MAJOR ARTICLE

Protective Anti-V Antibodies Inhibit *Pseudomonas* and *Yersinia* Translocon Assembly within Host Membranes

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Pathogenic *Yersinia* species and *Pseudomonas aeruginosa* share a similar type III secretion/translocation system. The translocation system consists of 3 secreted proteins, YopB/PopB, YopD/PopD, and LcrV/PcrV; the latter is known to be a protective antigen. In an in vitro assay, the translocation system causes the lysis of erythrocytes infected with wild-type *(wt) P. aeruginosa. wt Y. enterocolitica* is not hemolytic, but a multiknockout mutant deprived of all the effectors and of YopN (ΔHOPEMN) is hemolytic. In the presence of antibodies against PcrV and *Y. pestis* LcrV, the hemolytic activity of *P. aeruginosa* was inhibited. Similarly, the hemolytic activity of ΔHOPEMN was inhibited in the presence of anti-LcrV antibodies. The assembly of the translocon, composed of PopB/D and YopB/D proteins, was disturbed in immunoprotected erythrocyte membranes, mimicking the phenotypes of V knockout mutants. Thus, protective antibodies against the V antigens of *Yersinia* species and *P. aeruginosa* act at the level of the formation of the translocon pore in membranes of infected host cells by blocking the function of LcrV/PcrV. The hemolysis assay could be adapted for high-throughput screening of anti-infectious compounds that specifically target the type III translocon.

Injectisomes are complex nanomachines that allow pathogenic or symbiotic bacteria to inject proteins across the membrane of eukaryotic host cells. They consist of a basal body that contains a type III secretion (T3S) apparatus and a stiff needle that can be extended by a flexible pilus or filament [1, 2]. Upon contact with a eukaryotic cell membrane, the injectisome secretes, presumably in a sequential manner, a set of proteins called "translocators" and "intracellular effectors" [3, 4]. The translocators are a group of, generally, 3 proteins that are absolutely required for the proper delivery of the effectors across the host cell membrane [3–12].

Pathogenic members of the genus *Yersinia*—for example, *Y. pestis* (the causative agent of plague) and the enteric pathogens *Y. enterocolitica* and *Y. pseudotuberculosis*—share a common injectisome called "Ysc" and a common set of translocators and effectors called "Yops." The translocators are called "YopB," "YopD," and "LcrV." The latter is a bona fide Yop; it has a different name because it was discovered as a soluble protective antigen linked to virulence (hence, it is named "V antigen") >40 years ago [13, 14], long before the concept of T3S had been shaped [15]. Interestingly, the opportunistic pathogen *Pseudomonas aeruginosa,* which is evolutionarily remote from enterobacteriaceae, has a very similar injectisome called "Psc" and a set of similar translocators called "PopB," "PopD," and "PcrV" [16–18]. Both sets of translocators have been shown to form pores in erythrocytes (red blood cells [RBCs]) [5, 9], in eukaryotic cell membranes [19, 20], and in liposomes [21]. Formation of pores is readily detectable with wild-type *(wt) P. aeruginosa* but not with *wt Y. enterocolitica.* Only *Y. enterocolitica* mutants that are devoid of the effectors or of the control protein YopN form readily detectable pores [5, 11, 20]. An interpretation of this observation is that translocated Yop effectors obstruct the translocation chan-

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Table 1. Bacterial strains.

Strains	Relevant genotype or phenotype	Reference
P. aeruginosa, CHA	Mucoid, cytotoxic cystic fibrosis isolate	[32]
Y. enterocolitica		
E40	Wild-type strain with the plasmid pYV40	[39]
ΔHOPEMN	pYV40 $yopE_{21}$ $yopH\Delta_{41–352}$ $yopO\Delta_{65–558}$ yopP_{23} $yopM_{23}$ $yopN_{45}$	[11]
ΔHOPEMNV	pYV40 $yopE_{21}$ $yopH\Delta_{41–352}$ $yopO\Delta_{65–558}$ yopP_{23} $yopM_{23}$ $yopN_{45}$ $lcrV\Delta_{6–319}$	[11]
ΔHOPEMNVQ	pYV40 $yopE_{21}$ $yopH\Delta_{41–352}$ $yopO\Delta_{65–558}$ yopP_{23} $yopM_{23}$ $yopN_{45}$ $lcrV\Delta_{6–319}$ $yopQ_{17}$	[20]
ΔHOPEMNB	pYV40 $yopE_{21}$ $yopH\Delta_{41–352}$ $yopO\Delta_{65–558}$ yopP_{23} $yopM_{23}$ $yopN_{45}$ $yopB\Delta_{89–217}$	[20]
ΔHOPEMND	pYV40 $yopE_{21}$ $yopH\Delta_{41–352}$ $yopO\Delta_{65–558}$ yopP_{23} $yopM_{23}$ $yopN_{45}$ $yopD\Delta_{121–165}$	[20]
ΔN	pYV40 $yopN_{45}$	[6]

