Immunoblot analysis of human IgM, IgG and IgA responses to plasmid-encoded antigens of *Yersinia enterocolitica* serovar O3

T. H. STÅHLBERG, KAISA GRANFORS and AULI TOIVANEN*

*Departments of Medical Microbiology and * Medicine, Turku University, SF-20520 Turku 52, Finland*

Summary. Human IgM, IgG and IgA responses after infection with *Yersinia enterocolitica* serovar O3 were studied by immunoblotting sera against whole-cell homogenates of a plasmid-containing strain of *Y. enterocolitica* O3 and a plasmid-free strain derived from it; each strain was grown in conditions expressive for the plasmid. The antibodies observed were directed against several plasmid-encoded polypeptides. The response against different bacterial components decreased uniformly with time and the persisting antibody production was directed against several epitopes. Strong reactions to the prominent plasmid-specified antigens of mol. wts (10^3) 26, 34, 45 and 52.5 were found more often with IgG-class antibodies than with IgM or IgA; the latter immunoglobulins recognised, respectively, antigens of mol. wt (10^3) 26 and 45 (IgM) and 26, 34 and 52.5 (IgA). Immunoblotting of sera from patients with yersinia-triggered reactive arthritis did not reveal any antigens that were involved additionally or specifically. However, IgA-mediated recognition of certain antigens of mol. wts (10^3) 26, 34 and 52.5 tended to persist longer in the arthritic patients.

Introduction

The virulence of *Yersinia enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* is clearly associated with plasmids of mol. wt (10^6) 40–45 that are known to encode several polypeptides (Martinez, 1983; Heesemann *et al.*, 1984; Portnoy *et al.*, 1984; Bölin *et al.*, 1985; Mazza *et al.*, 1985; Skurnik, 1985). Studies of the immune responses against plasmid-encoded antigens, however, have been restricted to immunised animals or to the IgG responses of a limited number of patients (Martinez, 1983; Mazza *et al.*, 1985; Skurnik, 1985).

Among yersiniae, *Y. enterocolitica* serovar O3 is the most important enteropathogen in Scandinavian countries. When, previously, the immune response against chromosomally coded antigens of this bacterium was studied by immunoblotting of sequentially collected patients' sera, it appeared that more or less the same antigens were recognised by all immunoglobulin classes. Antigens recognised early or late in the course of infection were essentially the same and the responses to the individual antigens decreased uniformly with time; this suggested that the total antigenic repertoire of the bacterium was responsible for persisting antibody production (Toivanen *et al.*, 1985).

Despite some contradictory reports (Mazigh *et al.*, 1985; Simonet *et al.*, 1985), it is well-established that the plasmid-specified proteins are important for the virulence of yersiniae (Zink *et al.*, 1980; Heesemann *et al.*, 1983 and 1984; Portnoy *et al.*, 1984; Bölin *et al.*, 1985; Portnoy and Martinez, 1985). We considered it important, therefore, to study which of the plasmid-encoded proteins were antigenic during natural infection in man and how the proteins were recognised by antibodies of different immunoglobulin classes. Furthermore, we wished to clarify whether the early and late responses were directed against different antigens. Accordingly, sera obtained from patients in the acute or convalescent stages of infection (0–2 and 10–12 months, respectively, after onset of infection) were immunoblotted against a plasmid-containing strain of *Y. enterocolitica* serovar O3 and a plasmid-cured strain derived from it, with both strains grown in conditions favouring plasmid expression.

Materials and methods

Patients

A diagnosis of infection by *Y. enterocolitica* serovar O3 was established by bacterial isolation, or serologically with a serotype-specific enzyme-linked immunosorbent

assay (ELISA) (Granfors, 1979) or both. The clinical features of the 16 patients included are summarised in table I. The average age (SD) of the non-arthritis patients was 37 (13) years and of the arthritic patients was 44 (13) years. Specimens of serum, obtained from patients 0–2 months and 10–12 months after onset of the infection, were stored at -20°C . A further six specimens of serum from uninfected persons without a history of yersiniosis were studied as controls.

