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Binding to Collagen by *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*: Evidence for *yopA*-Mediated and Chromosomally Encoded Mechanisms

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Binding of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* strains to type I, II, and IV collagens has been studied. Wild-type strains which harbored the 40- to 50-megadalton virulence plasmid specifically bound all three types of collagen. Curing of the virulence plasmid or Tn*5* insertion in the *yopA* gene encoding the temperature-inducible outer membrane protein YOP1 abolished the binding of all three collagen types to *Y. enterocolitica* and type I and II collagens to *Y. pseudotuberculosis*. Full binding capacity was restored by introduction of the *yopA* gene into nonbinding *Yersinia* strains. Binding of type I, II, and IV collagens was expressed in *Escherichia coli* constructs harboring the *yopA* gene of either *Y. enterocolitica* or *Y. pseudotuberculosis*. The interaction of bacterial cells with type I collagen could be blocked by nonradiolabeled native collagens or denatured collagen but not with other serum and connective-tissue proteins. Unlabeled collagen could not displace bound radiolabeled collagen. The binding was inhibited by YOP1-specific polyclonal antibodies, in contrast to normal rabbit serum. The interaction was rapid and was quite resistant to heat treatment, to proteolytic enzymes, to various pHs in both acidic and alkaline ranges, and to the chaotropic agent urea. We propose that this newly identified interaction may be involved both in the first steps of the pathogenesis and in the complications of *Yersinia* infections affecting connective tissue.

Members of the genus *Yersinia* are invasive pathogens which are able to adhere to and penetrate eucaryotic cells both in vitro and in vivo (19, 23). Recent data indicate that cell adherence can be mediated by the *Yersinia* outer membrane protein 1 (YOP1) (8). This outer membrane protein is one of the temperature-inducible YOPs and is encoded by the 40- to 50-megadalton virulence plasmid common to virulent *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* strains (2, 19, 20). YOP1 expression has also been shown to be associated with the properties of autoagglutination, agglutination of guinea pig erythrocytes, and expression of surface fibrillae in both *Y. enterocolitica* and *Y. pseudotuberculosis* (1, 12, 22). Resistance of these *Yersinia* spp. to killing by human serum is also associated with the expression of YOP1 in *Y. enterocolitica* but not in *Y. pseudotuberculosis* (1, 17). Invasion of cultured mammalian cells by *Y. enterocolitica* is mediated by at least two different gene products encoded by the chromosomal loci *inv* (10, 11, 16, 21) and *ail* (16). In *Y. pseudotuberculosis*, the *inv* gene product, called invasin, has been identified as a 103-kilodalton protein localized on the bacterial surface (10, 11).

Since infection by *Y. enterocolitica* and *Y. pseudotuberculosis* frequently causes subsequent complications involving connective tissue, we have initiated an investigation of their interaction with well-characterized components of such tissue. We also studied the possibility that virulence-associated surface structures might be involved in such a binding process. In this report, we describe the binding to collagen types I, II, and IV by *Y. enterocolitica* and *Y. pseudotuberculosis* outer membrane protein YOP1 and partially characterize type I collagen binding. In addition, we present data suggesting that *Y. pseudotuberculosis* also possesses the ability to bind type IV collagen by a chromosomally encoded factor.

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MATERIALS AND METHODS

Bacterial strains. *Yersinia* spp. and *Escherichia coli* strains used in the study are listed in Table 1.

Chemicals. Porcine fibronectin (lot no. FP 108) was a kind gift of BioInvent, Lund, Sweden. Bovine type I collagen (Vitrogen 100, lot no. 87 J239) was purchased from Collagen Corporation, Palo Alto, Calif., and murine type IV collagen (lot no. 88 1403) was from Collaborative Research Inc., Bedford, Mass. Bovine type II collagen was a generous gift from P. Speziale, Pavia, Italy. Trypsin, proteinase K, papain, ovalbumin, and hemoglobin were from Sigma Chemical Co., St. Louis, Mo. Human immunoglobulin G and fibrinogen were from Kabi Vitrum, Stockholm, Sweden. Bovine serum albumin was from Boehringer GmbH, Mannheim, Federal Republic of Germany. Iodine was obtained from Amersham Corp., United Kingdom. Iodobeads were from Pierce Chemical Co., Rockford, Ill., and were used according to the instructions of the manufacturer.