NOTE. *P. aeruginosa, Pseudomonas aeruginosa; Y. enterocolitica, Yersinia enterocolitica.*

nel [5, 20]. However, it has also been shown that the Rho-GAP activity of YopE prevents membrane damage in cells infected with *wt* bacteria [22]. When YopN is missing, secretion is contact independent and effectors are not efficiently translocated [11]. The structure of the translocation pore, the stage during which the translocation pore is inserted, and how it is connected to the needle of the injectisome are not clear yet. It is tempting to consider the translocation pore as an integral part of the injectisome, but, unlike the needle, it could be assembled only after contact with a target cell. It has been shown that PopB and PopD are able to oligomerize in vitro and that their interaction with lipids promotes the formation of ringlike structures with external and internal diameters of 80 and 40 Å, respectively [23]. Although PcrV is unable to interact with lipids either in vitro or in vivo [9, 23], it is required for proper assembly of the PopB/D translocon in membranes of infected erythrocytes [9].

Y. pestis LcrV possesses a highly protective antigenic character, and antibody therapy against bubonic and pneumonic plague has been shown to be effective when tested in animal models of disease [24–26]. Similarly, in animal models, active immunization with PcrV or passive immunization with anti-V antibodies provides a high level of protection against lethal *P. aeruginosa* infections [7, 27]. Furthermore, administration of anti-PcrV $F(ab')_2$ in a *P. aeruginosa*–provoked sepsis model reduced the inflammatory response and bacteremia levels [28]. Although the mechanism of action is not known, even in vitro, anti-V antibodies prevent the cytotoxicity of bacteria toward cultured cells [7, 29, 30].

In the present study, we examined the action of anti-V antibodies on formation of the translocation pore in *P. aeruginosa* and *Y. enterocolitica* hemolysis assays. It has already been shown with *P. aeruginosa* that a high level of hemolysis can be obtained at a low MOI (MOI, 1) within 1 h of coincubation. Another advantage of this model is that RBCs are not a limiting factor, and their membranes can be isolated after infection and analyzed for translocon proteins without being contaminated with infecting bacteria [9, 31]. In the present study, we applied the RBC assay to *Yersinia* species by using poly-Yop effector mutants of *Y. enterocolitica* [20]. We demonstrate that the antibodies against the V antigens, through their direct interaction with V proteins, inhibit bacteria-induced hemolysis by acting at the level of type III translocon assembly in host cell membranes, for both pathogenic species.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in the present study are listed in table 1. The *P. aeruginosa* strain used was the cytotoxic cystic fibrosis isolate CHA [19, 32]. *P. aeruginosa* were grown either on *Pseudomonas* isolation agar (Difco) plates or in liquid Luria broth (LB) medium at 37°C with agitation. All the mutants of *Y. enterocolitica* used were derived from the strain E40, a strain from serotype O:9 [4, 11]. *Y. enterocolitica* strains were inoculated to an OD_{600} of 0.1 and cultivated in brain-heart infusion broth (ICN) for 3 h at 37°C. In vitro, secretion of *Y. enterocolitica* was triggered by sodium oxalate [11].

Production and purification of antibodies. The production and purification of the rabbit-derived anti-PcrV, anti-PopB, and anti-PopD polyclonal antibodies (PAbs) have been described elsewhere [9, 23]. MAb166 [27] was provided by D. W. Frank (Medical College of Wisconsin). The LcrV coding sequence was obtained from genomic DNA of the *Y. pestis* strain EV76 (gift from E. Carniel, Pasteur Institute, Paris) by polymerase chain reaction (PCR) using the primers 5′-GATAAGAATTCGAGCCTACGAACAAAACCCA-3′ and 5′-AAGGATCGTCGACTTACATAATTACCTCGTGTCA-3′. The PCR product was digested with *Eco*RI and *Sal*I and cloned into the expression plasmid pGEX-6P-2 (Amersham Biosciences). A soluble glutathione-S-transferase (GST)–LcrV fusion was produced in *Escherichia coli* and purified on GSTrap (Amersham Biosciences). The GST-LcrV protein fusion bound on the column was cleaved with PreScission Protease (Amersham), resulting in the elution of purified LcrV. Three CD1 mice were immunized with 100-μL intramuscular injections of 10 μg of purified LcrV in PBS con-

