DETAILED METHODS

Bacterial growth conditions. Bacteria were grown overnight in 5 ml of MedECa [1] or Luria-Bertani (LB) broth at 37°C without shaking. Antibiotics were added to the growth medium at the following concentrations: kanamycin (Km) 100 μg/ml in agar plates, and 20 μg/ml in broth; tetracycline (Tet), 12.5μg/ml.

Antibodies and antisera. The antibodies used in this work were: peroxidase-conjugated rabbit anti-mouse (DAKO) and goat anti-rabbit (BioRad) immunoglobulins, Alexa 488 donkey anti-rabbit and Alexa 546 goat anti-mouse immunoglobulins (Invitrogen), rabbit antiserum against purified human serum apoA-I, IgG fractions of rabbit antisera (R283, R297, R315, R322) against human apoA-I, pre-immune rabbit IgGs, monoclonal antibodies (mAbs) specific for human apoA-I: 8G8 (epitope unknown, generated in our laboratory), 4H1 (aa 2-8), 3G10 (aa 98-121), M9 (aa 137-144), 8A4 (aa 151-167), 7C5 (aa 151-167), 4.1 (aa 211-220, a gift from Dr. Dmitri Sviridov, Melbourne, Australia), and anti-LPS mAbs Tom A6 and 2B5 (specific for *Y. enterocolitica* O:3 O-ag and outer core hexasaccharide, respectively) [2]. Mouse IgG1 mAbs to human C5 (clone 137-76), and the isotype-matched control (clone G3-510, anti-HIV gp120) were obtained from Genentech. The anti-C5 antibody 137-76 binds C5, prevents its cleavage, and blocks the formation of C5b-9 [3]. MAbs 4H1, 3G10 and M9 are a gift from Dr. Yves Marcel, Ottawa, Canada, and mAbs 8A4 and 7C5 from Dr. Carlos Calvo, Concepcion, Chile.

Y. enterocolitica cross-reactive antibodies were removed from the anti-apoA-I antisera by adsorption with live *Y. enterocolitica* bacteria as described [4].

Isolation of lipopolysaccharide. LPSs from smooth *Y. enterocolitica* serotype O:3 strains grown at 10°C (YeO3 6748/81) or 37°C (Ye 75S) were extracted by the hot phenol/water method of Westphal *et al.* [5]. LPSs from the rough *Y. enterocolitica* O:3 strain Ye 75R grown at 37°C and from the outer core mutants (YeO3-trs11, YeO3-trs22, and YeO3-trs24) grown at 22°C or 37°C were extracted by the phenol/water method [5] followed by the phenol/chloroform/petroleum ether method as described [6].

Construction of YeO8-c-R2/pAY100:Tet strain. The pBR322-based plasmid pAY100 carries the O-ag gene cluster of *Y. enterocolitica* O:3 [7]. To introduce the ampicillin resistant (Amp^R) pAY100 into *Y. enterocolitica* O:8 strain (also Amp^R) we swapped the pAY100 Amp^R to tetracycline resistance (Tet^R) by subcloning the O-ag gene cluster-containing MscI fragment of pAY100 into ScaI-digested pBR322. The resulting plasmid, named pAY100:Tet, and the vector control pBR322, were electroporated into the *Y. enterocolitica* O:8 strain YeO8-c-R2. The surface expression of the O:3 O-ag by YeO8-c-R2/pAY100:Tet was verified as sensitivity of the strain to the O:3 O-ag - specific bacteriophage ϕYeO3-12 as described [8].

Serum bactericidal assay. NHS was used at a final concentration of 10 %. Duplicates or triplicates of bacterial suspensions (~500 bacteria in 10 μl) were mixed with (i) NHS, (ii) NHS pre-incubated on ice for 10 min with (1) anti-apoA-I antiserum (12 μg/reaction), R283 IgGs (2, 4, 6, 8, or 12 μg/reaction), or 12 μg of mAb against apoA-I (2) 5 μg/ml of anti-C5 or anti-gp120 antibodies, (3) 0.5 mM EDTA, (4) EDTA to which 2 mM $CaCl_2$ and 0.5 mM $MgCl_2$ were added, (5) EDTA and supplemented with $CaCl_2$/$MgCl_2$ to which 0.15 mg/ml of apoA-I was added, (6) LPS_{YeO3} (0.5, 2, 5, 8, or 10 μg/reaction), (7) LPS to which 0.15 mg/ml of the wild type or mutated apoA-I, HDL_2, or HDL_3 were added, (8) LPS to which 0.11 mg/ml of LDL, or VLDL were added, (9) LPS to which anti-C5 or anti-gp120 antibodies (1.5, 2.5, 5 μg/ml) were added, or (iii) HIS, in a final volume of 30μl. Serum was pre-incubated with LPS, EDTA, or antibodies on ice for 10 min. The bacteria-serum mixtures were incubated at 37°C for 30 min, after which 70 μl of brain heart infusion broth was added to stop complement. The reactions were kept on ice and plated on appropriate LA plates. The serum bactericidal effect was calculated as the survival percentage taking the bacterial counts obtained with bacteria incubated in HIS as 100 %. The killing experiment was repeated for each strain at least two times, in duplicates, starting from independent cultures.