Binding assays and radiolabeling of proteins. Proteins were radiolabeled by the chloramine T method (5) to a specific activity of 1×10^6 to 3×10^6 cpm/μg. Bacteria were grown on colonization factor antigen agar for 24 h at 37°C if not otherwise stated. The cultures were harvested and suspended in phosphate-buffered saline (PBS; 0.15 M, pH 7.2), and adjusted to a cell density of 10^{10} cells per ml. One hundred microliters of the above-mentioned cell suspension was added to 50 μl (ca. 25,000 cpm) of the protein solution

TABLE 1. Binding of ^{125}I-labeled collagen by *Yersinia* spp. and *E. coli* strains

Organism	Serogroup	Presence of virulence plasmid	Presence of YOP1 expression	% binding of collagen			Source or reference
				Type 1	Type II	Type IV	
Y. enterocolitica							
8265 P^+	O:3	Yes	Yes	67.2	68.5	52.7	Wild type (13)
8265 P^-	O:3	No	No	3.9	0.1	2.4	Derivative of 8265, P^+
NY 87-71 P^+	O:8	Yes	Yes	56.2	43.5	52.9	Wild type (13)
NY 87-71 P^-	O:8	No	No	5.5	1.9	8.5	Derivative of NY 81-71, P^+
3315 P^+	O:9	Yes	Yes	65.2	58.8	56.5	Wild type (13)
3315 P^-	O:9	No	No	4.5	0.6	0.3	Derivative of 3315, P^+
96-P	O:9	Yes	Yes	65.0	59.9	46.2	Wild type (6)
96-C	O:9	No	No	5.1	0.4	4.1	Derivative of 96-P
96-P^-	O:9	Yes	No	6.9	0.3	3.9	Tn*5* mutant of 96-P (7)
96-9-4	O:9	No	Yes	57.0	21.1	21.5	96-C with cloned *yopA* (8)
NF-O	O:5	No	No	9.6	1.2	3.9	Environmental strain (9)
NF-9	O:5	Yes	Yes	69.3	64.3	50.9	NF-O with plasmid of 96-P (8)
NF-P^-	O:5	Yes	No	6.8	0.4	1.3	Tn*5* mutant of NF-9 (8)
NF-9-4	O:5	No	Yes	71.0	12.1	38.2	NF-O with cloned *yopA* (8)
NF-8	O:5	Yes	Yes	63.0	nt	44.1	NF-O with plasmid of *Y. enterocolitica* serotype O:8; J. Heesemann, unpublished data
Y. pseudotuberculosis							
YPIII(pIB1)	III	Yes	Yes	71.3	25.9	54.4	Wild-type strain (2)
YPIII	III	No	No	4.4	6.1	62.6	Cured derivative of YPIII(pIB1)(2)
YPIII(pIB102)	III	Yes	No	3.7	nt	56.7	Tn*5* mutant of YPIII(pIB1) (4)
YPIII(pYMS2)	III	No	Yes	45.1	15.1	42.4	YPIII with cloned *yopA* of YPIII(pIB1); Skurnik and Wolf-Watz, unpublished data
YP-I	I	Yes	Yes	65.7	56.1	58.1	Wild type; J. Heesemann unpublished data
YP-I-C	I	No	No	15.8	6.5	62.4	Derivative of YP-I; J. Heesemann unpublished data
E. coli							
PM191		No	No	6.6	1.1	4.1	Laboratory strain (14)
PM191(pYMS1)		No	No	7.1	1.1	2.7	PM191 with *yopA* of *Y. pestis* 019; Skurnik and Wolf-Watz, in press
PM191(pYMS2)		No	Yes	71.3	47.7	66.1	PM191 with *yopA* of YPIII; Skurnik and Wolf-Watz, in press
PM191(pYMS3)		No	Yes	58.7	37.7	31.9	PM191 with *yopA* of *Y. enterocolitica* 8081; Skurnik and Wolf-Watz, in press
PM191(pYMS4)		No	Yes	71.4	53.1	41.8	PM191 with *yopA* of *Y. enterocolitica* 6471/76; Skurnik and Wolf-Watz, in press

(in PBS containing 0.5% bovine serum albumin). The tubes were incubated at room temperature for 1 h if not otherwise stated, and the reaction was stopped by the addition of 2 ml of PBS containing 0.5% (vol/vol) Tween 20, precooled to 4°C. The suspension was then centrifuged for 25 min at 1,350 × *g*, and the supernatant was carefully aspirated. Radioactivity associated with the pellet was determined in a gamma counter (LKB Wallac Clingamma, Turku, Finland). Background values (labeled proteins incubated with buffer only) were subtracted. All binding assays were performed in duplicate.