taining 2% alhydrogel (Superfos Biosector) and 10 μg of CpG oligonucleotide [33] as adjuvant. In each mouse, after 2 immunizations separated by a 2-week interval, the ELISA titer (the highest dilution giving twice the optical density obtained with naive CD1 serum) of anti-LcrV serum antibodies was >1:50,000. For production of anti-LcrV PAbs, the best-responding mouse was given an intraperitoneal (ip) booster injection of the same respective amounts of LcrV, alhydrogel, and CpG, followed 5 days later by a final ip injection containing LcrV, CpG, and 5×10^5 TG180 sarcoma cells. The ascitis fluid was collected 1 week later, and IgG was purified on protein A (Mab Trap; Amersham). The BCA protein assay kit (Pierce) was used to determine the concentration of purified antibodies.

Hemolysis assays and immunoprotection experiments. Hemolysis assays were performed as described elsewhere [9]. Briefly, sheep RBCs (Eurobio) were washed 3 times in PBS (pH 7.4) (150 mmol/L NaCl) and resuspended in RPMI 1640 medium (Sigma) at 5×10^8 RBCs/mL at 4°C. Bacteria were grown in LB to an OD_{600} of 1.0, centrifuged, and resuspended in RPMI 1640 at 5×10^8 bacteria/mL. Hemolysis assays were initiated by mixing 100 μL of RBCs and 100 μL of bacteria in round-bottom 96-well plates, which were then centrifuged at 2000 *g* for 10 min and incubated for 1 h at 37°C. The release of hemoglobin was measured, and the percentage of hemolysis was calculated as described elsewhere [31]. Immunoprotection experiments were performed by incubating bacteria for 45 min

