Persistence and in vivo effects of *Yersinia enterocolitica* 0:3 endotoxin in rats

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Abstract

In vivo effects of *Yersinia enterocolitica* 0:3 lipopolysaccharide (prepared from bacteria grown at 25°C and 37°C) were investigated after intraperitoneal (i.p.) and intraarticular (i.a.) injection in rats during 30 days of examination. The persistence of endotoxin in the peritoneal and the synovial cavities was demonstrated by the immunofluorescence technique. Peritoneal and synovial exudative cell infiltration, as well as changes in some parameters (glycolytic and acid phosphatase activity, and killing ability of peritoneal cells; lactate dehydrogenase concentration in synovial fluid) were studied. The results indicated that endotoxin could persist longer in the synovial than in the peritoneal cavity. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Endotoxin; *Yersinia enterocolitica*; Peritoneal response; Synovial response

1. Introduction

Bacterial lipopolysaccharides (LPS), in contrast to most protein antigens, are relatively resistant to digestion and may persist in the host for relatively long periods of time, retaining their biological activity [7]. Granfors [10] reported that *Yersinia enterocolitica* antigens were not properly eliminated during acute inflammation in humans, and could persist continuously. The prolonged persistence of *Yersinia* antigens in synovial cells may be a primary cause of maintenance of inflammatory host responses in the joints of patients with reactive arthritis due to *Yersinia* infection [15]. Recent studies of many authors [5,8,9, 11,12,27,29] contribute to the elucidation of mechanisms for the development of reactive arthritis. The structures found in the synovial fluid phagocytes of patients with reactive arthritis after *Yersinia* infection have usually been the LPS characteristic of the causative bacterium [14]. It is currently not known exactly how long *Yersinia* antigens could persist in the absence of live bacteria and what its exact role is in the pathogenesis of reactive arthritis. Of special interest are the chromosome-encoded temperature-
inducible differences in LPS structures of *Y. enterocolitica*. The O-side chain of the LPS of *Y. enterocolitica* 0:3 has been shown by chemical analysis to be shorter when synthesized at 37°C than at 25°C [2,17,28]. The percentage of neutral sugars in *Y. enterocolitica* cells grown at 37°C was much lower than that in *Y. enterocolitica* cells grown at 25°C and the ratio of 6-deoxy-L-altrose to heptose in LPS synthesized at 37°C was also lower than that in LPS synthesized at 25°C [2]. With respect to immunological complications after *Yersinia* infections the temperature-dependent LPS variations may be of clinical importance for their immunogenic properties.

In order to study the role of *Y. enterocolitica* LPS in the pathogenesis of reactive arthritis, respectively its fate (persistence and biological activity in vivo) in two different compartments (peritoneal and synovial cavities) we established a model using rats intraperitoneally (i.p.) and intraarticularly (i.a.) injected with *Y. enterocolitica* LPS (prepared from bacteria grown at 25°C and 37°C).

2. Materials and methods

2.1. Bacterial strain and LPS extraction

Virulent plasmid-bearing *Y. enterocolitica* 0:3 201/86 strain was used (received from Dr. G. Kapperud, Oslo University, Norway) [16]. Bacteria were grown at 25°C or 37°C on tryptic soy agar (Difco, USA) in Roux bottles for 48 h. The LPS from *Y. enterocolitica* grown at 25°C or 37°C was isolated from washed whole bacteria by the trichloroacetic acid extraction procedure of Boivin and Mesrobeanu [3]. After precipitation (0.5 N trichloroacetic acid) the LPS was dialyzed extensively against water for 96 h and then washed twice in frozen ethanol (99.8%, Merck, Germany) and in acetone, and lyophilized. The protein content of this LPS preparations was 15% (w/w) and was measured with bovine serum albumin as standard by the method of Lowry et al. [19]. The carbohydrate content was determined by the phenol-sulfuric acid method with glucose as standard [6]. To confirm the differences in O-side chain length of LPS produced by *Y. enterocolitica* 0:3 at 25°C and 37°C (a fact substantially proven by other authors [2,18,22,28]), we carried out additional gas chromatography analysis of the content of the O-specific sugar 6-deoxy-L-altrose by the method of Acker et al. [1]. It was found that LPS isolated from bacteria grown at 25°C contains about 29% (w/w) 6-deoxy-L-altrose, while LPS isolated from bacteria grown at 37°C contained 17% (w/w) of 6-deoxy-L-altrose. LPS, made up fresh for each experiment, was suspended in 0.15 M phosphate buffered saline (PBS).