Blocking and displacement assays. (i) Blocking with proteins. One hundred-microliter portions of bacterial suspensions were incubated with 100 μl of the blocking compounds to be tested (100 μg/ml), or with PBS as a control, at room temperature for 1 h. The cells were then washed in 2 ml of PBS, and the binding assay was performed as described above.

(ii) Displacement assay. Bacteria were incubated first with the radiolabeled protein as described for the binding assay, followed by incubation with 100 μl of nonradiolabeled collagen (100 μg/ml) at room temperature for 1 h. The reaction was stopped and proceeded as described for the binding assay.

(iii) Blocking with bacterial sonicates. Bacterial suspensions of the same cell density used for the binding assays were sonicated on ice by five 20-s bursts at 70% output (Branson Sonic Power Company, Danbury, Conn.) with 1-min intervals. The lysate was centrifuged at 13,000 × *g* for 15 min, and the supernatant was membrane filtered (0.45-μm pore size; Millipore Corp., Bedford, Mass.). For the blocking assay, radiolabeled type I collagen was incubated with 100 μl of the lysates for 1 h at room temperature before adding to the bacterial suspension.

(iv) Blocking of type I collagen binding with antibodies specific to YOP1. Polyclonal rabbit antibodies were obtained with electroeluted YOP1 protein as immunogen (7). One hundred-microliter portions of bacterial suspensions were incubated with serial 10-fold dilutions of the antibodies (100 μl) at room temperature for 1 h; the cells were then washed

in 2 ml of PBS, and the binding assay was performed. Bacteria were also incubated with normal rabbit serum as a control.

(v) Resistance of collagen binding to proteolytic enzymes, heat, pH, and urea. Treatment of bacterial cells with proteolytic enzymes was performed at 37°C for 30 min with 2 μg of the respective enzymes, and the reaction was stopped by repeatedly washing the cells in PBS.

Sensitivity to heat was investigated by heating the bacteria either at 80°C for 20 min or at 100°C for 60 min before the cells were subjected to the binding assay.

The affinity of the bacteria for bound proteins was also analyzed as follows. After the conventional binding assay had been performed, the bacterial pellet was suspended in 2.0-ml portions of PBS with pH values ranging between 3.0 and 10.0 or with 2.0 ml of 1, 3, or 6 M urea in PBS and incubated for 15 min at room temperature. The cells were then pelleted again, and after aspiration of the supernatant the radioactivity of the pellet was measured as described.

RESULTS

Collagen-binding capacity of YOP1-positive and -negative bacterial cells. Wild-type, virulent *Y. enterocolitica* strains of serotypes O:3, O:8, and O:9, which were grown on colonization factor antigen agar at 37°C, bound high levels of collagen types I, II, and IV (Table 1).

The virulence-plasmid-free isogenic pairs of these strains bound basal levels of these collagen types. Thus, collagen binding by *Y. enterocolitica* seemed to be a novel virulence-plasmid-associated property. Of the virulence-plasmid-encoded proteins, only YOP1 was likely to be expressed under the growth conditions used here, in which calcium was not limiting, as the other YOPs are stringently calcium regulated (3). We therefore decided to more closely examine the role of YOP1 in collagen binding. The Tn*5* insertion mutant, *Y. enterocolitica* 96-P$^-$ did not bind collagen. The site of Tn*5* insertion in this strain is the structural gene *yopA* for YOP1, and thus it is unable to produce YOP1. The strain cured of the virulence plasmid retained most of the collagen-binding capacity when the cloned *yopA* gene was mobilized into it (strain 96-9-4; Table 1).

The role of the virulence plasmid and the *yopA* gene in collagen binding by *Y. enterocolitica* was further studied with the serotype O:5 strains. The wild-type serotype O:5 strain (NF-O; Table 1) is of environmental origin and does not carry the virulence plasmid. Mobilization of either the serotype O:8 or O:9 virulence plasmid into this strain conferred on it collagen-binding ability (strains NF-8 and NF-9; Table 1). This phenotypic property was also abolished by insertional inactivation mutation of the *yopA* gene (strain NF-P$^-$; Table 1). Mobilization of the *yopA* gene into NF-O also gave this strain the collagen-binding phenotype (strain NF-9-4; Table 1).