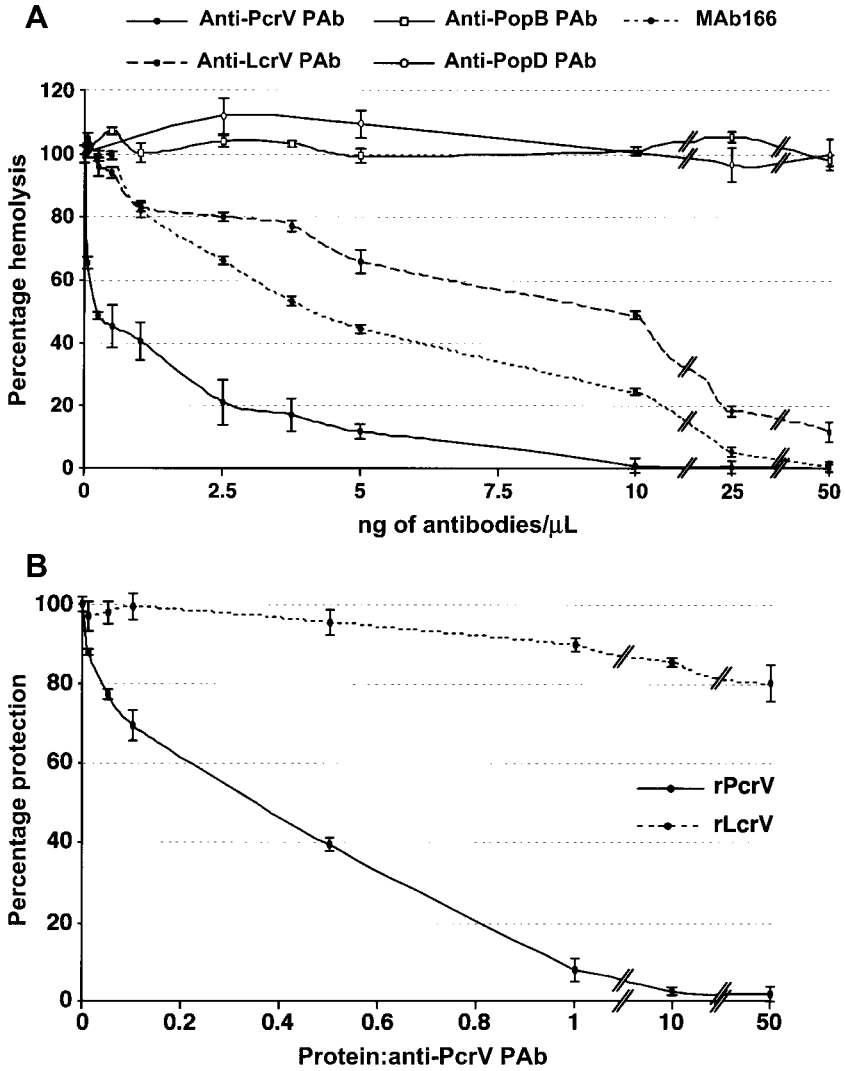

Figure 1. Immunoprotection of red blood cells (RBCs) against *Pseudomonas aeruginosa*–induced hemolysis. *A,* Lysis of RBCs by type III secretion of *P. aeruginosa* (MOI, 1) at 37°C, after 1 h in the presence of different antibodies added to the bacteria before coincubation. Anti-PcrV and anti-PopB polyclonal antibodies (PAbs) were affinity purified from rabbit serum, anti-LcrV PAbs were purified on protein A from mouse serum, and anti-PopD was total rabbit serum. MAb166 is a protective monoclonal antibody against PcrV [27]. *B,* Titration of protective antibodies by recombinant LcrV (rLcrV) or PcrV (rPcrV). The hemolysis assay was performed in the presence of immunoprotective anti-PcrV PAbs added in quantities necessary to achieve 90%–100% protection. Purified rPcrV and rLcrV proteins were added to the assay, and hemolysis was measured after 1 h of incubation.

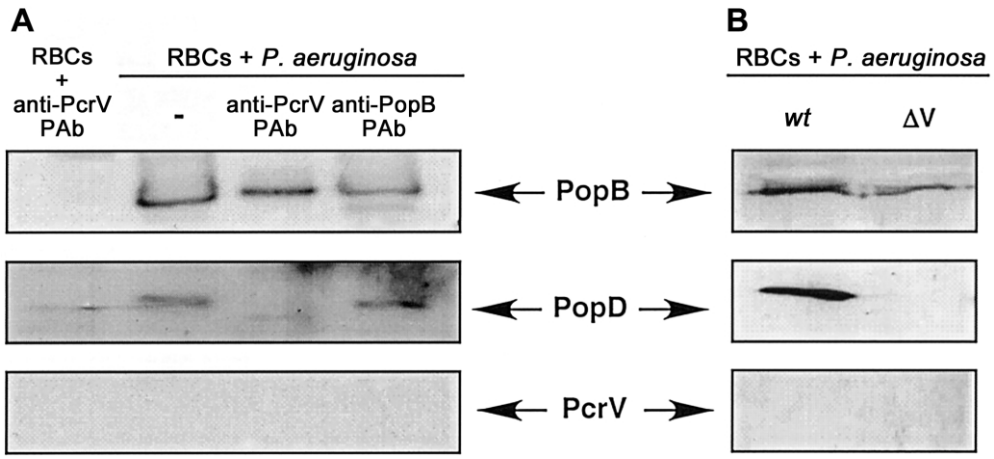

Figure 2. Assembly of the *Pseudomonas aeruginosa* PopB/D translocon in immunoprotected red blood cell (RBC) membranes. *A,* RBC membranes isolated on sucrose density gradients after performance of hemolysis assays in the presence of either protective anti-PcrV polyclonal antibodies (PAbs) or anti-PopB PAbs. *B,* RBC membranes isolated after hemolysis assay with the wild-type *(wt)* and the *pcrV* mutant (ΔV) [9]. The translocon content of PopB, PopD, and PcrV within membranes was analyzed by Western blotting.

with different concentrations of antibodies at room temperature, before the standard hemolytic assay. Negative controls included corresponding preimmune rabbit serum. In titration experiments, a constant concentration of 5 ng/μL PAbs was added to bacteria, then increasing amounts of the recombinant PcrV or LcrV were incubated with the bacteria-PAb mixture for 45 min at room temperature.

