



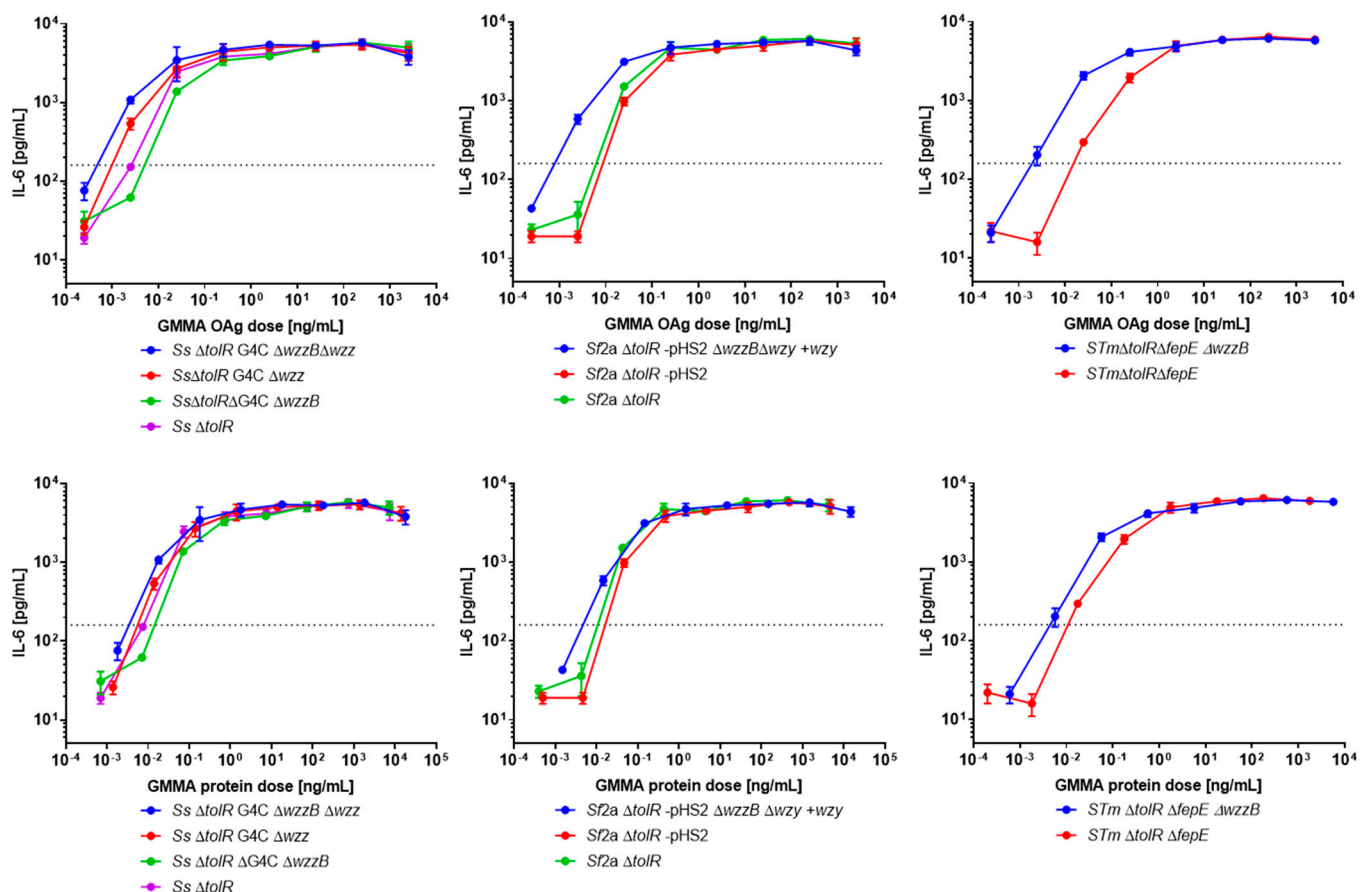
Article

# Effects of Chain Length Regulation of O-Antigen Polysaccharides on the Immunogenicity of Generalized Modules for Membrane Antigens (GMMA)-Based Vaccines

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## Supplementary Materials



**Figure S1.** Monocyte Activation Test (MAT): IL-6 released by human PBMC (Biopredic) after stimulation with each GMMA, normalized based on the OAg dose (upper panels) or the GMMA protein dose (lower panels). The dashed horizontal black line represents a 10-fold increase over background.

