Supplemental Data

Experimental Procedures

Extracellular matrix protein (ECM) binding assays- Binding of WA-314 *ΔyadA* strains to immobilized collagen, fibronectin and matrigel was performed as described previously (70). Briefly, 50 µl of type I collagen (20 µg/ml), fibronectin (10 µg/ml) and matrigel (10 µg/ml) (Sigma-Alderich, Taufkirchen, Germany) were coated onto Microlon 600 96-well plates (Greiner, Frickenhausen, Germany) overnight at 4 °C. Nonspecific binding sites were blocked with 200 µl 0.5 % bovine serum albumin in PBS for 1 h at 37 °C. After five washing steps with 0.1 % Tween in PBS, the wells were incubated with bacteria (OD_{600} ~ 0.5) in PBS for 1 h at 37 °C. After additional five washing steps binding was analyzed by immunostaining with anti-rabbit WA-c Serum (1:10000) for 1 h at 37 °C and subsequent incubation with goat anti-rabbit IgG alkaline phosphatase conjugate (1:2000). 1 mg of *p*-nitrophenyl phosphate per ml H_2O was added as substrate and the reaction was stopped with 0.5 M H_2SO_4. The absorbance was determined at 491 nm.

Binding studies with solube CEACAM-GFP constructs and yadA-, nadA-expressing yersiniae- To analyze interaction of human CEA-related adhesion molecules with NadA, the pathogen-host-receptor binding assay according to Kuespert and Hauck (71) was used. Cell culture supernatants containing the GFP-tagged amino-terminal immunoglobulin-variable (Igv)-like domains derived from distinct CEACAMs were prepared according to (72) and were provided by C.R. Hauck (Universität Konstanz, Germany). Thus, $4x10^6$ bacteria of *Y. enterocolitica* strain WA-c *Δinv*(p*nadA*), WA-c *Δinv*(p*yadA*), WA-c *Δinv*(p), non-piliated, non-opaque *N. gonorrhoeae* (Ngo Opa-), or non-piliated, Opa_{CEA}-expressing *N. gonorrhoeae* (Ngo Opa_{CEA}) were incubated with 250 µl cell culture supernatants containing GFP-tagged Igv-like domains of CEACAM1, CEACAM3, CEA, CEACAM6, or CEACAM8 for 30 min at 20 °C with head-over-head rotation. Bacteria were washed twice with PBS and analyzed by flow cytometry.

Figure Legends

Fig. 1. Binding of *nadA*-expressing yersiniae to extracellular matrix proteins. Comparison of binding capacity of different WA-314 *ΔyadA* strains to matrigel (10 µg), fibronectin (10 µg) and collagen type I (20 µg) tested by ELISA. *yadA*: positive control; *ΔyadA*: negative control. Data are expressed as the mean ± standard error of at least three independent experiments carried out in triplicate.

Fig. 2. Interaction of *nadA*-expressing yersiniae with soluble CEACAM-GFP fusion proteins. Different WA-c *Δinv* and *N. gonorrhoeae* strains were incubated with cell culture supernatants containing GFP-tagged CEACAM1, CEACAM3, CEA, CEACAM6 or CEACAM8 and analyzed by flow cytometry. Ngo Opa- (non-opaque *N. gonorrhoeae* MS11; negative control); Ngo Opa_{CEA} (Opa_{CEA}-expressing *N. gonorrhoeae* MS11-B2.1: positive control), WA-c *Δinv*(p): negative control (*ΔyadA, ΔnadA*); WA-c *Δinv*(p*yadA*) (*yadA*-positive); WA-c *Δinv*(p*nadA*) (*nadA*-positive). The mean fluorescence intensity (MFI) of 10.000 events counted in a representative experiment is shown.

Supplemental Figures

Figure 1

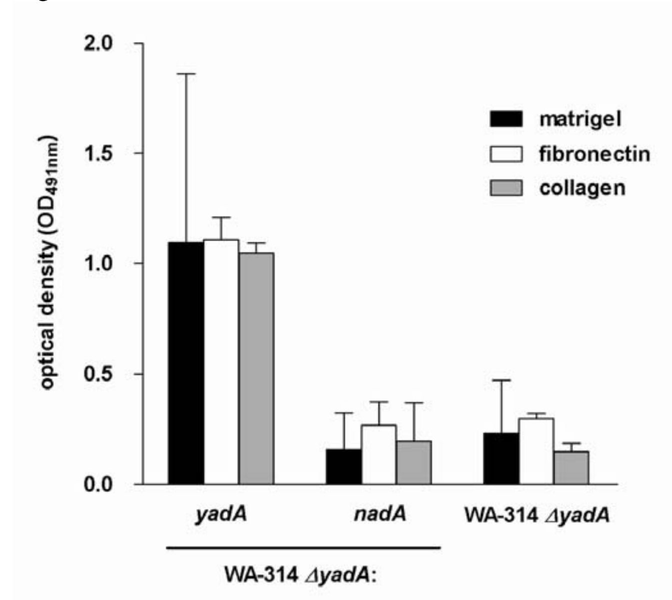

Figure 2