RBC membrane isolation. RBCs were resuspended in Tris-saline (30 mmol/L Tris and 150 mmol/L NaCl [pH 7.5]) at 1×10^{10} cells/mL. Before the infection, 3×10^{9} bacteria were incubated with 180 μg of PAbs in 700 μL of Tris-saline buffer for 45 min at room temperature. Then, hemolytic reactions were prepared in 50-mL conical tubes. The mixture of bacteria and antibodies was mixed with 300 μL of RBCs at 1×10^{10} cells/mL and 1 mL of a 2× protease inhibitor cocktail (Complete; Roche). Samples were centrifuged at 2000 *g* for 10 min at room temperature and incubated for 1 h at 37°C. Hemolysis was assessed spectrophotometrically, as described above. Then, 3 mL of distilled water at 4°C was added to each sample to lyse all RBCs, and these were vortexed and centrifuged again to remove bacteria. The RBC membranes were isolated by flotation on a sucrose density gradient, as described elsewhere [9]. The material at the 44%/25% sucrose interface was collected, diluted in Tris-saline, and concentrated by centrifugation in a TLA-100.3 rotor (Beckman) at 450,000 *g* for 20 min at 4°C. The pellets were resuspended in Laemmli sample buffer and analyzed by Western blotting.

Immunoblotting analysis. Immunoblotting analysis was performed with primary PAbs against YopB (gift from Å. Forsberg, Umeå University), PopB, PopD, and PcrV [9]; monoclonal antibodies (MAbs) against YopD [20] and LcrV [11]; and a secondary antibody conjugated to horseradish peroxidase (Sigma). Membranes were developed by use of an enhanced chemiluminescence kit (Amersham Biosciences).

RESULTS AND DISCUSSION

Since PcrV has been found to be necessary for assembly of PopB/D pores in RBC membranes [9], we asked whether antibodies against PcrV would interfere with the PopB/D-dependent hemolysis induced by cytotoxic *P. aeruginosa* strain CHA. Affinity-purified PAbs against translocator proteins PopB and PopD did not inhibit hemolysis. Concentrations of both antibodies up to 50 ng/μL had no protective effect. In contrast, affinity-purified anti-PcrV PAbs [9] mixed with either bacteria or RBCs before infection inhibited hemolysis in a dose-dependent manner (figure 1*A*). Concentrations of antibodies as low as 0.25 ng/μL were able to reduce *P. aeruginosa*–induced lysis by 50%. Increasing the concentration of antibodies up to 10 ng/μL in the infection assay resulted in complete protection of RBCs from lysis. MAb166 is an anti-PcrV MAb that has been shown to protect mice from lethal *P. aeruginosa* infection [27]. In the hemolysis assay, MAb166 prevented hemolysis, but an 18-fold greater concentration of MAb166 than of PAbs was required to obtain 50% inhibition. The higher neutralization activity of the PAbs could be due to the binding of several PcrV epitopes or to a higher affinity of some antibody component, compared with MAb166. The former hypothesis is favored because, in a competition ELISA, a 100-fold excess of PAb did not hinder the binding of MAb166 to recombinant PcrV (rPcrV) (data not shown). This could have practical importance, because efficient neutralization of PcrV by MAbs would then require a combination of several MAbs, as seems to be the case for botulinum toxin type A [34].

LcrV from *Yersinia* species and PcrV from *P. aeruginosa* share 41% amino acid identity [16], and they most likely share the same function in the assembly of the translocon. We raised mouse PAbs against recombinant LcrV (rLcrV) from *Y. pestis*. Similar to anti-PcrV PAbs and MAb166, anti-LcrV PAbs inhibited *P. aeruginosa*–induced hemolysis (i.e., 50% inhibition was obtained with 10 ng/μL anti-LcrV PAbs). In summary, these data show that the PAbs against PcrV and LcrV, as well as the protective MAb166, hinder cytotoxic *P. aeruginosa* from lysing RBCs.

LcrV and PcrV are surface-exposed antigens ([29, 35] and I.A., unpublished data) and, therefore, are probably accessible to antibodies. To further confirm that the protection against hemolysis was due to the direct interaction between antibodies and the antigen during the infection process, we set up competition experiments in which the antibodies necessary to achieve 90%–100% protection were kept constant and increasing amounts of the recombinant antigen were added to the infection mixture. With rPcrV, as few as 3–4 molecules/10 molecules of antibodies were sufficient to restore 50% hemolysis, showing that the protection is due to direct PcrV/antibody interaction (figure 1*B*).

