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Supplemental Information

Outer membrane vesicles as a platform for the discovery of antibodies to bacterial pathogens

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Supplemental Table S1 Polyclonal antibody responses generated by immunization with bacterial OMVs

Bacteria and OMV source(s)	**Organism(s) immunized**	**Immunization method(s)**	**pAb response(s) and target(s), if identified**	**Reference(s)**
Acinetobacter baumannii ATCC 19606	C57BL/6N mouse	s.c, 0.2 or 2 µg OMVs with 2 mg/mL alum adjuvant at 0, 2 and 4 weeks	Western blot evidence of IgG to OmpP1/FadL/ TodX family protein, OprB, OmpA, YbgF, PBP1b, CarO, putative exported protein, Omp25, Omp22 and OmpW	Huang et al. (2019)
Acinetobacter baumannii ATCC 19606	C57BL/6 mouse	i.m., 5 µg of OMVs with aluminum phosphate adjuvant on days 0 and 14	Measured IgG1 and IgG2 against *A. baumannii* OmpA, CarO, OmpW	McConnell et al. (2011)
Bacillus anthracis Sterne 34F2	BALB/c mouse	i.p., day 0 with FCA adjuvant and day 14 with FIA	Measured IgM against PA, LF, EF, ALO	Rivera et al. (2010)
Bacteroides thetaiotaomicron expressing chimeric *Yersinia pestis* LcrV or F1 fused to *B. thetaiotaomicron* OmpA leader	Rhesus macaque	i.n., with 12.5-50 µg of LcrV and F1 containing OMVs, or oral with 50 µg of OMVs on days 0 and 28	Detected IgG against F1, IgG and IgA against LcrV	Carvalho et al. (2019b)
Bacteroides thetaiotaomicron expressing chimeric *Salmonella enterica* ser. *Typhimurium* SL 1334 SseB or OmpA fused to *B. thetaiotaomicron* OmpA leader	C57BL/6 mouse	Oral or i.p. with 70 µg of OMV at months 0, 1 and 2, or i.n. with 70 µg of OMV at days 0, 14 and 21	Measured IgG and IgA titers against SseB and OmpA	Carvalho et al. (2019a)

Bordetella bronchiseptica 9.73	BALB/c mouse	s.c., on day 0 and 14	Detected IgG against *B. bronchiseptica* O-Ag and lipid A-Kdo	Bottero et al. (2018)
Bordetella pertussis B1917	BALB/c mouse	i.n. or s.c., 1-4 µg of OMV on days 0 and 28	Detected IgG, IgM and IgA against OMVs, *B. pertussis* B1917 cells, LPS, BrkA, GroEL, Fim2, Fim3, FHA, PRN, PT and Vag8 by western blot + mass spectrometry	Kanojia et al. (2018); Raeven et al. (2016); Raeven et al. (2018); Raeven et al. (2015); Raeven et al. (2020)
Bordetella pertussis W28 9K/129G	BALB/c mouse	i.p., 2.5 µg of OMV with aluminum hydroxide at weeks 0, 4 and 8	Detected IgG binding to recombinant BrkA, Vag8 and BipA expressed on *Escherichia coli* cells	Gasperini et al. (2018)
Burkholderia pseudomallei 1026b	BALB/c mouse	s.c., 5 µg of OMV at days 0, 21 and 42	Detected IgM, IgG1, IgG2a and IgG3 against OMV, LPS and CPS	Nieves et al. (2014)
Burkholderia pseudomallei 1026b	Rhesus macaque	s.c., 25 µg, 50 µg, and 100 µg of OMV with 400 µg of CpG adjuvant on days 0, 28 and 56, respectively	Measured IgG against LPS and CPS	Petersen et al. (2014)
Enterotoxigenic *Escherichia coli*	CD1 mouse	i.n., 20 µg OMV on days 0, 14 and 28	Detected IgG against recombinant EtpA, CexE and LT on western blot	Roy et al. (2011)
Enterotoxigenic *Escherichia coli,* chitosan encapsulated	BALB/c mouse	s.c., 10 µg OMV or oral, 50 µg	Measured IgG against LT subunit B	Noroozi et al. (2018)
Escherichia coli O1, O2 and O78	Arbor Acres broiler chicken	i.m., 10, 50 and 100 µg of OMVs on days 0, 7 and 14	Detected IgG against OMVs and LPS	Hu et al. (2020)