Wild-type virulence-plasmid-harboring *Y. pseudotuberculosis* YPIII(pIB1) and YP-I (serogroups III and I, respectively) bound high levels of type I, II, and IV collagens (Table 1). In contrast, however, to *Y. enterocolitica*, curing of the virulence plasmid (strains YPIII and YP-1-C) reduced only type I and II collagen binding. Thus, *Y. pseudotuberculosis* seems also to have a chromosomally encoded factor for type IV collagen binding. As YOP1 appeared to be involved in the collagen binding of *Y. enterocolitica*, the possibility of a similar role for YOP1 of *Y. pseudotuberculosis* was studied next. The Tn*5* mutation in the *yopA* region of the wild-type strain [strain YPIII(pIB102)] abolished binding to type I collagen (type II collagen binding was not tested in this case), and mobilization of the *yopA* gene of the wild-type strain into the plasmid-cured derivative [strain YPIII(pYMS2); Table 1] substantially restored the binding capacity.

Not only are putative virulence factors such as invasin and the products of the *ail* loci of *Y. enterocolitica* and *Y. pseudotuberculosis* expressed in *E. coli*, but the constructs also function in in vitro assays (10, 11, 16). The possibility of a similar relationship between YOP1 expression and collagen binding was studied by using *E. coli* strains harboring cloned *yopA* DNA sequences from various *Yersinia* species. Clones harboring the *yopA* gene of either *Y. enterocolitica* [PM191(pYMS3) and PM191(pYMS4)] or *Y. pseudotuberculosis* [PM191(pYMS2)] bound significant levels of the three collagen types tested, compared with those of the background *E. coli* PM191. The latter finding indicates that the *yopA* gene product of *Y. pseudotuberculosis* also binds type IV collagen (Table 1). Strain PM191(pYMS1) harbors the *yopA* region of *Yersinia pestis*. A single base-pair deletion in the early sequence of this *yopA* gene resulted in the loss of YOP1 expression (M. Skurnik and H. Wolf-Watz, Mol. Microbiol., in press). The collagen-binding capacity of this strain is within the range of the background strain (Table 1).

Since expression of YOP1 is temperature inducible, this effect was investigated with growing cells of *Y. enterocolitica* 96-P, 96-9-4, 96-41, NF-9, NF-9-4, and NF-9-41 at 25°C, and the binding assay for type I collagen was performed. This culture temperature, restrictive for YOP1 expression, caused a significantly reduced binding within the range of 10 to 23%, compared with the 57 to 71% binding range for cells grown at 37°C.

Kinetics of collagen binding. The kinetics of collagen binding were tested for type I collagen with isolates of wild-type *Y. enterocolitica* 8265 P$^+$, NY 81-71 P$^+$, and 3315 P$^+$. The binding was almost complete within 30 min (58 to 68%), and only a limited increase could be observed up to 4 h (68 to 81%) after incubation.

Blocking and displacement of type I collagen binding. Blocking experiments were performed on *Y. enterocolitica* NF-9-4. The relative blocking capacity of nonradiolabeled type I collagen was 99%. Collagen types II and IV abolished 80 and 38% of binding, respectively. Denatured collagen (gelatin) blocked 47% of the binding. Fibronectin, bovine serum albumin, hemoglobin, fibrinogen, ovalbumin, human immunoglobulin G, and laminin exhibited only a limited blocking capacity, ranging between 0 and 9%. Nonradiolabeled type I collagen could displace only 1% of the previously bound radiolabeled protein.

Sensitivity of collagen binding to proteolytic enzymes and heat. The effect of proteolytic enzymes and heat on type I collagen-binding capacity was evaluated for the wild-type strains NY 81-71 P$^+$, 3315 P$^+$, and 8265 P$^+$. Strain NY 81-71 showed marked decreases in binding after treatment with proteinase K (60%), papain (25%), and trypsin (18%). Binding of type I collagen by the other two strains was not affected by any of the above-mentioned enzymes.

Heat treatment at 80°C for 20 min did not decrease the binding capacity of any of the three strains, while boiling for 60 min resulted in 65 (strain 8265 P$^+$), 71 (strain 3315 P$^+$), and 77% (strain NY 81-71 P$^+$) decreases in the binding.

The effect of various pH values and urea on collagen binding. Urea (1 M) or pH values between 5.0 and 10.0 did not facilitate the release of bound type I collagen to *Y. enterocolitica* NF-9-4. Urea (3 M) abolished 32% and 6 M

TABLE 2. Blocking of type I collagen binding to *Yersinia* strains by anti-YOP1 and normal rabbit sera

Organism and serum	Dilution	% Blocking
Y. enterocolitica NF-9-4		
Normal	1:100	3
	1:200	0
	1:400	0
	1:800	0
Anti-YOP1	1:200	96
	1:400	88
	1:800	56
Y. pseudotuberculosis		
Normal	1:500	7
Anti-YOP1	1:500	74

urea abolished 97% of the binding, while 31% of the bound protein was released at pH 4.0 and 62% was released at pH 3.0.