2.2. Experimental animals

Male and female Lewis rats weighing 200–250 g (10–12 weeks old) were used in all experiments. The animals were obtained from a local breeder (National Centre for Laboratory Animals, Sofia, Bulgaria). Rats were free of specific pathogens. The animals were divided into four experimental groups according to the mode of application (i.p. or i.a.) and the type of LPS (isolated from bacteria grown at 25 or 37°C). Two groups of rats were inoculated with 0.1 ml PBS containing 25 µg LPS (isolated from bacteria grown at 25 or 37°C) into the ankle joint and, another two groups with 0.5 ml PBS containing 200 µg LPS (isolated from bacteria grown at 25 or 37°C) into the peritoneal cavity. Three to five animals were used at each experimental time point in all experiments. The experiments were repeated three times. Our previous dose-response experiments showed that these doses were optimal for the present investigations [26]. An injection of these doses was not lethal for rats. Control animals were i.p. injected with 0.5 ml of PBS and i.a. with 0.1 ml of PBS.

The experiments described in this article were performed in accordance with National Institutes of Health guidelines on the use of experimental animals. All experiments were performed in accordance with protocols approved by the Sofia State University Animal Use Committee.

2.3. Harvesting of peritoneal cells (PC)

The PC were harvested by lavage of the peritoneal cavity of rats with 25 ml of cold Hanks solution per rat at periodic intervals following i.p. injection of LPS (3, 7, 14, 21, and 30 days). The lavage fluid (about 20 ml) from each individual animal was centrifuged at 150×g for 20 min, resuspended in 1 ml of
medium and counted with the Cobas Helios cell counting system (Hoffman La Roche), based on the electronic conductive principle. The cell suspension used for the metabolic assays was collected from three animals, washed with saline three times and resuspended to a final concentration of $20 \times 10^6$ cells ml$^{-1}$. Purity of PC was $\cong 85\%$, and viability, as determined by trypan blue exclusion, was $\cong 90\%$ in all cases.

2.4. Determination of metabolic activity of PC

2.4.1. Glycolytic activity

Glycolysis was assayed according to Barker and Summerson with some modifications [24]. Lactic acid production was measured in the reaction mixture with the following components in 2.5 ml: 80 $\mu$mol of Tris buffer (pH 7.2), 60 $\mu$mol of glucose, 25 $\mu$mol of KCN and $20 \times 10^7$ PC. After a 2-h incubation at 37°C without shaking, 0.5 ml (for each sample) of 30% trichloroacetic acid was added. The amount of lactic acid was determined in protein-free supernatant. In the presence of sulfuric acid and p-hydroxybiphenyl, the lactic acid is turned into a purple compound. The intensity of this coloration was measured at a wavelength of $\approx 540$ nm in a Zeiss Spekol model 11 spectrophotometer (Jena, Germany). The results are expressed as $\mu$g of lactic acid released by $20 \times 10^6$ cells in 2 h.

2.4.2. Acid phosphatase activity

Acid phosphatase was assayed essentially as described by Radoucheva et al. [25]. The cell suspensions with a density of $20 \times 10^6$ cells ml$^{-1}$ were incubated for 5 h at 37°C in the presence of sodium $\beta$-glycerophosphate (0.05 M) at pH 5.4 (0.2 M sodium acetate-citric acid buffer). The amount of inorganic phosphate was determined spectrophotometrically in protein-free supernatant. The inorganic phosphate reacted with ammonium molybdate and 1-amino-2-naphthol-4-sulfonic acid. The obtained product has a blue color. The intensity of this coloration was measured at a wavelength of $\approx 650$ nm in a Zeiss Spekol model 11 spectrophotometer (Jena, Germany). The results are expressed as $\mu$g of inorganic phosphate released by $20 \times 10^6$ cells in 5 h.

All chemicals used in metabolic activity determinations were from Sigma Chemicals Co. (USA).