Escherichia coli O78	Lohmann chicken	i.m., 75 µg OMVs at days 0 and 14	Detected IgG against LPS	Wang et al. (2019)
Escherichia coli BL21(DE3) expressing chimeric *Chlamydia muridarum* HtrA fused to *E. coli* OmpA leader	BALB/c mouse	i.m., 50 µg OMV with aluminum hydroxide at weeks 0, 2 and 4	Detected IgG against HtrA-OMV and recombinant HtrA, confirmed IgG binding to *C. muridarum* elementary bodies	Bartolini et al. (2013)
Escherichia coli BL21(DE3) expressing chimeric *Streptococcus pyogenes* SLO, Bla, SpyCEP, Spy0269 and SAM_1372 fused to *E. coli* OmpA leader	CD1 mouse	i.p., 25 µg of OMV on days 0, 21 and 35	Measured IgG1 and IgG2 against *S. pyogenes* SLO, SpyCEP and SAM_1372	Fantappie et al. (2014)
Escherichia coli BL21(DE3)Δ60 expressing chimeric *Staphylococcus aureus* FhuD2- HLA_{H35L}, $ClfA_{Y338A}$- LukE and SpA_{KKAA}-HLA_{H35L} fused to an *E. coli* Lpp leader	CD1 mouse	i.p. and i.m., 20 µg of OMV with alum on days 0, 14 and 28	Detected IgG against *S. aureus* FhuD2, LukE, SpA and HLA	Konig et al. (2021)
Escherichia coli CLM37 displaying *Streptococcus pneumoniae* CPS serotype 14 or *Campylobacter jejuni* heptapolysaccharide *N*-glycan	BALB/c mouse for OMVs displaying *S. pneumoniae* CPS Leghorn chicken for OMVs displaying *C. jejuni* heptapolysaccharide *N*-glycan	Mice: i.p., 2 µg of OMV at days 0, 14 and 21 Chickens: oral, 0.5 µg of OMV days 0 and 14	Detected mouse IgG against whole cell *S. pneumoniae* serotype 14 and chicken serum IgY against BSA conjugated to *C. jejuni* heptapolysaccharide *N*-glycan	Price et al. (2016)

Escherichia coli DH5α expressing chimeric *Acinetobacter baumannii* ATCC 17978 Omp22 fused to *E. coli* W-15 ClyA	ICR mouse	s.c., 5-50 µg of Omp22-ClyA containing OMV or wtOMV on days 0 and 14	Measured IgG against OMVs and Omp22	Huang et al. (2016)
Escherichia coli JC8031 displaying *Francisella tularensis* subsp. *holarctica* O-Ag	BALB/c mouse	i.p., 10 µg OMV on days 0, 14 and 28	Detected IgG against *F. tularensis* LPS	Chen et al. (2016)
Escherichia coli JC8031 displaying *E. coli* PNAG, deacetylated PNAG	BALB/c mouse	s.c., 10 µg OMV on weeks 0, 3 and 6	Detected IgG and IgM against PNAG	Stevenson et al. (2018)
Escherichia coli K12 expressing *E. coli* MC001 LpxR	BALB/c mouse	i.p., 10 µg OMV with alum on day 0, 5 µg OMV on days 20 and 34	Detected IgG against *E. coli* MC001 LpxR	Rojas-Lopez et al. (2019)
Escherichia coli KPM404 expressing chimeric *E. coli* Lpp-OmpA-enhanced monoavidin; OMVs conjugated to GFP or *Chlamydia muridarum* MOMP	BALB/c mouse	s.c., 20 µg of OMV on days 0, 21 and 42	Detected IgG against GFP and MOMP	Weyant et al. (2023)
Escherichia coli MG1655 expressing chimeric *E. coli* $OmpA_{1-155}$-SpyCatcher; OMVs conjugated to chimeric *Staphylococcus aureus* SpyTag-EsxA, SpyTag-Sbi or SpyTag-SpA	BALB/c mouse	s.c., 50 µg of OMV conjugate on days 0, 14 and 28	Detected IgG against *S. aureus* EsxA, Sbi and SpA	Sun et al. (2023)
Escherichia coli Nissle 1917 displaying serotype	BALB/c mouse	s.c., 1 µg OMV on days 0 and 21	Detected IgG and IgM against serotype 14 CPS	Nakao et al. (2022)