Rabbit antiserum against Y. enterocolitica serovar O3

This serum, kindly supplied by Dr H. Wolf-Watz, Umeå, Sweden, was raised by immunising a rabbit with a live plasmid-containing strain of *Y. enterocolitica* serovar O3, grown in conditions that allow plasmid expression. Three injections, each containing 10^8 bacteria, were given intravenously at 2-week intervals. The rabbit was bled 4 days after the final injection.

Bacterial strains and cultural conditions

Strain 4147 of *Y. enterocolitica* serovar O3, an isolate from the faeces of a patient who developed reactive arthritis following infection, contains a virulence-associated plasmid (type II of Pulkkinen *et al.*, 1986) designated hereafter as pYV+ (plasmid of *Yersinia*, associated with virulence). A plasmid-cured derivative strain pYV– was obtained from single colonies growing at 37°C on the

magnesium oxalate-containing agar medium of Higuchi and Smith (1961). The presence or absence of virulence plasmid was verified by an autoagglutination test (Laird and Cavanaugh, 1980) and by plasmid purification (Pulkkinen *et al.*, 1986). Stock cultures were maintained at -70°C in Trypticase Soy Broth (Difco) containing glycerol 20% v/v. For optimal expression of the plasmid-specified polypeptides, the following procedure (Dr H. Wolf-Watz, personal communication) was used: bacteria were grown overnight at room temperature in Nutrient Broth (Oxoid); pelleted bacteria were resuspended in a small amount of saline (NaCl, 0.9% w/v) and inoculated in Higuchi Minimal Medium supplemented with Tryptone (Difco) 1% w/v, Yeast Extract (Oxoid) 0.5% w/v and glucose 0.2% w/v; cultures were incubated with shaking (250 rpm) at 37°C to an optical density of 0.3 at 540 nm; bacteria were washed twice in phosphate-buffered saline (PBS; pH 7.4) and aliquots of the resultant suspension were centrifuged in an Eppendorf centrifuge for 10 min; the pellets obtained were stored at -20°C until required.

SDS-PAGE and immunoblotting

The bacterial pellet was suspended in sample buffer (0.06 M Tris-HCl, pH 6.8) containing glycerol 10% w/v, sodium-dodecyl sulphate 5% w/v and 2-mercaptoethanol 5% v/v, and boiled for 5 min. Disrupted sample preparations were separated on a vertical sodium-dodecyl sulphate polyacrylamide-slab gel (SDS-PAGE) (Laemmli, 1970), with a stacking gel of acrylamide 5%

Table I. Clinical features of the patients*

Patient no.	Age (y)	Sex	HLA-B27	Bacterial isolation†	Clinical symptoms				
					Abdominal pain	Diarrhoea	Arthritis‡	Other sequelae	Highest ESR value (mm/h)
1	17	M	–	–	+	–	–	–	80
2	25	M	–	–	+	+	–	–	46
3	28	F	–	–	+	+	–	–	12
4	31	F	–	+	+	+	–	–	...
5	35	M	+	+	+	–	–	–	91
6	38	F	–	+	+	+	–	–	64
7	49	F	–	–	+	+	–	–	24
8	57	F	–	–	–	+	–	–	59
9	32	M	–	+	–	–	+	–	13
10	36	F	+	+	+	+	+	–	45
11	37	M	–	–	+	+	+	–	64
12	39	M	+	+	+	+	+	–	66
13	56	M	+	–	+	–	+	Iritis	27
14	56	M	+	–	+	+	+	–	30
15	56	M	+	–	+	+	+	–	23
16	65	F	–	–	–	+	+	–	59

* All patients showed a clear response in terms of specific IgM-, IgG- or IgA-class antibodies against *Y. enterocolitica* O3 as detected by ELISA.

