Maltose-binding protein is a potential carrier for oral immunizations

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INTRODUCTION

In humans and most animal species such as pigs, vaccination via the oral route is a prerequisite for induction of a protective immunity against enteropathogens. Hereto, live attenuated microorganisms can be used. However, these microorganisms often are either too attenuated to induce sufficient intestinal immunity or are still too virulent resulting in clinical signs. We previously demonstrated that it is possible to induce immunity against enteropathogens by targeting antigen towards enterocytes.

Maltose-binding protein (MBP) is part of the maltose/maltodextrin system of *Escherichia coli.* MBP is a relatively small protein (42.5 kDa) approximately 3 × 4 × 6.5 nm in size with surface residues capable of both hydrogen bonding interactions and hydrophobic interactions. Recombinant proteins are often fused to MPB to improve their yield and to increase their solubility. In mice, these fusion proteins showed an enhanced immunogenicity following systemic immunization. More recently, this has been attributed to interaction of MBP with TLR4 on dendritic cells (DCs). TLR4 is also expressed in the enterocytes of the gut. Therefore, we examined if oral administration of MPB-FedF to 4-week-old pigs could be used to induce an immune response against F18+ verotoxigenic *E. coli* in pigs. Also we examined if the oral administration of MBP to pigs is able to induce an immune response. In both experiments cholera toxin was used as oral adjuvant.

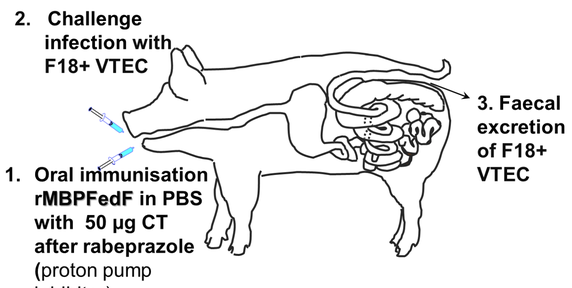
RESULTS

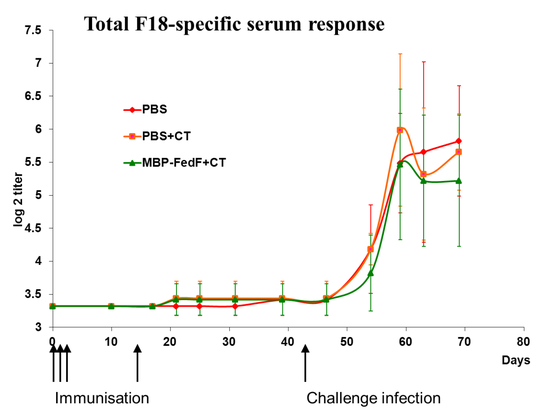
Does MPB-FedF induce a protective immune response against F18+ VTEC ?

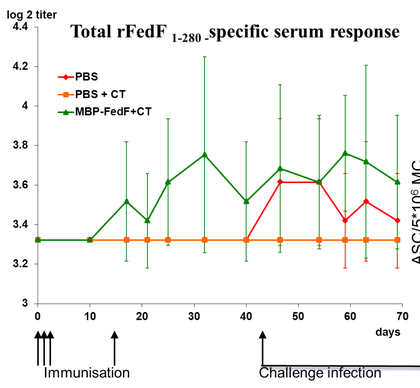
Materials & Methods

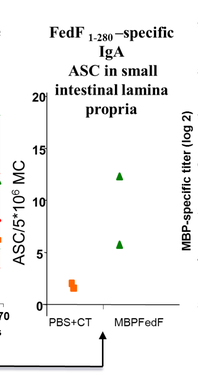
Two weeks after weaning, six pigs were orally immunised with 3 mg MBPFedF in 10 mL PBS. The mucosal adjuvant cholera toxin (CT, Sigma) was used. As negative controls six pigs were orally given 10 mL PBS and five pigs 50 µg CT in 10 mL PBS. Five days following the third immunisation, the local immune response was analysed in two pigs of the MBPFedF and the PBS + CT group. Monomorphonuclear cells (MC) were isolated from a mid-jejunal intestinal segment without Peyer's patches (Lamina propria, LP). The $FedF_{1-280}$- and the MBPFedF-specific IgA antibody secreting cells (ASC) in the LP were enumerated by ELIspot as described by Tiels *et al.* (2008). One week following the third immunisation, the remaining pigs were orally challenged with the F18⁺ *E. coli* strain F107/86 and the faecal excretion of F18⁺ *E. coli* was determined from 2 till 10 days after the first infection as described by Verdonck *et al.* (2004).