14 *Streptococcus pneumoniae* CPS				
Haemophilus influenzae 019-R	BALB/c mouse	i.n. or i.p., 2-25 µg OMV on days 0, 14 and 28	Detected IgG against Hup, BamA, HbpA, OmpP1, OmpP2, OmpP5 and OmpP6	Roier et al. (2012)
Helicobacter pylori 60190	BALB/c mouse	Oral, 50 µg OMV with CTB adjuvant on days 0, 7, 14 and 21	Detected IgG against Lpp20	Keenan et al. (2000)
Mannheimia haemolytica 89010807N	BALB/c mouse Holstein calf	s.c., 10-50 µg OMV with or without FIA on days 0 and 14 for mice, 150 µg OMV with FIA for calves	Detected IgG against LKT in both animals	Ayalew et al. (2013)
Mycobacterium bovis BCG	BALB/c mouse	i.p., 50 mg proteoliposome with FIA at days 0 and 21	Detected IgG against BCG whole cells, *M. bovis* cell wall fractions, soluble cell wall proteins, LAM, Acr, Ag85B, Mce1A, HBHA and RplL (L7/L12)	Reyes et al. (2013)
Mycobacterium bovis BCG *Mycobacterium tuberculosis* H37Rv	C57BL/6 mouse	s.c., 2.5 µg of either OMV on days 0 and 21	Detected IgG and IgM to *M. tuberculosis* H37Rv protein extracts, identified LpqH, LppX, VapC6 and PstS1	Prados-Rosales et al. (2014)
Neisseria flavescens 2830 expressing *Neisseria meningitidis* NspA	NIH mouse	s.c., 10 µg of OMV on days 0, 20 and 27	Detected IgG against NspA	O'Dwyer et al. (2004)
Neisseria lactamica NIP3 and NIP2 *Neisseria sicca* NsP1 and NsP3	BALB/c mouse	i.p., 20 µg OMV with FCA on day 0, FIA on day 14 and no adjuvant on day 28	Detected IgG against RmpM	Troncoso et al. (2001)

Neisseria meningitidis NZ98/254 *Escherichia coli* BL21(DE3) expressing chimeric *N. meningitidis* PorA, Opc, NspA, PilQ, OmpH, Omp85, MafA, OmpP1, LbpA, or FrpB fused to *E. coli* OmpA leader	CD1 mouse	i.p., 8 µg of OMV with aluminum hydroxide adjuvant on days 0, 21 and 35	Detected IgG against PorA, Opc, NspA, PilQ, OmpH, Omp85, MafA, OmpP1, LbpA and FrpB	Viviani et al. (2023)
Neisseria meningitidis NZ98/254	Human	1 dose of 4CMenB on days 0 and 28	Detected IgG against NHBA	Perrett et al. (2015)
Neisseria meningitidis (multiple strains)	CD1 mouse	i.p., 1.25-5 µg OMV with alum adjuvant on days 0, 21 and 42	Detected IgG and IgM against GNA1870 fHbp	Hou et al. (2005); Koeberling et al. (2007)
Neisseria meningitidis (multiple strains)	OF-1 mouse	i.m., 10 µg of OMV with 100 µg of aluminum hydroxide on days 0, 21 and 28	Detected IgG against NhhA derived from strain H44/76	Peak et al. (2013)
Neisseria meningitidis (multiple strains)	BALB/c and CD-1 mice Rhesus macaque	i.p., 2.5 µg of OMV with alum adjuvant on days 0 and 21 for BALB/c mice i.p., 2-5 µg of OMV with alum adjuvant on days 0, 21 and 42 for CD-1 mice i.m., 25 µg of OMV with alum adjuvant on days 0 and 42 for macaques	Detected IgG in all immunized animals against NadA, fHbp v1 and v2, and LOS	Beernink et al. (2019a); Beernink et al. (2012); Beernink et al. (2019b); Granoff et al. (2015); Koeberling et al. (2011a); Koeberling et