2.5. Assay for intracellular bacterial survival (‘killing’ ability)

The number of PC was adjusted to $1 \times 10^6$ cells ml$^{-1}$. ‘Killing’ ability was determined according to the method described by Van Furth et al. [30]. A total of 1 ml of the cell suspension ($1 \times 10^6$ cells ml$^{-1}$) was added to Leighton test tubes. The cells were allowed to attach for 1 h at 37°C under 5% CO$_2$. Following attachment, cultures were washed with Hanks solution to remove non-adherent cells. To the monolayers was added 0.1 ml of bacterial suspension ($5 \times 10^8$ cfu ml$^{-1}$) of $Y. \text{enterocolitica}$ 0:3 and after 30 min incubation at 37°C (zero time for starting the killing assay), the monolayers were washed 15 times with cold Hanks solution (4°C) to remove extracellular bacteria. At time zero and after 2 h, 1 ml of distilled water was added to the Leighton monolayers. The numbers of viable bacteria before and after incubation were determined by dilution and lysis of the reaction mixtures in sterile distilled water and plating on tryptic soy agar (Difco, USA). The plates were incubated at 37°C for 48 h and colonies were enumerated. Results are expressed as the ratio of initial bacterial number at time zero and the bacterial number after 2 h incubation with PC.

2.6. Assessment of synovial response

Synovial fluid samples were obtained from rats killed at 1, 3, 7, 14, 21 and 30 days after administration of LPS. Synovial fluid was aspirated from ankle joints after injection of 0.1 ml PBS. Synovial cell count was measured with the Cobas Helios cell counting system (Hoffman La Roche). Lactate dehydrogenase activity (LDH) in synovial fluid samples was measured automatically with a Cobas Mira analyzer using the kinetic UV test according to the recommendations of the German Clinical Chemistry Association. Determination of LDH activity involved a system containing 0.6 mmol l$^{-1}$ sodium pyruvate, 0.18 mmol l$^{-1}$ NADH, and 50.0 mmol l$^{-1}$ phosphate buffer (pH 7.5). The rate of decrease of the NADH concentration was determined photometrically at 340 nm every minute for 3 min and was directly proportional to the LDH activity in the sample material.
Arthritis was defined as visible joint swelling and the joint diameter was estimated with calipers (made in the Central Instrumental Laboratory of the Bulgarian Academy of Sciences, Sofia, Bulgaria).

2.7. Preparation of specific antisera

Inbred BALB/c mice received three times weekly i.p. 0.1-ml (in the first week) and 0.2-ml (in weeks 2, 3, 4, and 5) injections of *Y. enterocolitica* 0:3 antigen suspension (10⁹ bacteria ml⁻¹) prepared from a 48-h culture on tryptic soy agar at 25°C or 37°C. The suspensions were washed with sterile saline and inactivated at 100°C for 1 h before injection. Six weeks after immunization, the titer of antibodies was measured by the indirect immunofluorescence technique. The titer of antibodies against *Y. enterocolitica* 0:3 grown at 25°C was 1:640 and against *Y. enterocolitica* 0:3 grown at 37°C 1:340. Sera from five animals were pooled, mixed and frozen in aliquots at −70°C until use.

2.8. Indirect immunofluorescence (IF) assay

Thin smears from synovial and peritoneal fluid samples were prepared on glass slides, dried in air and fixed with cold acetone at 4°C for 10 min. Slides were then stored at 4°C until use. For immunofluorescence staining, the slides were overlaid with specific mouse anti-*Y. enterocolitica* 0:3 serum at a dilution of 1:16 and incubated at 37°C in humidified chambers for 30 min. The slides were then washed three times with PBS and incubated at 37°C for 30 min with a mixture of fluoresceinated goat anti-mouse IgG (1:6) and labeled with rhodamine B isothiocyanate bovine serum albumin (counter-stain for non-specific fluorescence). After three washes, the slides were dried, mounted with PBS/glycerol (1:9 v/v) and finally read under a fluorescence microscope Fluo-wall (VEB, Zeiss Jena, Germany). The preparations showed a clear, sharp fluorescence of the LPS. As controls we used: (i) slides made in the same way from animals injected i.p. and i.a. with saline; (ii) slides prepared without treatment with specific anti-*Y. enterocolitica* 0:3 serum, only with FITC; (iii) slides prepared with antisera first absorbed with immunizing antigen.