Blocking of type I collagen binding by sonicated bacterial lysates. The effect on binding of sonicated extracts was tested with lysates of variants of *Y. enterocolitica* NF serotype O:5 either harboring only the *yopA* region (and expressing YOP1) of the virulence plasmid (strain NF-9-4) or lacking only *yopA* (strain NF-P$^-$). The sonicate of the YOP1-positive clone blocked 85% of the binding to the homologous bacterial cells, while the lysate of the YOP1-negative, but otherwise homologous, strain did not inhibit. The YOP1-positive lysate had a 95% blocking effect on the binding capacity of *Y. enterocolitica* 96-9-4 serotype O:9. This strain harbors the same *yopA* DNA sequence as that in strain NF-9-4 but in a serologically different background (8).

Blocking of type I collagen binding by YOP1-specific antibodies. The blocking effect of specific antibodies to YOP1 was studied by using polyclonal antibodies raised in rabbit serum against a purified YOP1 preparation from a *Y. enterocolitica* serotype O:9 strain (7). The 50% blocking dilution of this YOP1-specific serum was about 1:800 when tested with *Y. enterocolitica* NF-9-4 O:5 harboring a recombinant plasmid with *yopA* DNA sequence (8). Dilutions of normal rabbit serum from 1:100 to 1:800 had no significant influence on the binding (Table 2). To check whether the blocking effect of the anti-YOP1 serum was independent of the species of the *Yersinia* strain and the origin of the *yopA* gene, the blocking experiment was also performed with *Y. pseudotuberculosis* YPIII(pIB1). In this case, 1:500 dilutions of the YOP1-specific and normal rabbit sera were applied (Table 2). In summary, the anti-YOP1 serum exhibited a remarkable blocking capacity, while the normal rabbit serum had no effect on binding in this experiment.

DISCUSSION

As attachment to the host cell surface is likely to be the first step of *Yersinia* infection, it is crucial to study the mechanism of this interaction. It is known that both plasmid-mediated and chromosomally encoded surface proteins are involved in the process of invasion (8, 10–12, 15). Mobilization of the *yopA* gene encoding the *Yersinia* outer membrane protein YOP1 into a nonadherent *Y. enterocolitica* strain has been shown to confer adherence for epithelial cells (8). The chromosomally encoded invasin, homologous in *Y. enterocolitica* and *Y. pseudotuberculosis*, and the product of the *ail* locus in *Y. enterocolitica* have been reported to promote cell attachment and enable the bacterial cells to invade several tissue culture cell lines (10, 11, 15). In this report, we present evidence that one of the above-mentioned surface proteins, YOP1, is involved in the interaction between bacterial cells and various types of collagen.

The involvement of YOP1 in collagen binding was demonstrated in several ways. All of the YOP1-positive strains tested exhibited a high degree of binding to collagen types I, II, and IV. Inactivation of the *yopA* locus by transposon mutagenesis abolished the binding capacity, and introduction of the *yopA* gene into a nonbinding host resulted in a high binding ability. A bacterial lysate containing YOP1 blocked binding of type I collagen to YOP1-positive strains regardless of the YOP1 being expressed in a homologous or heterologous background. Lysate of a *yopA* transposon mutant expressing no YOP1 did not inhibit the binding. YOP1-specific polyclonal antibodies, but not normal rabbit serum, exhibited a strong blocking capacity. The specificity of the binding was supported also by the fact that only native and denatured collagens inhibited the interaction, while several other proteins, including plasma and connective-tissue proteins, failed to cause a significant blocking effect.

In addition to the high affinity, the binding was essentially irreversible, as only a negligible portion of the bound protein could be displaced by excess amounts of the nonradiolabeled homologous compound. Two lines of evidence argue against the possibility that radiolabeling collagen might have altered its properties, thus causing such irreversibility. Unlabeled type I collagen was 99% effective in blocking the binding of labeled collagen, indicating that the former was an equally well-recognized ligand. Also, we have been able to reproduce binding patterns (Table 1) in a qualitative assay based on binding of bacteria to unlabeled type I collagen immobilized in microdilution wells (data not shown). Since the results of such assays correspond well to the binding assay with radiolabeled protein, the iodination is unlikely to have dramatically altered the binding properties.