				al. (2009); Koeberling et al. (2008); Koeberling et al. (2011b); Pajon et al. (2013)
Neisseria meningitidis 44/76	OF1 mouse	i.m., 5 µg OMV on days 0, 21 and 28	Detected IgG against LOS, specifically the LNnT epitope	Weynants et al. (2009)
Neisseria meningitidis 44/76	NIH/RIVM mouse	s.c., 0.33-10 µg of OMV on days 0 and 28	Detected IgG against PorA, Rmp, Opa/Opc and a 20 kDa OMP	Peeters et al. (1999)
Neisseria meningitidis 44/76	BALB/c mouse	i.n. or rectal, 25-250 µg OMV with CTB or FIA adjuvant on days 0, 7, 14 and 21	Detected IgG and IgA against PorA, PorB, RmpM and Opc	Dalseg et al. (1999)
Neisseria meningitidis 44/76	NIH/OlaHSD mouse	s.c., 2.5 µg of OMV on days 0 and 28	Detected IgG against PorA	Nagaputra et al. (2014)
Neisseria meningitidis 44/76	Human	i.m., 25 µg of OMV with alum on months 0, 2 and 4	Detected IgG against PorA, PorB, Opc and Opa	Wedege et al. (2003)
Neisseria meningitidis 44/76	Human	i.n., 250 µg OMV on days 0, 7, 14 and 21	Detected IgG and IgA against LPS, PorA, PorB, RmpM and Opc	Haneberg et al. (1998)
Neisseria meningitidis 44/76, 8570 and B16B6	CD1 mouse Rhesus macaque	i.m., 1-3 µg OMV on days 0 and 14 for mice, 12-37.5 µg OMV on days 0, 56 and 112 for macaques	Detected IgG against fHbp v1, fHbp v2 and PorA in mice and rhesus macaques	Zhang et al. (2016)
Neisseria meningitidis 44/76-SL or NZ98/254	Human	i.m., 25-50 µg of OMV from NZ98/254 or 25 µg	Detected IgG against Omp85, FetA, PorA, PorB, FbpA, RmpM, Opc, NspA and LPS	Wedege et al. (2007)

		of OMV from 44/76-SL on days 0, 42 and 84		
Neisseria meningitidis 8750	Human	i.m., 25-75 µg of OMV at days 0, 42 and 84	Detected IgG against OMV, Opc, fHbp and LPS	Keiser et al. (2011)
Neisseria meningitidis 9162	New Zealand White rabbit	i.m. or oral, 300 µg of OMV on day 0	Detected IgG against LOS	Shoemaker et al. (2005)
Neisseria meningitidis 99M, M986 OMVs conjugated to CPS of group B *N. meningitidis*	Rhesus macaque	i.m., 5 µg of OMV conjugate in MPL+TDM on days 0, 42 and 98	Detected IgG and IgM against CPS of group B *N. meningitidis*	Devi et al. (1997)
Neisseria meningitidis B:4:P1.9, B:8:P1.6	BALB/c, A/Sn mouse	s.c., 2 µg of OMV on days 0, 20 and 35	Detected IgG against PorA and Opa	Trzewikoswki de Lima et al. (2020)
Neisseria meningitidis CH501, Cu385 or H355	New Zealand White rabbit	i.m., 25 µg OMV on months 0, 1 and either 2 or 6	Detected IgGs against PorB by western blot	Bash et al. (2000)
Neisseria meningitidis F91	BALB/c mouse	s.c., 10 µg of OMVs measured on days 0, 14 and 28	Detected IgG against PorA	Arigita et al. (2003)
Neisseria meningitidis H44/76 SMenPF1.2	NIH/OlaHsd mouse New Zealand White rabbit	For mice, s.c., 1 to 10 µg of OMVs with alum adjuvant on days 0, 21, 42 and 63 For rabbits, s.c., 25 µg of OMVs with alum adjuvant on days 0 and 21	Detected mouse and rabbit IgG against OMVs, PorA and FetA	Sanders et al. (2015)
Neisseria meningitidis H44/76 ST32 complex	Human	i.m., 25 to 50 µg of OMV on day 0, 21 and 42	Detected IgG against several *N. meningitidis* proteins *via* 2D western blot + mass spectrometry	Williams et al. (2014)

Neisseria meningitidis H44/76Lpxl1	C57BL/6 mouse	i.m., 2.5 or 5 µg of OMVs with alum adjuvant on days 0 and 28	Detected mouse IgG against OMVs, *N. meningitidis* cells, fHbp and PorA	Daniels-Treffandier et al. (2016)
Neisseria meningitidis HB-1 expressing chimeric *Borrelia burgdorferi 297* OspA fused to truncated forms of *N. meningitidis* fHbp	BALB/cOlaHsd mouse	s.c., 1 or 4 µg OMVs at days 0 and 28	Detected IgG against *B. burgdorferi* OspA	Salverda et al. (2016)
Neisseria meningitidis M986	C57BL/6 mouse	i.p., 75 µg of LOS in OMVs on days 0, 1 and 2	Detected IgG against LOS	Quakyi et al. (1999)
Neisseria meningitidis M986 NCV-1 serotype 2a	Human	50 ug of OMV on days 0 and 28	Detected IgG, IgA and IgM against PorA and several unidentified proteins by western blot	Wedege and Froholm (1986)
Neisseria meningitidis MC58	BALB/c mouse	i.p. or s.c. on days 0, 21 and 42	Detected cross reactive IgG against *Neisseria gonorrhoeae* PilQ, BamA, MtrE, NHBA, PorB and Opa by western blot + mass spectrometry	Leduc et al. (2020)
Neisseria meningitidis MC58	BALB/cAnNCr mouse	i.p., 12.5 µg OMV with alum on days 0, 14 and 28	Detected IgG against PorA, PorB, RmpM and LOS	Matthias et al. (2020)
Neisseria meningitidis MC58	BALB/cAnNCr mouse	i.p., 12.5 µg OMV with alum on days 0, 14 and 28	Detected IgG against PorB, Rmp, PilQ, MtrE, GroL, AceF, GuaB and NlpD by co-immunoprecipitation and mass spectrometry	Matthias et al. (2022)
Neisseria meningitidis Mk 83/94, F8238, Mk 686/02, Mk 760/02, Mk196/02 and Mk222/02	BomTac:NMRI mouse	s.c., 1-10 µg of OMV on days 0 and 21	Detected IgG against *N. meningitidis* F8238 OMVs, Mk 686/02 live cells, NspA by ELISA, PorA, PorB, NadA, RmpM, Opc, Opa and LOS	Norheim et al. (2005); Norheim et al. (2004);