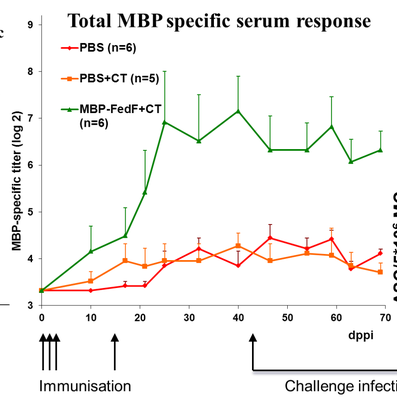
For detection of F18-specific antibodies, the F18 fimbriae were coated directly onto the plates (2 µg/mL in 50 mM sodium bicarbonate pH 9.4). The plates were blocked with PBS + 0.2% Tween® 80. Subsequently, the plates were sequentially incubated with twofold serial dilutions of pig sera, with goat anti-pig HRP (Fitzgerald) and with ABTS.
The FedF-specific serum response was analysed using a direct sandwich ELISA. Briefly, an optimal dilution of rabbit polyclonal antibodies against $FedF_{1-280}$ was coated on the maxisorb plates, 1% BSA was used to block the plates. Thereafter, 5 µg/mL $FedF_{1-280}$ was added, followed by the pig sera and goat anti-pig HRP (Fitzgerald).
For the MBP-specific serum response, MBP (New England Biolabs, Frankfurt, Germany) was coated onto the plates. The following steps were similar to the F18-specific ELISA.

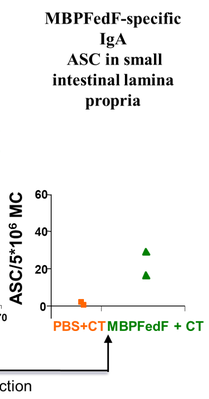


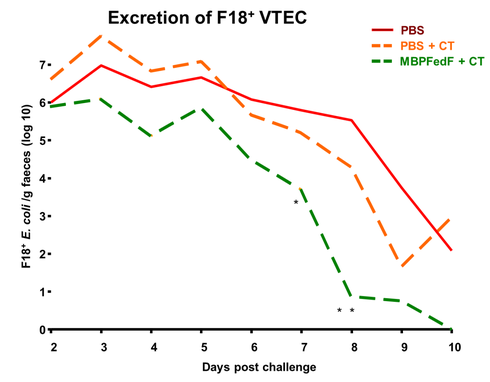












Systemic immune response ↑

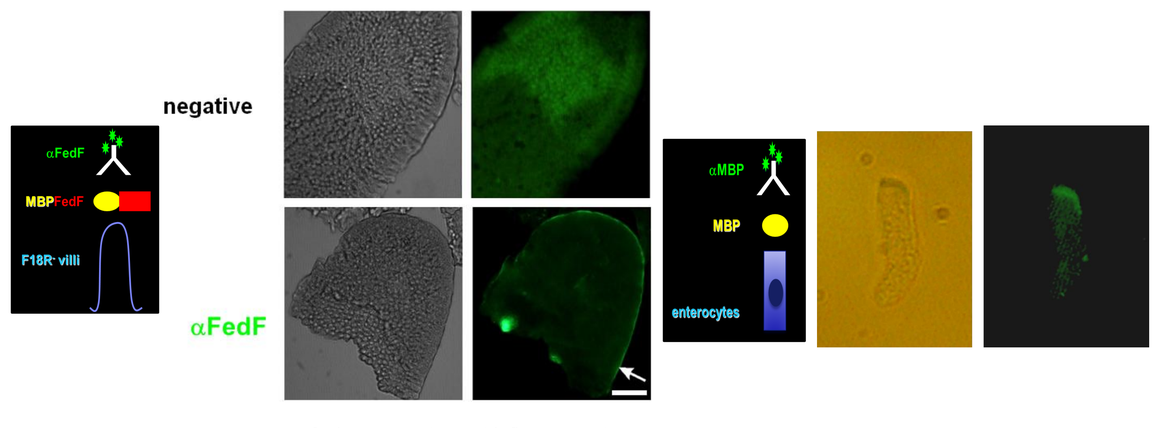
Mucosal immune response ↑

Faecal excretion ↓

Binding of MBP to the intestinal brush border?