**Table S1.** List of bacterial strains and primers used in this study.

Bacterial Strains	
Name	Genotype
<i>S. sonnei</i> wild type	<i>S. sonnei</i> 53G $\Delta$ virG::cm
<i>S. sonnei</i> $\Delta$ tolR	<i>S. sonnei</i> ATCC 25931 $\Delta$ tolR::kan $\Delta$ msbB2::cm
<i>S. sonnei</i> $\Delta$ tolR $\Delta$ G4C $\Delta$ wzzB	<i>S. sonnei</i> ATCC 25931 $\Delta$ tolR::erm $\Delta$ msbB2::cm $\Delta$ ept-etk::tet $\Delta$ wzzB::kan
<i>S. sonnei</i> $\Delta$ tolR $\Delta$ G4C $\Delta$ wzz	<i>S. sonnei</i> ATCC 25931 $\Delta$ tolR::kan $\Delta$ ept-etk::tet $\Delta$ wzz::cm
<i>S. sonnei</i> $\Delta$ tolR $\Delta$ G4C $\Delta$ wzz $\Delta$ wzzB	<i>S. sonnei</i> ATCC 25931 $\Delta$ tolR::erm $\Delta$ ept-etk::tet $\Delta$ wzz::cm $\Delta$ wzzB::kan
<i>S. flexneri</i> 2a wild type	<i>S. flexneri</i> 2a 2457A
<i>S. flexneri</i> 2a $\Delta$ tolR	<i>S. flexneri</i> 2a 2457A $\Delta$ tolR::frt
<i>S. flexneri</i> 2a pHS2-cured $\Delta$ tolR	<i>S. flexneri</i> 2a 2457A $\Delta$ tolR::frt pUC19-pHS2ori
<i>S. flexneri</i> 2a pHS2-cured $\Delta$ tolR $\Delta$ wzzB	<i>S. flexneri</i> 2a 2457A $\Delta$ tolR::frt $\Delta$ wzzB::frt pUC19-pHS2ori
<i>S. flexneri</i> 2a pHS2-cured $\Delta$ tolR $\Delta$ wzzB $\Delta$ wzy	<i>S. flexneri</i> 2a 2457A $\Delta$ tolR::frt $\Delta$ wzzB::frt $\Delta$ wzy::kan pUC19-pHS2ori
<i>S. flexneri</i> 2a pHS2-cured $\Delta$ tolR $\Delta$ wzzB $\Delta$ wzy pACYC-wzy	<i>S. flexneri</i> 2a 2457A $\Delta$ tolR::frt $\Delta$ wzzB::frt $\Delta$ wzy::kan pUC19-pHS2ori pACYC-Duet_wzy
<i>S. Typhimurium</i> wild type	<i>S. enterica</i> serovars Typhimurium isolate D23580
<i>S. Typhimurium</i> $\Delta$ tolR	<i>S. enterica</i> serovars Typhimurium isolate 1418 $\Delta$ tolR::frt
<i>S. Typhimurium</i> $\Delta$ tolR $\Delta$ fepE	<i>S. enterica</i> serovars Typhimurium isolate 1418 $\Delta$ tolR $\Delta$ fepE::cm
<i>S. Typhimurium</i> $\Delta$ tolR $\Delta$ fepE $\Delta$ wzzB	<i>S. enterica</i> serovars Typhimurium isolate 1418 $\Delta$ tolR $\Delta$ fepE::cm $\Delta$ wzzB::kan
Primers	
Name	5'-3' Sequence
<i>Shigella</i> tolR KO Fwd (aph/frt)	ACCGCCAGGCGTTTACCGTTAGCGAGAGCAACAAGGGGTAAGCCATGGCCG TGTAGGCTGGAGCTGCTTC
<i>Shigella</i> tolR KO Rev (aph/frt)	ACCCGCTCTCTTTCAAGCAAGGGAACGCAGATGTTTAGATAGGCTGCGTCA TATGAATATCCTCCTTAG
<i>Shigella</i> tolR KO Fwd (erm)	ACCGCCAGGCGTTTACCGTTAGCGAGAGCAACAAGGGGTAAGCCATGGCCA GAGTGTGTTGATAGTGCAGTATC
<i>Shigella</i> tolR KO Rev (erm)	ACCCGCTCTCTTTCAAGCAAGGGAACGCAGATGTTTAGATAGGCTGCGTAC CTCTTAGCTTCTTGGAAGCT
<i>Salmonella</i> tolR KO Fwd (aph/frt)	CCAGGCGTTTACCGTAAGCGAAAGCAACAAGGGGTAAGTGTAGGCTGGAG CTGCTTC
<i>Salmonella</i> tolR KO Rev (aph/frt)	CCTGTTACTCGCCGCTCTTTCAAGCCAACGGGACGCAGACTCATATGAATATC CTCCTTAG
<i>msbB2</i> KO Fwd (cat)	TAAAATATTAATGATGATTATGGTAGGGGCATTTCGCACTAAATAATGAAAG TGTAGGCTGGAGCTGCTTC
<i>msbB2</i> KO Rev (cat)	ACAACACTAGTGGAATAACCTGTACTTTATAATTTCAAGGGTACGGGTCCGCCA TATGAATATCCTCCTTAG
<i>Shigella</i> wzzB KO Fwd (aph/frt)	AGAGTAGAAAATAATAATGTTTCTGGGCAAACCATGACCCGGAACAGATG TGTAGGCTGGAGCTGCTTC
<i>Shigella</i> wzzB KO Rev (aph/frt)	CTTCGCGTTATAATTACGCAGAGCGTTACGCCCCAGCACAAATCCCCGCGCCA TATGAATATCCTCCTTAG
<i>Salmonella</i> wzzB KO Fwd (aph)	GATTGATTTGATTGAGTTATTGCTACAGTTATGGCGTGGGAAGATGACCAGT GTAGGCTGGAGCTGCTTC
<i>Salmonella</i> wzzB KO Rev (aph)	TCCCGGCACCGATCATCCCACCCAGCAATACAGCCAGCACAAAGGGTAATGC ATATGAATATCCTCCTTAG
<i>Salmonella</i> fepE KO Fwd (cat)	TTCACTGCCGCCCGCCAACAGTCATGAAATCGATTTGTTAGCCTTATAGGT GTAGGCTGGAGCTGCTTC