				Norheim et al. (2012)
Neisseria meningitidis Mk499/03 and Mk222/02	BALB/c mouse	s.c., 2.5 µg of each strain of OMV with 5 µg PSA, adsorbed to alum adjuvant on day 0 and 21	Detected IgG against *N. meningitidis* CPS	Romeu et al. (2014)
Neisseria meningitidis serogroup B conjugated to fHbp variant 3	C57BL/6 and CD1 mice	i.m. or i.p., 0.625-100 µg OMV with or without alum adjuvant on days 0, 21 and 35	Detected IgG1, IgG2a, IgG2b and IgG3 against fHbp	Alfini et al. (2022); Piccioli et al. (2023)
Neisseria meningitidis serogroup B expressing chimeric *Borrelia burgdorferi* B31 OspA fused to truncated *N. meningitidis* fHbp	C3H/HeN mouse	40 µg of OMVs at days 0, 14 and 28	Measured IgG1 and IgG2a against *B. burgdorferi* OspA	Klouwens et al. (2021)
Neisseria meningitidis serogroup B NZ98/254	Human	i.m., 25-50 µg of OMV at weeks 0, 6 and 12	Detected IgG against OMV antigens by western blot, validated binding to PorA by mutational analysis	Martin et al. (2006)
Neisseria meningitidis serogroup W	CD-1 mouse	i.p., 0.2 to 5 µg of OMV with alum adjuvant on days 0, 14 and 28 or days 0 and 28	Detected IgG against fHbp	Koeberling et al. (2014); Marini et al. (2017)
Neisseria meningitidis H44/76 CE2001	OF-1 mouse	i.m., 5 µg OMV with alum adjuvant on days 0, 21 and 35	Detected IgG against LbpA	Pettersson et al. (2006)
Neisseria meningitidis N44/89 conjugated to *N. meningitidis* serogroup C CPS	C3H/HePas mouse	i.p., 2.5 µg of CPS on OMVs with alum adjuvant on days 0, 14 and 28	Detected IgG against serogroup C CPS and PorA by western blot	Fukasawa et al. (1999)

Pasteurella multocida P4881 *Mannheimia haemolytica* SH789	BALB/c mouse	i.n., 25 µg OMV on days 0, 14 and 28	Detected IgA and IgG against OMVs from *P. multocida* and *M. haemolytica*. Immunoprecipitation using pooled sera from *P. multocida* OMVs identified outer membrane proteins OmpA, OmpH and P6. Pooled sera from *M. haemolytica* OMVs identified SSA-1, TbpA, OmpD15, OmpP2 and OmpA	Roier et al. (2013)
Porphyromonas gingivalis ATCC 33277	BALB/c mouse	i.n.,1 µg of OMVs and 10 µg of poly(I:C) on weeks 0 and 3	Detected IgG against *P. gingivalis* LPS and whole cells	Bai et al. (2015)
Pseudomonas aeruginosa PAO1	BALB/c mouse	i.m., 30 µg of OMV and 30 µg aluminum phosphate adjuvant on days 0, 14 and 21	Detected IgG1 and IgG2a against *P. aeruginosa* PAO1 OMVs, cell lysates and FliC	Zhang et al. (2018)
Salmonella enterica ser. Paratyphi A NVGH308 displaying *Salmonella Typhi* Vi antigen	C57BL/6 mouse	s.c., OMV containing ~5 µg of O-Ag and ~5 µg of Vi antigen at days 0 and 28	Detected IgG against Vi antigen as well as *S. enterica* Paratyphi A O:2 and O9 O-Ags	Gasperini et al. (2021a)
Salmonella enterica ser. Typhimurium 1418 *ΔtolR* conjugated to *Neisseria meningitidis* MenB fHbp, MenC or MenA oligosaccharides *N. meningitidis* Men B ΔsynX, Δctra, Δgna33, ΔlpxL1 conjugated to MenC or MenA oligosaccharides, *Haemophilus influenzae* type b oligosaccharides	CD1 mouse	s.c. or i.m., OMV conjugates with aluminum hydroxide on days 0 and 28 *S. enterica* OMVs: 0.75 µg fHbp, 1 µg MenC and MenA oligosaccharides *N. meningitidis* OMVs: 1 µg MenC, MenB, or *H. influenzae* type b oligosaccharides	Detected IgG against *Salmonella* O-Ag, *N. meningitidis* fHbp, MenA, MenC, MenC polysaccharide, *H. influenzae* polysaccharide, *E. coli* FdeC and SslE, and *S. sonnei* LPS	Micoli et al. (2020)