† *Y. enterocolitica* serovar O3 was (+), or was not (–), isolated.

‡ Definite synovitis, confirmed by a physician, was (+), or was not (–), present.

and a resolving gradient gel of 10–12.5%. Standards of known mol. wt were included in each gel run (Electrophoresis Low Molecular Weight Calibration Kit, Pharmacia Fine Chemicals AB, Uppsala, Sweden). Bromophenol Blue was used as the tracking dye. Proteins were usually visualised with Coomassie Brilliant Blue R 250, although some gels were silver stained with a commercially available system (Bio-Rad Silver Stain, Bio-Rad Laboratories, Richmond, CA, USA).

Immunoblotting was performed by a modification of the method of Towbin *et al.* (1979). After electrophoresis, gels were transferred immediately to the electrotransfer apparatus (Transphor, LKB-Bromma, Bromma, Sweden). The separated components were transferred to nitrocellulose sheets (HAHY 00100, pore size 0.45 µm, Millipore SA, France) in pre-chilled Tris-glycine buffer (pH 8.3), at 1.0 A for 1.5 h. Non-specific binding sites were blocked by overnight incubation at 4°C of the nitrocellulose sheet in PBS (pH 7.4) containing horse serum 10% v/v (HS-PBS). After five washes with PBS, the sheets were cut into vertical strips and allowed to react overnight at 4°C with a 1 in 100 dilution of patient's serum in HS-PBS. The strips were washed as before and incubated for 3 h at room temperature with horseradish peroxidase-labelled rabbit anti-human IgM, IgG or IgA, specific for heavy chains (DAKO Immunoglobulins, Copenhagen, Denmark), and diluted in HS-PBS to 1 in 1500, 1 in 1000, and 1 in 1500, respectively. After three washes, the strips were developed with 4-chloro-1-naphthol (Sigma, St Louis, MO, USA) according to the method of Hawkes *et al.* (1982).

Immunoblotting of the rabbit antiserum against *Y. enterocolitica* O3 was performed in the same way. The rabbit serum was diluted 1 in 300, and the peroxidase-conjugated swine immunoglobulins to rabbit immunoglobulins (DAKO Immunoglobulins) were diluted 1 in 1000, in HS-PBS.

HLA typing

The HLA-B27 lymphocyte antigen was determined by a cytotoxicity method with Histognost®-B27 (Behring Institut, Marburg, FRG).

Results

Demonstration of plasmid-encoded antigens

The presence of a virulence-associated plasmid in strain pYV+ of *Y. enterocolitica* serovar O3 was established by the autoagglutination test and by plasmid purification. The plasmid-free strain pYV- derived from it by differential growth on magnesium-oxalate agar at 37°C was not autoagglutinable and plasmids were no longer detectable on purification. The presence or absence of plasmid-specified antigens was always confirmed by autoag-

glutination. As a further control, the major polypeptides from whole-cell lysates of strains pYV+ and pYV- were separated by SDS-PAGE and stained by Coomassie Brilliant Blue. A major band of mol. wt 150 000 was observed in strain pYV+ only (data not shown).

When a rabbit antiserum raised against *Y. enterocolitica* serovar O3 was immunoblotted against both plasmid-containing and plasmid-free strains grown at 37°C, several plasmid-specified antigens were recognised (fig. 1); the most prominent of these were of mol. wts (10^3): 12.5, 22, 26, 32, 34, 38, 45, 49, 52.5 and 150. In addition, the

Fig. 1. Immunoblot analysis of rabbit antiserum raised against *Y. enterocolitica* O3 in tests with strain pYV+ of *Y. enterocolitica* that contained plasmid (+) or strain pYV- that was plasmid-free (-). Bacteria were grown at 37°C or at room temperature (RT). C = control serum from a non-immunised rabbit in tests with strain pYV+.

following minor antigens were detected of mol. wts (10^3): 40, 42, 70 and 80. Altogether, at least 14 plasmid-specified antigens were observed. It was of interest that an antigen of mol. wt 28 000 was apparently present in the plasmid-cured strain only. These differences were not found when the plasmid-containing and plasmid-free strains were grown at room temperature.