RBC lysis occurs by osmotic shock following the formation of PopB/D pores within erythrocyte membranes. Since PcrV is required for formation of functional Pop pores, we addressed the question of whether PopB/D inserts into membranes in the presence of anti-V antibodies. The hemolysis assay was scaled up, and antibodies were added at levels necessary to achieve 90%–100% protection. After 1 h of incubation, the RBCs were lysed by the addition of water, and the membranes were purified by sucrose density gradient, as described elsewhere [9]. The purified membranes were examined for the presence of Pop translocators by Western blotting. *P. aeruginosa*–infected RBC membranes contained both translocators, PopB and PopD, as reported elsewhere (figure 2) [9]. Notably, in the presence of anti-PcrV PAbs, PopD protein was absent from infected RBC membranes (figure 2), corroborating the phenotype of the PcrV knockout bacteria that were unable to assemble the functional translocon (figure 2) [9]. It should be noted that systematically less PopB could be detected in membranes purified from these immunoprotection experiments. Antibodies against PopB were not able to disturb PopB/D pores inserted into host membranes (figure 2), which is in agreement with the incapacity of these antibodies to immunoprotect infected RBCs. These results show that anti-PcrV antibodies either block the proper insertion of pore-forming proteins or destabilize them within membranes. That PopB was still found in immunoprotected membranes, although it was unable to form functional pores, suggests that protective antibodies do not prevent contact between the host cell and the bacterial injectisome.

Since the serum raised against the *Y. pestis* rLcrV prevented, to some degree, *P. aeruginosa* T3S–induced hemolysis, we applied the hemolysis test to several *Y. enterocolitica* strains. As

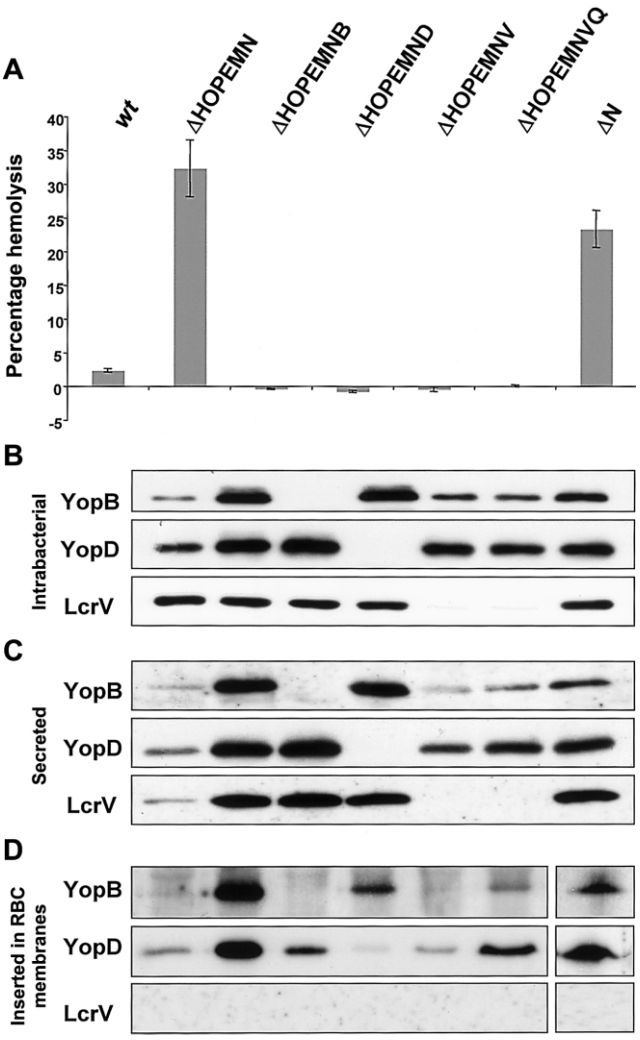

Figure 3. Lysis of red blood cells (RBCs) by the Ysc-Yop type III secretion system from *Yersinia enterocolitica*. The removal of YopQ minimizes the negative effect of the *lcrV* deletion on synthesis of YopB and YopD [11]. *A,* Percentage hemolysis after 1 h of contact (MOI, 1). *B, C,* and *D,* Western blot analysis of YopB, YopD, and LcrV performed on intrabacterial proteins *(B),* the total amount of proteins secreted by the bacteria during the infection *(C),* and proteins inserted into the membranes of erythrocytes *(D). wt,* wild-type bacteria; ΔHOPEMN, a multi-effector Ca^{2+}-blind knockout [20]; ΔHOPEMNB, ΔHOPEMN lacking translocator YopB [20]; ΔHOPEMND, ΔHOPEMN lacking translocator YopD [20]; ΔHOPEMNV, ΔHOPEMN lacking translocator LcrV [11]; ΔHOPEMNVQ, ΔHOPEMNV lacking YopQ; ΔN, *wt* lacking regulator YopN [6].