Size-exclusion chromatography. Human apoA-I, HDL_2, or HDL_3 (300 μl, 1.5 mg/ml) alone or pre-incubated with smooth LPS (50 μg/ml) were passed through a Superose 6 column (HR 10/30; Pharmacia Biotech; 0.5 ml/min, 0.5 ml/fraction). The control contained the corresponding amount of

LPS incubated in the presence of PBS (300 µl). The fractions (500 µl) were analyzed for apoA-I and LPS_{YeO3} using immuno-dot blotting.

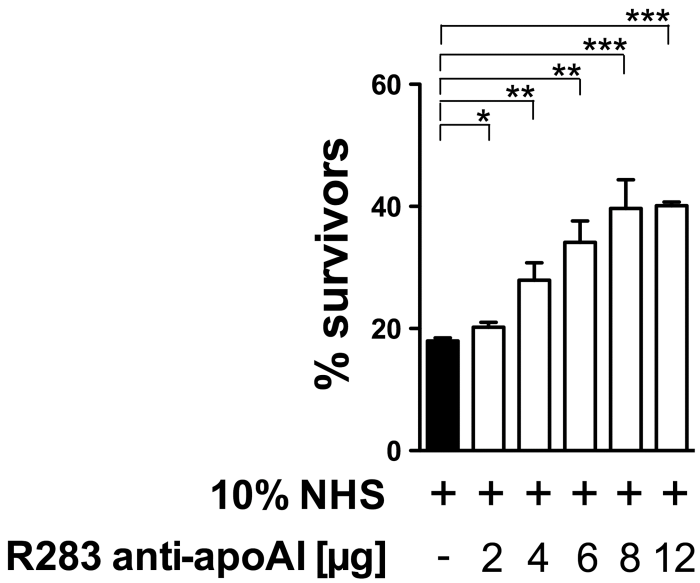
Immunoprecipitation analysis. Anti-apoA-I antibody (R322, 12 mg) was incubated with 600 µl of Protein G - Sepharose beads (Amersham Biosciences AB) o/n at +4°C with gentle rotation. The beads were washed with PBS and incubated with 500 µl of 10 % serum pre-incubated with either LPS_{YeO3} (10 µg/ml) or PBS (overnight at +4°C). The beads were washed with PBS, the bound material was eluted using 0.1M glycine pH 2.5, neutralized with 1M Tris pH 8.5, and chromatographed on Superose 12 column (HR 10/30, Pharmacia Biotech). Fractions were analyzed for apoA-I and LPS_{YeO3} using immune-dot blotting.

Immunofluorescence analysis. YeO3-O28 and YeO3-O28R strains (5×10^8) were washed with PBS and nonspecific binding sites were blocked with 1 % BSA-PBS for 50 min. The bacteria were incubated with 100 µl of 10 % HIS, apoA-I (0.15 mg/ml), HDL_3 (0.15 mg/ml), or PBS, for 40 min at 37°C. Following washes with 1 % BSA-PBS, the bacteria were incubated with 7.5 µg of R315 rabbit IgGs against apoA-I, 5 µg of 2B5 mAb, or 5 µg of TomA6 mAb, for 1 hour at 37°C. The bacteria were washed with 1 % BSA-PBS and incubated with Alexa 488 donkey anti-rabbit (1:200) or Alexa 546 goat anti-mouse (1:200) immunoglobulins for 1 hour at 37°C. The bacteria were washed with 1% BSA-PBS. The stained samples were mounted with Mowiol [9] and examined with an Olympus BX51 microscope equipped with a digital camera and DP Controller software (Olympus, Tokyo, Japan).