Materials & Methods

Porcine F18 receptor (F18R) positive enterocytes were isolated based on the method described by Ramirez *et al.* (2005). Approximately 10^7 cells were centrifuged during 3 min and washed twice with Krebs–Henseleit buffer. Subsequently, the cells were incubated during 1 h with 50 µg/mL MBPFedF or MBP, followed by incubation of the cells with mAb against MBP (New England Biolabs, Frankfurt, Germany) and with goat anti-mouse FITC (Molecular probes). Incubations occurred for 1 h at room temperature and after each incubation two washes were performed with Krebs–Henseleit buffer.

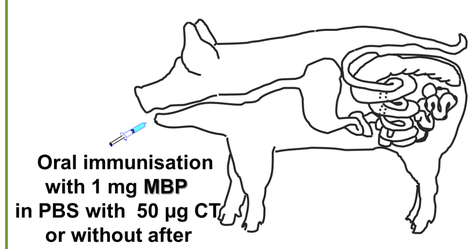


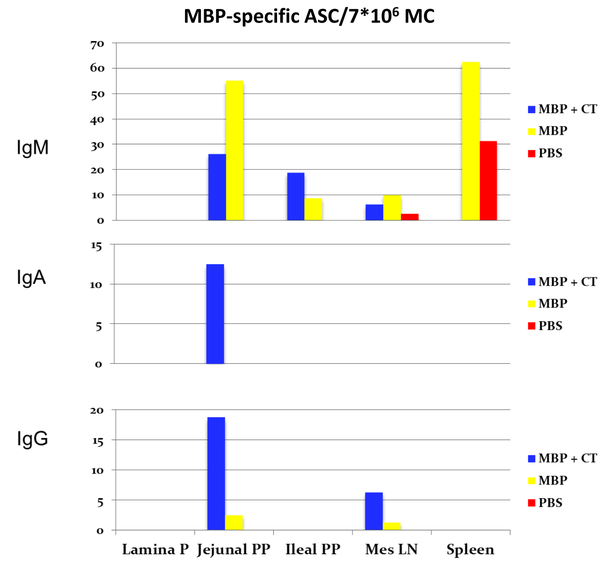
Left: phase contrast view Right fluorescence microscope
Bar = 10 µm.

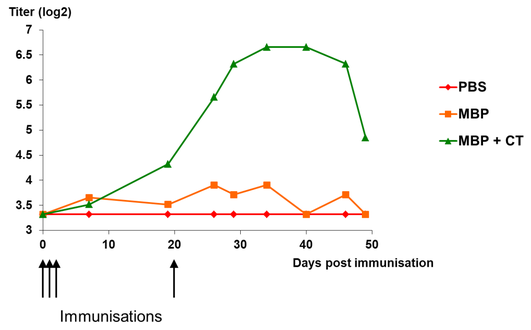
➔ **MBP binds to pig enterocytes**

Does MBP induce an immune reponse?

Materials & Methods
Two weeks after weaning, six pigs were orally immunised with 1.68 mg MBP in 10 mL PBS, three pigs also received CT as adjuvant. As negative controls two pigs were orally given 10 mL PBS. Five days following a fourth immunisation, the local immune response was analysed in one pig of each group.
The MBP-specific serum response MBP was analysed as previous described. The MBP-specific antibody secreting cells (ASC) were enumerated by ELIspot. Briefly cells were collected as described by Verdonck *et al.* 2002. Maxisorb 96-well plates were coated with MBP in PBS. Thereafter, MC suspensions at a concentration of 10^7 cells/mL in leukocyte medium were added to the plates (100 µL/well). Then the plates were incubated for 10 h at 37 °C in a humidified CO_2 atmosphere. PBS + 0.2% Tween® 20 was used to remove the cells from the plates. Subsequently, optimally diluted mouse anti-swine IgM/IgA/IgG mAbs were added to the wells followed by anti-mouse-HRP. The amount of IgM/IgA/IgG -specific ASCs per 5 × 10^6 MC was obtained by counting the spots in 7 wells (10^6 MC/well).







Systemic immune response ↑

Mucosal immune response ↑

CONCLUSION

Results showed an enhanced systemic and mucosal immune response against FedF and a significant decrease in the faecal excretion. It is demonstrated that MBP is able to bind to pig enterocytes and after oral uptake can induce a specific intestinal mucosal immune response. Therefore we suggest that the maltose-binding protein has the potential to be a targeting/carrier molecule for mucosal immunisations in pigs.

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