Shigella sonnei ΔtolR ΔvirG ΔhtrB 53G conjugated to *Escherichia coli* SslE and/or FdeC		*S. sonnei* OMVs: 5 µg FdeC, 5 µg SslE, or 5 µg FdeC + SslE		
Salmonella enterica ser. Typhimurium SGSC1418, *S. enterica* ser. Enteritidis SA618, genetically modified for hypervesiculation (ΔtolR) and to reduce LPS reactogenicity (ΔmsbB, ΔhtrB and ΔpagP)	CD1 mouse	s.c., OMV containing 1 µg of O-Ag with Alhydrogel adjuvant on days 0 and 28	Measured IgG against O-Ag	De Benedetto et al. (2017)
Salmonella enterica ser. Typhimurium SL3261 ΔtolRA expressing chimeric truncated *Escherichia coli* HbpD fused to truncated *Streptococcus pneumoniae* PspA or PLY	BALB/c mouse	i.n., 4 µg of OMV in 1-3 doses at days 0, 14 and 28	Detected IgG and IgA against *S. pneumoniae* PspA and PLY	Kuipers et al. (2015)
Salmonella enterica ser. Typhimurium χ9281 expressing chimeric *Streptococcus pneumoniae* PsP fused to a beta-lactamase signal sequence	BALB/c mouse	i.n., 50 µg of OMV at weeks 0, 1, 2 and 3	Detected IgG against OMVs, *Salmonella* LPS, *Salmonella* outer membrane protein extracts and *S. pneumoniae* PspA	Muralinath et al. (2011)
Salmonella enterica Enteritidis χ3744	BALB/c mouse	i.n. with 20 µg OMV or i.p. with 5 µg on days 0 and 30	Measured IgG and IgA against OMVs and LPS	Liu et al. (2017)

Salmonella enterica var. Typhimurium SL1418 ΔtolR	C57BL/6 mouse	i.p., 1 µg of OMV	Detected IgG and IgM against OMVs, LPS, OmpC, OmpF and OmpD	Schager et al. (2018)
Salmonella enterica Typhi C-6953 *S. enterica* Paratyphi A C-6915	BALB/c mouse	Oral, 25 µg of OMVs on days 0, 14 and 28	Detected IgG, IgA and IgM against OMVs, measured IgG against LPS	Howlader et al. (2018)
Salmonella enterica Typhimurium 14028	C57BL/6 mouse	i.m., 50 µg OMV on day 0, 5 µg OMV on days 21 and 42	Detected IgG against CPS	Sokaribo et al. (2021)
Salmonella enterica Typhimurium 1418 conjugated to *Streptococcus pyogenes* GAC	CD1 mouse New Zealand White rabbit	i.p., on days 0 and 28, or s.c., on days 0 and 22 for mice i.m., on days 0, 21 and 35 for rabbits	Detected IgG against *S. pyogenes* GAC in mice and rabbits	Palmieri et al. (2022)
Salmonella enterica Typhimurium NVGH2363	C57BL/6 mouse	s.c., 10 µg O-Ag in OMV with alum on days 0 and 70	Detected IgG and IgA against O-Ag (O:4,5)	Fiorino et al. (2021)
Salmonella enterica Typhimurium SGSC1418 expressing chimeric *Neisseria meningitidis* fHbp fused to *Escherichia coli* OmpA leader	CD1 mouse	s.c., with alum on days 0 and 28	Detected IgG against *S. enterica* Typhimurium O-Ag and *N. meningitidis* fHbp	Necchi et al. (2021)
Salmonella enterica Typhimurium χ3761 and χ9241 expressing chimeric *Streptococcus suis* SaoA	BALB/c mouse	i.p., 10 µg OMV on days 0 and 21	Detected IgG against *S. suis* SaoA	Li et al. (2023)