was already reported by several authors [5, 20], the *wt Yersinia* strains had very low hemolytic activity, even when the bacteria–host cell contact was forced with centrifugation. Indeed, no detectable hemolysis could be observed at 1 h after infection (figure 3*A*). Neyt et al. [20] and Marenne et al. [11] reported that, in contrast to the *wt* strain, the mutant strain ΔHOPEMN, which lacks T3S effectors (YopH, -O, -P, -E, and -M) as well as the control protein YopN [36], provokes macrophage cell lysis through the formation of pores presumably constituted

by the translocators YopB and YopD. Notably, in the standard RBC assay, ΔHOPEMN bacteria lysed ~30% of the erythrocytes within 1 h of coincubation (MOI, 1), a value lower than that observed with cytotoxic *P. aeruginosa* but still very significant (figure 3*A*). ΔHOPEMN-induced hemolysis was dependent on the presence of LcrV, since the mutant ΔHOPEMNV showed only a basal level of hemolysis. In addition, LcrV was not directly hemolytic to RBCs, since the mutants ΔHOPEMNB and ΔHOPEMND, which secrete the same amounts of LcrV as *wt* bacteria, were not hemolytic. This result is in agreement with data showing that *P. aeruginosa* strains that secrete PcrV are nonhemolytic [9]. *lcrV* mutants are known to produce less YopB and YopD, compared with *wt* bacteria [37–39], and this effect can be attenuated by also mutating *yopQ* (figure 3*C*) [11]. We thus also tested a ΔHOPEMNVQ mutant in the hemolysis assay, and it was also found to be nonhemolytic (figure 3*A*), showing that the lack of hemolysis observed with ΔHOPEMNV was not due to insufficient secretion of YopB and YopD but was directly due to the absence of LcrV (figure 3*C*).

Since PcrV is required for correct formation of PopB/D pores, we addressed the function of LcrV in *Y. enterocolitica*. RBC membrane fractions were examined for the presence of the translocators after infection with the same strains of *Y. enterocolitica* (figure 3*D*). Only YopD was readily detectable in membranes of RBCs infected with *wt* bacteria. In contrast, YopB and YopD were readily detectable in membranes of RBCs infected with ΔHOPEMN and ΔN bacteria. This difference reflects the fact that *wt* bacteria produce and secrete less Yops on contact with RBCs than do *yopN* mutant bacteria, which are deregulated for Yop secretion (figure 3*B* and 3*C*) [36]. This shows that centrifugation-achieved contact with RBCs does not trigger optimal Yop secretion of *wt* bacteria. Both YopB and YopD were detected in membranes of RBCs infected with ΔHOPEMNVQ, but the amount of both translocators, especially YopB, was reduced, compared with that in ΔHOPEMN or ΔN bacteria. This may simply reflect the fact that ΔHOPEMNVQ bacteria secrete less YopB/D than do ΔHOPEMN bacteria, but it should be noted that they secrete at least as much YopB as do *wt* bacteria (figure 3*C*). Nevertheless, although both YopB and YopD were detected in the membrane, no pore was formed. This suggests that, although LcrV is necessary for the proper assembly of the YopB/D pore in the host cell membrane, it is not necessary for the insertion of the translocators within host membranes.

Anti-LcrV antibodies and anti-PcrV antibodies were then tested for their capacity to protect erythrocytes from hemolysis by ΔHOPEMN. As can be seen in figure 4*A*, anti-LcrV PAbs were capable of protecting RBCs. The specific anti-LcrV serum was as immunoprotective as were the PcrV PAbs in the *P. aeruginosa*/RBC assay. However anti-PcrV PAbs had no protective effect on ΔHOPEMN-induced hemolysis, suggesting that, although some epitopes are shared by LcrV and PcrV, other neutralization epitopes are unique to LcrV.