We have shown the binding to be rapid and relatively resistant to heat, to various pH values spanning the acidic and alkaline range, and to the chaotropic agent urea. This may allow binding of *Y. enterocolitica* and *Y. pseudotuberculosis* to collagen in a wide range of in vivo conditions. It is difficult to adequately explain the apparent resistance of the collagen-binding proteins of the serotypes O:3 and O:9 of *Y. enterocolitica* strains to proteases. This may be due to different configurations of the YOP1 superstructure in the serogroups, since comparative sequence analysis has indicated significant differences in a number of *yopA* genes (Skurnik and Wolf-Watz, in press).

Collectively, the biochemical, immunological, and genetic evidence provide conclusive proof that YOP1 mediates a strong, specific binding to a limited number of collagen types. We have shown that the *yopA* gene product mediates binding to collagen types I, II, and IV, and we infer from our data that a separate chromosomal locus in *Y. pseudotuberculosis* exists which encodes a receptor for type IV collagen alone. The differential specificities of these bindings may be somewhat elucidated by reference to the properties of the relevant collagen types. The classical fiber-forming collagens are allocated, as a functional demarcation, to group I, while group II collagens (including types IV, VI, and VII) have distinctive sequences which do not allow the extensive lateral aggregation noted for group I collagens (15). There may be sufficient sequence homology between collagen types I and II to allow binding to both by YOP1, which must

also bind, via another part of the structure, to type IV collagen. This latter putative domain may be similar to the proposed chromosomally encoded type IV collagen receptor of *Y. pseudotuberculosis*. We are currently investigating these possibilities by molecular cloning techniques.

Type I collagen is distributed abundantly in the connective tissue matrix, including synovial membranes, type II collagen is an essential constituent of the cartilage, and type IV collagen is a major component of basement membranes. Binding of yersiniae or their products to these types of collagen may therefore have several pathological implications. YOP1 protein might be a colonization factor of *Y. enterocolitica* in mice after oral infection, as shown by prolonged excretion of YOP1-positive cells compared with excretion of isogenic YOP1-negative cells (12). *Y. pseudotuberculosis* seems to be different in this respect, since a *yopA* mutant was as virulent after oral infection in mice as its wild type (12). It is not clear whether *Yersinia* cells appearing in the intestinal content are derived from bacteria adhering to epithelial cells or from those invading the lamina propria (18). If the latter is true, intestinal colonization, as suggested by Kapperud et al. (12) may be attributed to survival in the lamina propria rather than to adherence to the epithelial lining. This view is supported also by the fact that resistance to killing by serum, which is necessary for bacterial survival and multiplication in the lamina propria (18), has been shown to promote intestinal colonization (12). The contribution of YOP1 to serum resistance (1) and the YOP1-mediated binding to basement membrane type IV collagen might, therefore, have an important role in the process leading to the colonization of the lamina propria. This may be true especially in the case of *Y. enterocolitica*, where serum resistance (1) and binding of various types of collagen, as shown in our study, are dependent on the expression of YOP1. However, resistance to human serum, adherence to HeLa cells, intestinal colonization, and the killing rate in experimental animals are not YOP1-related properties in *Y. pseudotuberculosis* (1, 4, 8, 12, 21). Since we have shown that *Y. pseudotuberculosis* may possess an additional, chromosomally encoded type IV collagen-binding site, a defect in the YOP1-promoted binding could be complemented by the chromosomally encoded one. This may be an explanation for the YOP1-independent virulence of *Y. pseudotuberculosis*. Indeed, an alternative colonization mechanism may be important, if one considers that the lack of YOP1 (or possibly also its in vivo down regulation) can be advantageous for the developing of a systemic *Y. pseudotuberculosis* infection (12, 21). Studies on the chromosomally mediated binding of type IV collagen may, therefore, contribute to a better understanding of the pathogenesis of *Y. pseudotuberculosis* infection.

Collagen binding by yersiniae might, however, have several other pathological implications. This interaction may be involved not only in the first colonization at the site of entry but also in a later settlement and subsequent colonization leading to infectious foci at various body sites. It is also possible that YOP1, or the chromosomally encoded type IV collagen-binding factor, may be released from lysed bacterial cells and deposited at collagen-containing structures either by themselves or as parts of immune complexes. By this mechanism, reactive disease sequelae (e.g., reactive arthritis, ankylosing spondylitis, and erythema nodosum) could be elicited.

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