fused to *Escherichia coli* Lpp leader				
Salmonella enterica Typhimurium 2189 *S. enterica* Enteritidis 618	C57BL/6 mouse	s.c., 1 ng to 10 µg with alum on days 0 and 28	Detected IgG against LPS O-Ag	Micoli et al. (2018)
Salmonella enterica var. Typhimurium χ3761 (various LPS mutants)	BALB/c mouse	i.n., 20 µg of OMV or i.p., 5 µg OMV on days 0 and 21	Detected IgG and IgA against OMVs, as well as IgG against FliC	Liu et al. (2016)
Shigella boydii BCH612	Swiss webster mouse	Oral, 32 µg OMV on days 0, 7, 14 and 21	Detected IgG against IpaB, IpaC and IpaD	Mitra et al. (2012)
Shigella boydii BCH612 *Shigella dysenteriae* NT4907 *Shigella flexneri* B294, C519 and C347 *Shigella sonnei* IDH00968	Swiss albino mouse	Oral, 50 µg of mixed OMVs on days 0, 7, 14 and 21	Detected IgG against OMVs, putatively identified VirG, IpaB, IpaC, IpaD and OmpA using western blot	Mitra et al. (2013)
Shigella flexneri *Shigella sonnei* (Multispecies)	CD1 mouse	s.c. or i.p., 1-500 ng of O-Ag in OMVs on days 0 and 28	Detected IgG against LPS and O-Ag	Arato et al. (2021); Mancini et al. (2023); Raso et al. (2020); Richardson et al. (2021)
Shigella flexneri 2a 2457A *Shigella sonnei* ATCC 25931	CD1 mouse	s.c., 500 ng of O-Ag in OMVs on days 0 and 28	Detected IgG against LPS and O-Ag for *Shigella* and *Salmonella*	Gasperini et al. (2021b)

Salmonella Typhimurium 1418				
Shigella sonnei 53G *Salmonella Typhimurium* 2192	C3H, BALB/c, and C57BL/6 mice	i.m., 100 ng of O-Ag in OMVs on days 0 and 28	Detected IgGs against LPS and O-Ag for *Shigella* and *Salmonella*	Piccioli et al. (2022)
Shigella sonnei 53G	CD1 mouse	i.n., 10 µg of OMV on days 0 and 28	Detected IgG against *S. sonnei* LPS, co-immunoprecipitated OmpA, OmpC, BamA, BamB, BamC and BamD	Mancini et al. (2021)
Shigella sonnei 53G NVGH1790 genetically modified by deletion of the late acyltransferase genes htrB and integration of *Escherichia coli* nadA and nadB genes, resulting in expression of penta-acylated LPS (O-Ag)	BALB/c mouse New Zealand White rabbit	i.p., 29-238 µg of OMV with 2% Alhydrogel on days 0 and 21 for mice i.m., with 100 µg, i.d., with 10 µg, or i.n., with 80 µg of OMV - Alhydrogel at weeks 0, 2, 4 and 6 for rabbits	Detected IgG against *S. sonnei* LPS in both animals	Gerke et al. (2015)
Shigella sonnei 53G NVGH1790 genetically modified by deletion of the late acyltransferase genes htrB and integration of *Escherichia coli* nadA and nadB genes, resulting in expression of penta-acylated LPS (O-Ag)	Human	1-100 µg OMV with alum on days 0, 28 and for some day 56	Detected IgG against LPS	Frenck et al. (2021); Launay et al. (2017); Launay et al. (2019); Micoli et al. (2021); Obiero et al. (2017)
Shigella sonnei 53G *Shigella flexneri* 2a 2457T, 1b NCTC5 and 3a NCTC6885	CD1 mouse	i.p., 2.3-600 ng O-Ag in OMVs on day 0	Detected IgG against *S. sonnei* LPS and *S. flexneri* O-Ag	Necchi et al. (2023)