2.9. Statistical analysis

The results are expressed as the mean±S.D. of values obtained from 3–5 animals. Data were analyzed by the method of Student-Fisher, involving determination of the statistical significance of the changes through the values of the *t*-criterion. The difference was considered to be significant at *P* < 0.05. The results were obtained from three separate experiments.

3. Results

3.1. Monitoring of peritoneal cell response

Samples of peritoneal washings were collected and evaluated at 3, 7, 14, 21 and 30 days after i.p. administration of LPS (Table 1). The cellular infiltrate response to LPS synthesized at 25°C found at 3 and 7 days (2.3 ± 0.2 and 2.5 ± 0.1 × 10³ ml⁻¹) did not differ significantly from that found to LPS synthesized at 37°C (2.0 ± 0.1 and 1.9 ± 0.2 × 10³ ml⁻¹) at the same time points. The cell number in the peritoneal cavity during the next 14, 21 and 30 days was reduced in both groups, reaching approximately the control values at day 30. The glycolytic activity of peritoneal cells increased after administration of LPS synthesized at 25°C from 30 ± 2.3 µg of lactic acid (control value) to 151 ± 4.5 µg on the 3rd day to 133 ± 2.6 µg on the 7th day, and after application of LPS synthesized at 37°C to 124 ± 5 µg (3rd day) and to 92.3 ± 3.2 µg (7th day). Similar to the dynamics of glycolytic activity, the acid phosphatase activity of peritoneal cells also increased on the 3rd and 7th days after administration of LPS and the enhancement of acid phosphatase activity was stronger in the group inoculated with LPS synthesized at 25°C than in the group inoculated with LPS synthesized at 37°C. The glycolytic and acid phosphatase activities of peritoneal cells decreased and reached the control values in both experimental groups during the next days of examination (14, 21 and 30). Most interesting was the finding that ‘killing’ ability of peritoneal cells after administration of LPS synthesized at 25°C was stronger than after application of LPS synthesized at 37°C on the 3rd and 7th days. ‘Killing’ ability values of peritoneal cells stimulated
by LPS synthesized at 25°C were 35 ± 3.5 on the 3rd day and 22.5 ± 1.4 on the 7th day, while ‘killing’ ability values of peritoneal cells stimulated by LPS synthesized at 37°C were 18 ± 1.9 on the 3rd day and 12.5 ± 2.2 on the 7th day. During the next days the ‘killing’ ability values in both groups were similar to the control ones.

### 3.2. Monitoring of synovial response

Rats injected i.a. with a dose of 25 µg per joint were checked at various time points for development of arthritis. Arthritis was defined as visible joint swelling and evaluated increase in joint diameter, determination of synovial cell number and LDH activity in synovial fluid (Table 2). On the 1st day after injection the paws of rats became red and swollen.

### Table 1
Dynamics of peritoneal cell response to i.p. application of LPS prepared from *Y. enterocolitica* 0:3 grown at 25 and 37°C

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Number of PC×10³ ml⁻¹</th>
<th>Glycolytic activity of PC (µg of lactic acid)</th>
<th>Acid phosphatase activity (µg of inorganic phosphate)</th>
<th>Killing ability of PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>1.2 ± 0.1</td>
<td>30 ± 2.3</td>
<td>4.5 ± 0.5</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>LPS synthesized at 25°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.3 ± 0.2 a</td>
<td>151 ± 4.5 a</td>
<td>25 ± 1.1 a</td>
<td>35.0 ± 3.5 a</td>
</tr>
<tr>
<td>7</td>
<td>2.5 ± 0.1 a</td>
<td>133 ± 2.6 a</td>
<td>23 ± 2.1 a</td>
<td>22.5 ± 1.4 a</td>
</tr>
<tr>
<td>14</td>
<td>1.4 ± 0.1</td>
<td>70 ± 3.5 a</td>
<td>6 ± 0.7</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>21</td>
<td>1.4 ± 0.1</td>
<td>34 ± 2.7</td>
<td>5 ± 1.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>30</td>
<td>1.3 ± 0.1</td>
<td>32 ± 2.4</td>
<td>5 ± 0.7</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>LPS synthesized at 37°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.1 ± 0.1 a</td>
<td>124 ± 5 a;b</td>
<td>16 ± 2.1 a;b</td>
<td>18.0 ± 1.9 a;b</td>
</tr>
<tr>
<td>7</td>
<td>1.9 ± 0.2 a</td>
<td>92 ± 3.2 a;b</td>
<td>10 ± 2.5 a;b</td>
<td>12.5 ± 2.2 a;b</td>
</tr>
<tr>
<td>21</td>
<td>1.2 ± 0.1</td>
<td>72 ± 2.1 a</td>
<td>6 ± 1</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>1.3 ± 0.2</td>
<td>31 ± 1.6</td>
<td>5 ± 0.9</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>a</td>
<td>Significantly different compared to saline control.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>Significantly different compared to LPS synthesized at 25°C.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2
Dynamics of synovial response to i.a. application of LPS prepared from *Y. enterocolitica* 0:3 grown at 25°C and 37°C