IgM and IgA antibodies

From each of 16 patients with yersinia infection, sera were obtained 0–2 and 10–12 months after onset of infection. By immunoblotting these 32 sera against strains pYV+ and pYV– of *Y. enterocolitica* serovar O3, IgM- and IgA-class antibodies against plasmid-specified antigens were recognised. IgM-class antibodies of several patients reacted with the plasmid-specified antigens of mol. wts (10^3) 26 and 45. IgA immunoglobulins recognised at least those antigens of mol. wts (10^3) 26, 34 and 52·5.

IgG antibodies

All patients had detectable levels of IgG antibodies to several antigenic determinants of both the pYV+ and pYV– strains, as illustrated in fig. 2, whereas the sera from the healthy controls responded to neither strain. There was considerable heterogeneity in the reactions obtained with individual sera with about 15 major and 20 minor bands revealed. The most pronounced bands were those of mol. wts (10^3) 14, 28, 33, 35, 50 and 67, present in both plasmid-containing and plasmid-free strains. The immunoblot pattern against the plasmid-containing strain showed a few other bands, the most strongly reacting of which had mol. wts (10^3) of 26, 34, 45 and 52·5.

Comparison of IgM, IgG and IgA responses

The different immunoglobulin classes generally recognised more or less the same antigens. IgM and IgA patterns were much weaker than those for IgG, and the total amount of visible IgM or IgA bands could not be counted because of intense background staining. As well as some weaker bands, the prominent plasmid-specified antigens—of mol. wts (10^3) 26, 34, 45 and 52·5—were recognised more frequently by IgG than by IgM or IgA; the latter immunoglobulins recognised, respectively, anti-

Fig. 2. Immunoblot analysis showing IgG responses against strains of *Y. enterocolitica* O3. Strain pYV+ contained plasmid (+); strain pYV– did not (–). Sera were from: a patient with uncomplicated yersiniosis, A–; a patient with reactive arthritis, A++; a healthy control, C.

gens of mol. wts (10^3) 26 and 45, and 26, 34 and 52·5 (table II).

Comparison of early and late responses

The same antigens were recognised in tests with acute and convalescent sera. It was the strength of the reactions only that varied; thus IgM showed a few, faint bands with convalescent sera whereas IgG- and IgA-reactive bands generally appeared to be quite strong at that same stage.

Table II. Reactions of different immunoglobulins with plasmid-encoded antigens of *Y. enterocolitica* serovar O3 in tests with sera from non-arthritic and arthritic patients

Immuno- globulin isotype	Arthritis in patient	Number of sera (of 8 tested) from acute (A) or convalescent (C) stages of infection* that reacted with plasmid-encoded antigens of <i>Y. enterocolitica</i> of mol. wt (10^3)							
		26		34		45		52.5	
		A	C	A	C	A	C	A	C
IgM	—	6	3	ND	ND	6	4	ND	ND
	+	4	1	ND	ND	4	2	ND	ND
IgG	—	5	2	6	1	7	3	5	0
	+	6	2	5	2	6	4	6	4
IgA	—	4	0	5	1	ND	ND	5	1
	+	4	4	7	4	ND	ND	5	4

ND = not detected.

* Sera were obtained 0–2 months (A) or 10–12 months (C) after onset of infection.

