al., 2009). The α subunit (CD11b) of CR3 is not yet identified, but two candidate molecules, namely CD11R1 and CD11R3, have been described (Dominguez et al., 2001). Both CD11R1 and CD11R3 dimerise with the β2 chain CD18, but their role in complement or β-glucan recognition is still unclear (Dominguez et al., 2001).

The involvement of dectin-1 and CR3 in mediating the biological activities of particulate β-glucans remains largely unresolved, but seems to be cell type-specific, structure-related and species-dependent (Adams et al., 2008; Bose et al., 2014; Huang et al., 2012; Qi et al., 2011; van Bruggen et al., 2009). Here, we investigate the role of these receptors in immunomodulating innate immune cells by different particulate β-glucans in order to elucidate the mechanism of β-glucan recognition and signalling in pigs.

2. Materials and methods

2.1. β-glucans

Six different β-glucans were used to stimulate porcine innate immune cells (Supplementary Table S1). Curdlan, zymosan and β-glucans purified from *Saccharomyces cerevisiae* and *Euglena gracilis* were purchased from Sigma (Bornem, Belgium). Scleroglucan and macrogard were kindly provided by INVE (Belgium) and Biotec Pharmacon ASA (Norway), respectively. A description and comparison of the carbohydrate structures as well as the preparation and storage of these β-glucans was previously published (Sonck et al., 2010). The endotoxin concentration present in each β-glucan preparation was determined by the Chromogenic Limulus Amebocyte Lysate (LAL) test (Cambrex Bio Science Walkersville, Inc.) and with exception of curdlan (47 endotoxin units/μg β-glucan), these levels were consistently lower than 0.5 endotoxin units/μg β-glucan. Laminarin was kindly provided by INVE. Depleted zymosan was purchased from Invivogen (France).

2.2. Isolation of porcine PBMCs and neutrophils

Pigs (between 4 and 23 weeks old) were housed under standard conditions as blood donors. All animal experiments were approved by the animal care and ethics committee of the Faculty of Veterinary Medicine, Ghent University (grant nr: EC2008/79). Peripheral blood (10 ml) was collected on heparin (100 μl) from the jugular vein. Subsequently, peripheral blood monomorphonuclear cells (PBMCs) and neutrophils were isolated as previously described by density gradient centrifugation on Lymphoprep (NYCOMED Pharma AS, Life Technologies, Belgium) or a discontinuous Percoll gradient (68% and 75%) (GE Healthcare, Sweden), respectively (Sonck et al., 2010).

2.3. Generation of monocyte-derived macrophages

Monocyte-derived macrophages (MDM) were generated from blood-derived monocytes, which were enriched from the PBMC fraction to a purity of >95% by positive immunomagnetic bead selection (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) using the CD172a-specific monoclonal antibody (clone 74-12-15a). These cells were seeded in a 96-well plate at a density of 2×10^5 cells/well in MDM medium (DMEM (Gibco, Merelbeke, Belgium) supplemented with 10% (v/v) foetal calf serum (FCS, Greiner), 100 IU/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco) and recombinant human M-CSF (R&D Systems; 3 ng/ml)). After 3 days' incubation (37 °C, 90% humidity, 5% CO_2) fresh MDM medium was added to obtain monocyte-derived macrophages at day 7–8.

2.4. β-glucan-induced PBMC proliferation

To examine if dectin-1 is involved in the β-glucan-induced PBMC proliferation in pigs, PBMCs were seeded in a 96-well plate at 5.0×10^5 cells/well (100 μl/well) in leukocyte medium (RPMI-1640 containing FCS (10%), non-essential amino acids (1%), Na-pyruvate (100 μg/ml), L-glutamine (292 μg/ml), penicillin (100 IU/ml), streptomycin (100 μg/ml) and kanamycin (100 μg/ml)) supplemented with β-mercapto-ethanol (50 μM). To block dectin-1, laminarin (1 mg/ml) was added to the cells (1 h). Subsequently, different concentrations of the six β-glucans (800, 200, 50 and 5 μg/ml) were added. As a positive control the cells were treated with 1 μg/ml concanavalin A (ConA, Sigma-Aldrich), while medium served as a negative control. The cells were incubated for 72 h at 37 °C in a humidified atmosphere with 5% CO_2. Next, the cells were pulse-labelled with 1 μCi of [H^3]-methyl-thymidine (Amersham ICN, UK) per well for 18 h at 37 °C and 5% CO_2, harvested onto glass fibre filters and measured incorporated radioactivity with a β scintillation counter (PerkinElmer, Life Science, Belgium). Each stimulation was performed in duplicate and the results are presented as counts per minute (cpm).

2.5. β-glucan receptor inhibitors

To inhibit dectin-1 or CR3, cells were pre-incubated with the inhibitors in the CO_2 incubator at 37 °C before exposure to the β-glucans. To inhibit dectin-1, laminarin was added to the cells. To inhibit CR3-mediated responses, monoclonal antibodies (mAbs; mouse IgG_1 isotypes) against CD18, CD11R1 and CD11R3 (AbD Serotec, Kidlington, UK) were added. These antibodies were dialysed against PBS to remove sodium azide. An isotype-matched mAb control (IgG_1) was added to the cells to measure background inhibition.