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Mean ankle diameter (mm)</th>
<th>Number of synovial cells per joint</th>
<th>LDH activity in synovial fluid (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>8.2 ± 0.1</td>
<td>100 – 200</td>
<td>197 ± 24</td>
</tr>
<tr>
<td>LPS synthesized at 25°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.0 ± 0.2</td>
<td>1300 ± 100 a</td>
<td>1078 ± 103 a</td>
</tr>
<tr>
<td>3</td>
<td>8.5 ± 0.2</td>
<td>550 ± 100 a</td>
<td>996 ± 48 a</td>
</tr>
<tr>
<td>7</td>
<td>8.5 ± 0.1</td>
<td>540 ± 100 a</td>
<td>865 ± 41 a</td>
</tr>
<tr>
<td>14</td>
<td>8.2 ± 0.2</td>
<td>430 ± 200 a</td>
<td>306 ± 53 a</td>
</tr>
<tr>
<td>21</td>
<td>8.2 ± 0.1</td>
<td>390 ± 200 a</td>
<td>293 ± 47 a</td>
</tr>
<tr>
<td>30</td>
<td>8.2 ± 0.2</td>
<td>310 ± 100</td>
<td>306 ± 39 a</td>
</tr>
<tr>
<td>LPS synthesized at 37°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.7 ± 0.2</td>
<td>1120 ± 200 a b</td>
<td>676 ± 35 a;b</td>
</tr>
<tr>
<td>3</td>
<td>8.5 ± 0.1</td>
<td>410 ± 200 a</td>
<td>1390 ± 95 a</td>
</tr>
<tr>
<td>7</td>
<td>8.5 ± 0.1</td>
<td>520 ± 200 a</td>
<td>610 ± 54 a;b</td>
</tr>
<tr>
<td>14</td>
<td>8.2 ± 0.1</td>
<td>580 ± 200 a</td>
<td>306 ± 34 a</td>
</tr>
<tr>
<td>21</td>
<td>8.2 ± 0.1</td>
<td>370 ± 100 a</td>
<td>338 ± 44 a</td>
</tr>
<tr>
<td>30</td>
<td>8.2 ± 0.2</td>
<td>410 ± 100 a</td>
<td>244 ± 45 a</td>
</tr>
<tr>
<td>a</td>
<td>Significantly different compared to saline control.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>Significantly different compared to LPS synthesized at 25°C.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The mean ankle diameter increased, whereas the control animals (injected with saline) it remained unchanged. The number of synovial cells increased from 100–200 per joint (control values) to 1300 ± 100 cells for LPS synthesized at 25°C and to 1120 ± 200 cells for LPS synthesized at 37°C. LDH activity in synovial fluid increased approximately 5 times in joints injected with LPS synthesized at 25°C and 3 times in joints injected with LPS synthesized at 37°C. The clinical arthritic symptoms remained mild for the next 7 days and after that they disappeared. Slight synovial cell infiltration and elevated level of LDH activity were observed to the 30th day of examination, which revealed the maintenance of inflammation in joints. No significant differences between the arthritogenic effects of LPS synthesized at 25°C and 37°C were found.