Comparison of sera from non-arthritic and arthritic patients

The qualitative responses with sera from non-arthritic and arthritic patients were directed in the same way against the individual antigens and there was no antigen, plasmid-specified or otherwise, that was exclusively detected with sera from either patient group. In an example presented in fig. 2, two antigens, the mol. wts of which were 88 000 and 92 000 were recognised exclusively with sera from arthritic or non-arthritic patients, respectively; these differences, however, were not consistent when complete groups of patients were compared. When individual bands were compared, it was obvious that their recognition with sera from the two patient groups was remarkably similar. A similar conclusion was reached when the bands were grouped and counted according to their mol. wts, as shown for IgG (fig. 3). The mean number of visible IgG bands was the same for sera from non-arthritic and arthritic groups: 34.8 (SEM 4.6) and 34.8 (SEM 5.2) at 1–2 months after onset of the infection, and 23.4 (SEM 5.3) and 23.5 (SEM 5.1) at 10–12 months, respectively. The only difference observed was that plasmid-encoded antigens of mol. wts (10^3) 26, 34 and 52.5 were more often recognised by the sera from arthritic than non-arthritic patients at 10–12 months after onset of the infection; the difference was, however, small and applied to IgA-class antibodies only (table II).

Fig. 3. IgG response (mean number of visible bands of different mol. wt) in sera of patients with uncomplicated yersiniosis (□ --- □) or with Yersinia-triggered reactive arthritis (●—●). Number of sera per group at each time point was 8; bar = $\pm$ SEM. Mol. wts (10^3) of bands were: (a) >94, (b) 67–94, (c) 43–67, (d) 30–43, (e) 20.1–30, (f) <20.

Discussion

In the present study, immunoblotting was applied to compare IgM-, IgG- and IgA-responses against the plasmid-encoded antigens of *Y. enterocolitica* serovar O3 after natural infection in man. The results obtained indicated that the antibody response was directed against several individual antigens, that different immunoglobulin classes recognised the different antigens similarly and that antibodies against the different antigens decreased uniformly with time. The IgA-response against some of the plasmid-specified antigens seemed to persist longer in arthritic than in non-arthritic patients.

The immunoblot patterns revealed that the expression of the antigens of *Y. enterocolitica* was influenced not only by the presence or absence of the virulence plasmid but also by growth temperature. Thus, several antigens were absent when the plasmid-containing pYV+ strain was grown at 37°C. Again, this phenomenon may depend on factors other than plasmid expression because a partial transition from smooth to rough lipopolysaccharides has been shown to occur at higher temperatures (Darveau *et al.*, 1983; Kawaoka *et al.*, 1983; Ogasawara *et al.*, 1985).

Recognition of the plasmid-encoded antigens was achieved by comparison of results with related plasmid-containing and plasmid free strains. First, only one prominent plasmid-encoded band could be detected by SDS-PAGE. Although this method is far less sensitive than immunoblotting, it provided, nevertheless, an appropriate control because it is known that all of the major plasmid-specified outer-membrane proteins are expressed in similar conditions (Portnoy *et al.*, 1984; Bölin *et al.*, 1985). Second, by immunoblotting a yersinia-specific rabbit antiserum against both strains, at least 14 plasmid-encoded antigens were revealed, a finding in agreement with that of Portnoy *et al.* (1984) who detected ≥ 20 plasmid-specified polypeptides by SDS-PAGE and autoradiography after plasmids of yersiniae were transformed into minicell-producing *Escherichia coli*. Again, Skurnik (1985) by immunoblotting rabbit anti-yersinia serum found ≥ 16 polypeptides apparently associated with the virulence plasmid; the mol. wt distribution of the bands was similar to that found in the present study. Perhaps because of varying growth conditions, fewer plasmid-encoded antigens have been reported by others (Portnoy and Martinez, 1985).

