to IFN-γ, bacteria were harvested at various growth phases after exposure to IFN-γ, and PA-I mRNA and protein were measured (7). Both transcription and translation of PA-I increased in response to IFN-γ, starting at early stationary phase of growth (Fig. 1E and F). PA-I protein expression was also dose dependent (Fig. 1G). Taken together, these results suggest that PA-I expression in P. aeruginosa is enhanced in the presence of IFN-γ in a growth-dependent manner.

To determine whether IFN-γ induced PA-I by activation of the QS signaling system, we measured rhl gene expression in response to IFN-γ (7). RhlR is the gene required for the synthesis of C4-HSL (C4-homoserine lactone), a core QS signaling molecule that plays a central role in the expression of PA-I (5). IFN-γ induced rhlR transcription in PA01 (Fig. 2, A and B), and C4-HSL synthesis increased significantly after exposure to IFN-γ (Fig. 2C). Activation of the QS system by IFN-γ also led to the increase of pyocyanin (PCN), another QS-dependent virulence product (13) (Fig. 2D). In addition, rhlR and rhlR were required for the production of PCN and PA-I expression in response to IFN-γ, because the increase of these two virulence factors by IFN-γ was abolished in mutant strains (Fig. 2, E and F). Finally, supernatant from P. aeruginosa exposed to IFN-γ, but not controls, altered the barrier function of cultured epithelial cells (fig. S1). Taken together, these data suggest that the QS system plays a key role in the response of P. aeruginosa to IFN-γ and that IFN-γ can shift the virulence of P. aeruginosa against epithelial cells.

We next hypothesized that IFN-γ may directly bind to a protein on the surface of P. aeruginosa, leading to virulence up-regulation. Consistent with this, we observed that IFN-γ avidly bound to whole fixed cells of P. aeruginosa in a dose-dependent manner (Fig. 3A). The vast majority of bacterial cells (73% ± 3.2% versus 8.5% ± 2.5%) bound IFN-γ (Fig. 3B and fig. S2). The binding capacity of the IFN-γ to P. aeruginosa was not affected significantly by the growth phase of bacteria (fig. S3A). To determine whether IFN-γ bound to membrane or cytosolic fractions of P. aeruginosa, equal protein concentrations of each fraction were prepared (7), and results showed that IFN-γ preferentially bound to membrane fractions by enzyme-linked immunosorbent assay (ELISA) (fig. S3B). Furthermore, IFN-γ binding to P. aeruginosa membranes was diminished upon proteinase K treatment (fig. S3C), which suggests that IFN-γ binds to a protein on the bacterial cell membrane. Binding was specific to IFN-γ, because no binding was observed with any other cytokines tested (fig. S3D). Taken together, these data indicate that IFN-γ binds specifically to a membrane protein (s) on P. aeruginosa.

P. aeruginosa membrane proteins solubilized with mild detergents (7) retained their binding capacity to IFN-γ (Fig. 3C), thus making it possible to isolate the putative binding protein by immunoprecipitation. Membrane proteins were next separated by non-denaturing gel electrophoresis, transferred to polyvinylidene difluoride membranes, and hybridized with IFN-γ followed by biotin-labeled antibody to IFN-γ; results revealed a single immunoreactive band at 35 kDa that was dependent on the dose of IFN-γ (Fig. 3D). Immunoprecipitation against the P. aeruginosa fractionated membrane protein isolated a 35-kDa protein that was IFN-γ dependent (Fig. 3E). Use of ESI-TRAP LC-MS-MS ion trap (electrospray ionization–tandem mass spectrometry) identified the 35-kDa protein to be the P. aeruginosa outer membrane porin OprF (Fig. 3F) (14). We next verified that OprF was a major binding site for IFN-γ by showing that solubilized membrane proteins from OprF mutant strains (15) displayed reduced binding to IFN-γ (Fig. 4A). Immunoprecipitation of solubilized membrane protein confirmed the role of OprF by showing complete loss of the ~35-kDa band in the OprF mutant strain (Fig. 4B). Further evidence supporting the role of OprF in the IFN-γ response was found when mutant strains failed to increase PA-I protein expression after exposure to an effective stimulating dose of IFN-γ as compared with the wild-type strain (Fig. 4, C and D). When OprF was reconstituted in the mutant strain 31899 using the plasmid pUCP24/OprF,