### 3.3. Immunofluorescence findings

*Y. enterocolitica* 0:3 antigens (from bacteria grown at 25°C and 37°C) were detected by indirect immunofluorescence microscopy until the 7th day in the peritoneal cavity (Fig. 1a,b). LPS structures fluoresced a bright yellow-green, and host cells had an orange color with the rhodamine counter-stain. The antigens from bacteria grown at 25°C were found in the synovial cavity until the 21st day and antigens from bacteria grown at 37°C until the 14th day after inoculation (Fig. 1c).

### 4. Discussion

In the present study, we used two experimental models (i.p. and i.a. administration of LPS in rats) to investigate the persistence and biological effects of LPS prepared from *Y. enterocolitica* grown at 25°C and 37°C. The fate of LPS inside the peritoneal and synovial cavities might reveal some of the mechanisms for its incidence and persistence, which are of potential critical importance to the pathogenesis of *Yersinia*-induced reactive arthritis. Generally, the action of purified LPS in experiments is focused on its effect at the cellular level and especially on the consequence of functional and metabolic changes in phagocytic cells. Glycolytic and acid phosphatase activities as real indicators of phagocyte cells recruitment and activation [20] were used to indicate changes (an increase) in the functional achievement of the cells after treatment with endotoxin. The results showed that LPS synthesized at 37°C manifested lower biological activity with respect to the peritoneal leukocytes than LPS synthesized at 25°C. Typical alterations in the metabolism (increased glycolytic and acid phosphatase activity) of peritoneal cells were observed after administration of LPS. Generally, LPS synthesized at 25°C was shown to induce an influx of peritoneal leukocytes with higher glycolytic and acid phosphatase activity and to provoke more enhanced ‘killing’ ability of peritoneal macrophages than LPS synthesized at 37°C. It has been reported that the temperature-dependent synthesis of chromosome- and plasmid-encoded surface structures (LPS, Yop proteins) determines, in part, the phlogistic and antigenic properties of *Yersiniae* [2,4,13,23]. The length of the LPS O-side chain of *Y. enterocolitica* 0:3 has been shown to be shorter when organisms are grown at 37°C than at 25°C [28]. Kawaoka et al. [18] found that the immunogenicity of LPS of *Y. enterocolitica* in vivo was similar to that of the bacteria grown in vitro at 25°C. Obviously, the length of the O-antigen, i.e. the number of repeating units, correlates with its biological activity and the reduced amount of O-polysaccharides in LPS synthesized at 37°C may well provide an explanation for its lower biological activity. Moran et al. [21] have suggested that the low immunological activity of *Helicobacter pylori* LPS in the gut might allow the bacteria to survive in vivo for longer periods of time than would a more aggressive pathogen. To this effect, the temperature-dependent partial smooth-rough transition of *Y. enterocolitica* LPS that occurs at 37°C versus 25°C may be thought of as

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Fig. 1. Immunofluorescence of *Y. enterocolitica* LPS synthesized at 25°C. A: LPS associated with peritoneal cells after 1 day of i.p. injection; arrows indicate small dots and clusters of LPS structures, located extracellularly. B: Attachment (arrows) of LPS structures to peritoneal cells after 7 days of i.p. injection. C: Attachment (arrows) of LPS structures to synovial cells after 14 days of i.a. injection. ×1000.
as a special strategic factor, which protects the bacteria from host defence. Of interest was the finding that \textit{Y. enterocolitica} LPS was detected within the peritoneal cavity retaining its immunoreactive activity during 7 days after i.p. administration. In contrast, \textit{Y. enterocolitica} LPS persisted inside the joints for longer (21 days after i.a. administration of LPS synthesized at 25°C and 14 days after i.a. application of LPS synthesized at 37°C). Analysis of synovial samples after i.a. injection of LPS revealed its arthritogenic effect as assessed by influx of inflammatory cells and the maintenance of joint inflammation until the 30th day post-LPS treatment. The increased LDH activity in synovial fluid was also an indicator for the prolonged inflammatory process in the joints.

We conclude that the ability of \textit{Y. enterocolitica} LPS to persist in the host varies according to the tissue localization, with higher rates of persistence in joints as compared to the peritoneal cavity. The complexity of interactions of LPS with host cells appeared to depend partially on the length of the LPS side chains, as well as on the type of host cells (of different tissue origin) and their ability effectively to eliminate LPS.

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