According to the nomenclature proposed by Bölin *et al.* (1985), the plasmid-specified major

outer-membrane proteins of *Yersinia* (YOPs) should be designated YOP1–5. Most of the YOPs were detected with the whole-cell lysate used in the present study. The YOP1 of mol. wt 150 000 was rarely seen in the immunoblot patterns with sera from most patients. However, the observed bands of mol. wt 52 500 most probably represented subunits of YOP1, degraded by the denaturing conditions used (Skurnik *et al.*, 1984). Likewise, the bands of mol. wts (10^3) 45, 34 and 26 detected with several of our sera corresponded to YOPs 2, 4 and 5, respectively. YOP3 of mol. wt 40 000 was not detected with the human sera used in this study; nor was it detected with sera against *Y. pestis* in the study of Mazza *et al.* (1985) who, nevertheless, showed antibody responses to the antigens of mol. wts 26 000 and 34 000. Thus, YOP3 is either poorly antigenic for man during natural infection or is denatured under the conditions used. The antigens of mol. wts (10^3) 45, 34 and 26 also corresponded to the released proteins studied by Heesemann *et al.* (1984). Furthermore, it seems likely that the YOPs and the released proteins are identical antigens; whether they are integrated into the outer membrane or released to the surrounding medium apparently depends on the growth conditions used.

Antibody response against the plasmid-specified antigens, whether considered as the total number of bands observed or as bands in regions of different mol. wt, appeared quite similar in patients without post-infection arthritis and in those developing reactive arthritis after yersinia infection. Examination of individual bands, either encoded by the plasmid or otherwise expressed at 37°C, revealed no clear differences between the two patient groups for any of the immunoglobulin classes studied. Special attention should be given, however, to the IgA-mediated recognition of bands of mol. wts (10^3) 26, 34 and 52.5 because these bands were recognised almost exclusively in tests with sera from arthritic patients taken in the convalescent stage. These bands most probably represent the plasmid-encoded YOPs and the subunit of YOP1, as described by Bölin *et al.* (1985). The present findings, taken together with previous results (Toivanen *et al.*, 1985), indicate that the differences in the antibody responses of non-arthritic and arthritic patients are quantitative rather than qualitative. The quantitative difference, particularly the strong and persisting IgA response as detected by ELISA, has been discussed previously (Granfors, 1979; Granfors *et al.*, 1980 and 1983; Toivanen *et al.*, 1985).

The excellent technical assistance of Mrs S. Tuomola is greatly appreciated. We are grateful to Dr J. Heesemann, Hamburg, and Dr H. Wolf-Watz, Umeå, for fruitful discussions, and to Mr L. Pulkkinen who provided the plasmid-free strain. This work was supported by grants from the US Public Health Service

(grant no. 5 RO 1 AM 33311 awarded by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases) and the Sigrid Jusélius Foundation which are gratefully acknowledged.