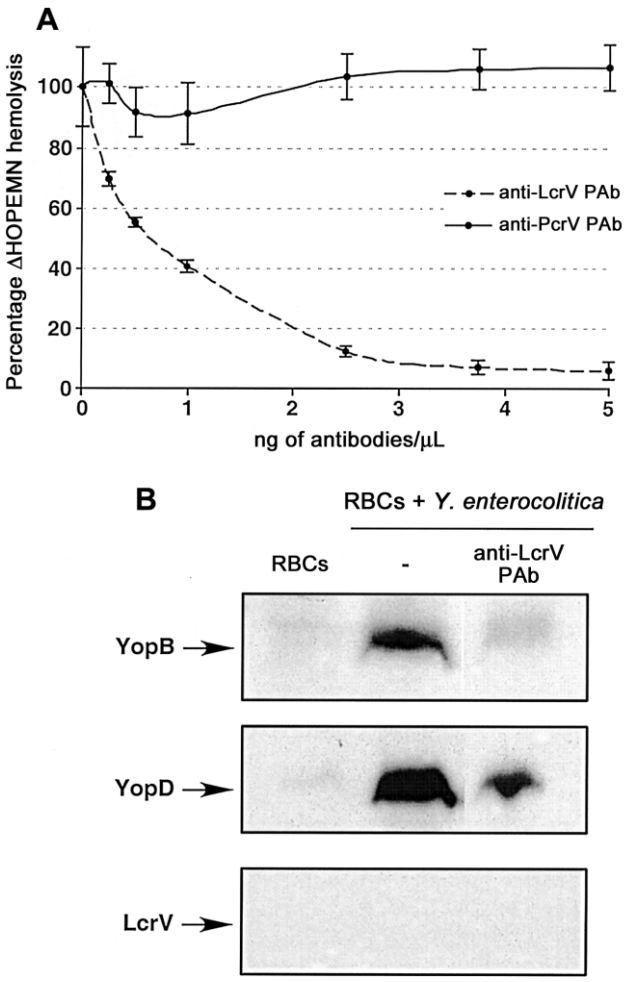

Figure 4. Immunoprotection of red blood cells (RBCs) from ΔHOPEMN-induced hemolysis, by use of anti-LcrV or anti-PcrV antibodies. *A,* Percentage hemolysis in the presence of various amounts of affinity-purified anti-PcrV polyclonal antibodies (PAbs) and protein A–purified anti-LcrV PAbs. *B,* Western blot analysis of YopB, YopD, and LcrV performed on the membranes of erythrocytes either not protected or protected by anti-LcrV antibodies. ΔHOPEMN, a multi-effector Ca^{2+}-blind knockout [20].

Next, we addressed the question of whether YopB/D pores are formed in the presence of anti-LcrV antibodies. ΔHOPEMN bacteria were preincubated for 30 min with antibodies at levels necessary to achieve 90%–100% protection. Immunoprotected membranes were purified and examined for the presence of the translocators by Western blotting. The amount of YopB was severely reduced in the presence of protective antibodies. It should be noted that less YopD could be detected in immunoprotected membranes (figure 4*B*). This suggests that anti-LcrV antibodies, similar to anti-PcrV antibodies, prevent the assembly of the functional translocation pore.

PcrV/LcrV protective antigens would thus act as extrabacterial chaperones or scaffolds in the sense that they would bind

unfolded PopB/YopB and PopD/YopD emerging from the bacterium and ensure their correct folding and assembly into a functional pore. This hypothesis, which is supported by the fact that the T3S apparatus exports unfolded proteins [40], is compatible with the known localization of LcrV at the bacterial surface [29, 35]. It implies that the V antigen interacts, at least transiently, with the B and D antigens, which is in agreement with the observation of Sarker et al. [39]. However, it does not imply that the V antigen remains bound to the assembled pore and, thus, is not contradictory to results showing the absence of direct interactions between PcrV and Pop proteins folded in vitro [9, 23]. Finally, although it is inconsistent with the idea that the purified LcrV forms channels on its own, the chaperone or scaffold hypothesis is compatible with the observation that LcrV determines the size of the pore [10, 18].

In conclusion, we have demonstrated that the protective antibodies against the V antigens (PcrV and LcrV) of *P. aeruginosa* and *Yersinia* species act by preventing the assembly of a functional type III translocon in host cell membranes. In addition, our work has shown that the hemolysis assay is well suited for study of the function of the type III translocon and could be adapted for screening of anti-infectious molecules that specifically target bacterial toxin translocons.

Acknowledgments

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