Staphylococcus aureus JE2	Swiss webster mouse	s.c., 5 µg of OMV on days 0, 14 and 28	Detected IgG against HLA and LukE	Wang et al. (2018)
Streptococcus mutans UA159	BALB/c mouse	i.n., 0.5 or 2.5 µg OMVs with 5 µg poly(I:C) adjuvant	Detected IgG and IgA against OMVs, partial validation of serum against GtfC	Nakamura et al. (2020)
Vibrio cholerae O1 Ogawa and O1 Inaba	BALB/c mouse	i.n., 25 µg of OMV on days 0, 14 and 18	Detected IgG1 against O1 Ogawa + Inaba OMVs, IgG1 and IgA against LPS	Bishop et al. (2010)
Vibrio cholerae AC53 expressing *Escherichia coli* H10407-S CfaB or chimeric FlaA-FliC fusion Enterotoxigenic *E. coli* H10407-S	BALB/c mouse	i.n., 25 µg of a single OMV or 12.5 µg of each of two OMV types combined on days 0, 14 and 28	Detected IgG, IgM and IgA responses against *V. cholerae* LPS as well as recombinant *E. coli* FliC and CfaB in animals immunized with *V. cholerae* OMV, and *E. coli* LPS in animals immunized with *E. coli* H10407-S OMV	Leitner et al. (2015)
Vibrio cholerae C7258, El Tor Ogawa C7258	BALB/c mouse	i.n., OMVs containing 25 µg of LPS at days 0 and 28	Detected IgG against Ogawa LPS	Perez et al. (2009)
Vibrio cholerae Inaba O1 ATCC 39315, chitosan encapsulated	BALB/c mouse	i.d., 10 µg of OMV on day 0 or orally, 50 µg of OMV on day 0, i.p., 10 µg of OMV on days 10, 20 and 30	Detected IgG against OMVs, LPS and CTB, detected sIgA against OMV	Adriani et al. (2018)
Vibrio cholerae O1	BALB/c mouse	i.n., 25 µg of OMVs on days 0, 14 and 28	Detected IgG to OMVs, LPS and TcpA	Leitner et al. (2013)
Vibrio cholerae AC53 expressing *Escherichia coli* PhoA	BALB/c mouse	i.n., 0.0025 - 25 µg of OMV at days 0, 14 and 28	Detected IgG against *E. coli* PhoA and OMVs	Schild et al. (2009)
Yersinia pseudotuberculosis YptbS44 *expressing*	BALB/c mouse	i.m., 50 µg OMV on days 0 and 21	Detected IgG and IgM against *P. aeruginosa* PcrV-HitA fusion antigen	Li et al. (2021)

chimeric Pseudomonas aeruginosa PcrV-HitA fused to a beta-lactamase signal sequence				

Abbreviations: ALO: anthrolysin O; BCG: bacillus Calmette-Guérin; BSA: bovine serum albumin; CEACAM: carcinoembryonic antigen-related cell adhesion molecule; CPS: capsular polysaccharide; CTB: cholera toxin B; EF: edema factor; ELISA: enzyme-linked immunosorbent assay; F1: F1 capsular antigen; FCA: Freund's complete adjuvant; FHA: filamentous hemagglutinin; fHbp: factor H binding protein; FIA: Freund's incomplete adjuvant; GAC: group A carbohydrate; GFP: green fluorescent protein; HBHA: heparin-binding hemagglutinin adhesin; HLA: alpha-hemolysin; Hup: haem-utilization protein; i.d.: intradermal; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; IgY: immunoglobulin Y; i.n.: intranasal; i.m.: intramuscular; i.p.: intraperitoneal; Kdo: 3-deoxy-d-manno-octulosonic acid; LAM: lipoarabinomannan; LF: lethal factor; Lf: flocculating units; LKT: leukotoxin; LOS: lipooligosaccharide; LNnT: lacto-N-neotetraose; LPS: lipopolysaccharide; LT: heat-labile toxin; MPL+TDM: monophosphoryl lipid A + trehalose dicorynomycolate adjuvant; MOMP: major outer membrane protein; NHBA: Neisserial heparin binding antigen; O-Ag: O-antigen; OMP: outer membrane protein; OMV: outer membrane vesicle; PA: protective antigen; pAb: polyclonal antibody; PLY: pneumolysin; PNAG: poly-N-acetyl-D-glucosamine; PRN: pertactin; PSA: polysaccharide A; PT: pertussis toxin; Sbi: *Staphylococcus aureus* binder of IgG; s.c.: subcutaneous; sIgA: secretory immunoglobulin A; SLO: streptolysin O; SpyCEP: *Streptococcus pyogenes* cell-envelope proteinase; SSA-1: serotype 1-specific antigen; wtOMV: wild-type outer membrane vesicle

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