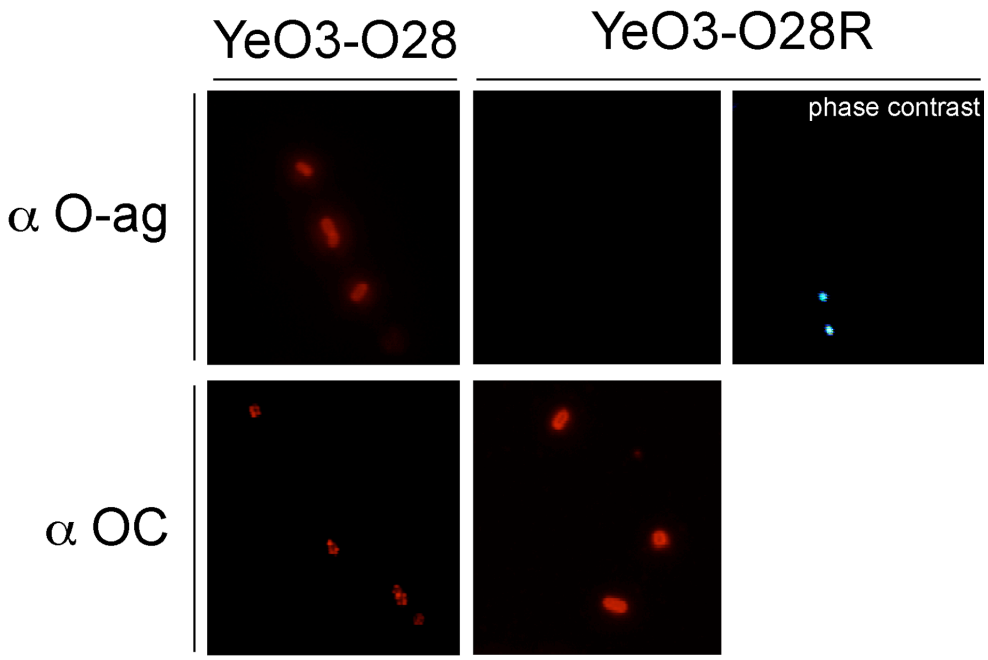
Binding of apoA-I to Y. enterocolitica. YeO3-O28 and YeO3-O28R strains (3×10^9) were washed with PBS and incubated with 40 µl of 50 % NHS for 5, 10, 15, or 20 min at 37°C. Bacteria incubated with PBS for 20 min at 37°C served as control. Following incubation, bacteria were washed with ice-cold PBS. Bacterial pellets were suspended in 30 µl of PBS, mixed with Laemmli buffer, and subjected to immunoblotting.

Immuno-dot blotting and Western blot. For the immuno-dot blotting analyses 5 µl of the gel filtration fractions or 1.5 µl aliquots of serial two-fold dilutions of LPSs extracted from *Y. enterocolitica* outer core mutants were spotted on nitrocellulose membrane. Western Blot analysis was performed on 4-12 % gradient Nu-PAGE Bis-Tris gels (Invitrogen) and transferred onto nitrocellulose membranes. Western blotting and immune-dot blotting analyses were carried out as described [1]. The following primary antibodies were used: R297, R315, mAb 8G8, and mAb TomA6. If needed, quantitative analysis of signal intensity was performed using NIH Image J software.

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS



Supplemental Figure 1. Anti-apoA-I antibody concentration-dependent survival of the *Y. enterocolitica* indicator strain. The bacteria were incubated in 10 % NHS alone or supplemented with increasing amounts of IgG fraction of rabbit monospecific anti-apoA-I antiserum (R283). Survival in 10 % HIS was set to 100 %. Data represent means and standard deviations from three separate experiments, each done in duplicate. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$; ns, not significant.



Supplemental Figure 2. Immunofluorescence analysis of LPS_{YeO3} O-ag and outer core expression by YeO3-O28 and YeO3-O28R strains. The bacteria were incubated with 5 µg of mAb 2B5, or 5 µg of mAb TomA6 for 1 hour at 37°C. Bound antibodies were detected with with Alexa 546 goat anti-mouse immunoglobulins.

Supplemental Table 1. Bacterial strains, plasmids, and bacteriophages used in this study

Bacterial strain	Description	Reference
Y. enterocolitica strains		
6471/76 (YeO3)	Serotype O:3, patient isolate, wild type	[10]
YeO3-R2	Spontaneous rough derivative of YeO3	[11]
YeO3-028	Δ*yadA::*Km-GenBlock, derivative of YeO3, Km^R, indicator strain in this study	[1]
YeO3-028-R1	Spontaneous rough derivative of YeO3-O28, Km^R	[1]
8081-c-R2	Serotype O:8, virulence plasmid-cured (pYV), O-ag-negative derivative of 8081	[12]
E.coli strains		
S17-1λ_{pir}	*thi pro hsdR*$^-$ *hsdM*$^+$ *recA*::RP4-2-Tc::Mu-Km::Tn*7*, Str^R (*λpir*)	[13]
Plasmids		
pBR322	Cloning vector; Amp^R, Tet^R	[14]
pAY100	A 12.5 kb genomic fragment of *Y. enterocolitica* serotype O:3 carrying the O-ag gene cluster cloned into BamHI site of pBR322; Amp^R	[7]
pAY100:Tet	A 12.5 kb genomic fragment of *Y. enterocolitica* serotype O:3 carrying the O-ag gene cluster cloned into ScaI site of pBR322; Tet^R	This study
Bacteriophage		
ϕYeO3-12	O-ag-specific phage of *Y. enterocolitica* O:3	[7]

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