<i>Salmonella fepE</i> KO Rev ( <i>cat</i> )	CCATCGCGTGACGCAGTAATACGCCGCCGAGGCCATCATACCGCCAATCC <b>ATATGAATATCCTCCTTAG</b>
<i>ept-etk</i> KO Fwd ( <i>tet</i> )	TTACTCTTTCTCGGAGTAACTATAACCGTAATAGTTATAGCCGTAAGTGTAC <b>CTGAAGTCAGCCCC</b>
<i>ept-etk</i> KO Rev ( <i>tet</i> )	AATATCTATCCCGTCACGCCAGGATTGATTGATCAGTTGCGCGCCAAACCT <b>CCAATTCTTGGAGTGGT</b>
<i>wzz</i> KO Fwd ( <i>cat</i> )	TCAAAAGCATCTGAACCACAACAGACCCCTTATCTGATCCCGCAAGGGGTG <b>TCTTGAGCGATTGTGTAGG</b>
<i>wzz</i> KO Rev ( <i>cat</i> )	ATTAACCAAAAAGAGTATTAACATTTTGACCAAATCATCAAGACTTAATGTC <b>CTCCTTAGTTCCTATTCC</b>
<i>wzy</i> KO Fwd ( <i>aph</i> )	TTTTGCTCCAGAAGTGAGGTTATTACTAATTTGGATATTTTCTATAGAAAGTG <b>TAGGCTGGAGCTGCTTC</b>
<i>wzy</i> KO Rev ( <i>aph</i> )	TATTGGTGGTGGTGGAAAGATTACTGGAGCCATTGGGAATATTCCCTTGCCA <b>TATGAATATCCTCCTTAG</b>
pHS2-Ori Fwd XbaI	TTGAGATCATTCCCTTACAGCCG
pHS2-Ori Rev XmaI	CTGAACGCGATTCTGCCG
<i>wzy</i> Fwd BamHI	<b>CGCGGATCCACGGCTTGGTTTGGTGAGAA</b>
<i>wzy</i> Rev XhoI	<b>CCGCTCGAGCTTCGGCCTTGACCAAAGC</b>