REFERENCES

- Bölin I, Portnoy D A, Wolf-Watz H 1985 Expression of the temperature-inducible outer membrane proteins of *Yersinia*. *Infection and Immunity* **48**: 234–240.
- Darveau R P, Charnetzky W T, Hurlbert R E, Hancock R E W 1983 Effects of growth temperature, 47-megadalton plasmid, and calcium deficiency on the outer membrane protein porin and lipopolysaccharide composition of *Yersinia pestis* EV76. *Infection and Immunity* **42**: 1092–1101.
- Granfors K 1979 Measurement of immunoglobulin M (IgM), IgG, and IgA antibodies against *Yersinia enterocolitica* by enzyme-linked immunosorbent assay: persistence of serum antibodies during disease. *Journal of Clinical Microbiology* **9**: 336–341.
- Granfors K, Isomäki H, von Essen R, Maatela J, Kalliomäki J L, Toivanen A 1983 *Yersinia* antibodies in inflammatory joint diseases. *Clinical and Experimental Rheumatology* **1**: 215–218.
- Granfors K, Viljanen M, Tiilikainen A, Toivanen A 1980 Persistence of IgM, IgG, and IgA antibodies to *Yersinia* in *Yersinia* arthritis. *Journal of Infectious Diseases* **141**: 424–429.
- Hawkes R, Niday E, Gordon J 1982 A dot-immunobinding assay for monoclonal and other antibodies. *Analytical Biochemistry* **119**: 142–147.
- Heesemann J, Algermissen B, Laufs R 1984 Genetically manipulated virulence of *Yersinia enterocolitica*. *Infection and Immunity* **46**: 105–110.
- Heesemann J, Keller C, Morawa R, Schmidt N, Siemens H J, Laufs R 1983 Plasmids of human strains of *Yersinia enterocolitica*: molecular relatedness and possible importance for pathogenesis. *Journal of Infectious Diseases* **147**: 107–115.
- Higuchi K, Smith J L 1961 Studies on the nutrition and physiology of *Pasteurella pestis*. VI. A differential plating medium for the estimation of the mutation rate to avirulence. *Journal of Bacteriology* **81**: 605–608.
- Kawaoka Y, Otsuki K, Tsubokura M 1983 Growth temperature-dependent variation in the bacteriophage-inactivating capacity and antigenicity of *Yersinia enterocolitica* lipopolysaccharide. *Journal of General Microbiology* **129**: 2739–2747.
- Laemmli U K 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685.
- Laird W J, Cavanaugh D C 1980 Correlation of autoagglutination and virulence of *Yersinia*. *Journal of Clinical Microbiology* **11**: 430–432.
- Martinez R J 1983 Plasmid-mediated and temperature-regulated surface properties of *Yersinia enterocolitica*. *Infection and Immunity* **41**: 921–930.
- Mazigh D, Chalvignac M A, Quilici M L, Mollaret H H 1985 Lack of correlation between plasmid-encoded outer membrane proteins and virulence of *Yersinia enterocolitica*. *Annales de L'Institut Pasteur (Microbiologie)* **136B**: 39–47.
- Mazza G, Karu A E, Kingsbury D T 1985 Immune response to plasmid- and chromosome-encoded *Yersinia* antigens. *Infection and Immunity* **48**: 676–685.
- Ogasawara M, Granfors K, Kono D H, Hill J L, Yu D T Y 1985 A *Yersinia enterocolitica* serotype O:3 lipopolysaccharide-specific monoclonal antibody reacts more strongly with bacteria cultured at room temperature than those cultured at 37°C. *Journal of Immunology* **135**: 553–559.
- Portnoy D A, Martinez R J 1985 Role of a plasmid in the pathogenicity of *Yersinia* species. *Current Topics in Microbiology and Immunology* **118**: 29–51.
- Portnoy D A, Wolf-Watz H, Bölin I, Beeder A B, Falkow S 1984 Characterization of common virulence plasmids in *Yersinia* species and their role in the expression of outer membrane proteins. *Infection and Immunity* **43**: 108–114.
- Pulkkinen L, Granberg I, Granfors K, Toivanen A 1986 Restriction map of virulence plasmid in *Yersinia enterocolitica* O:3. *Plasmid* **16**: 225–227.
- Simonet M, Berche P, Mazigh D, Veron M 1985 Protection against *Yersinia* infection induced by non-virulence-plasmid-encoded antigens. *Journal of Medical Microbiology* **20**: 225–231.
- Skurnik M 1985 Expression of antigens encoded by the virulence plasmid of *Yersinia enterocolitica* under different growth conditions. *Infection and Immunity* **47**: 183–190.
- Skurnik M, Bölin I, Heikkinen H, Piha S, Wolf-Watz H 1984 Virulence plasmid-associated autoagglutination in *Yersinia* spp. *Journal of Bacteriology* **158**: 1033–1036.
- Toivanen A, Granfors K, Lahesmaa-Rantala R, Leino R, Ståhlberg T, Vuento R 1985 Pathogenesis of *Yersinia*-triggered reactive arthritis: Immunological, microbiological and clinical aspects. *Immunological Reviews* **86**: 47–70.
- Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the USA* **76**: 4350–4354.
- Zink D L *et al.* 1980 Plasmid-mediated tissue invasiveness in *Yersinia enterocolitica*. *Nature* **283**: 224–226.