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Yersinia enterocolitica O:8 and O:5 lipopolysaccharide arthritogenicity in hamsters

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Abstract

Objective. To assess the arthritogenicity of *Yersinia enterocolitica* O:8 and O:5 lipopolysaccharide (LPS) administered separately as single antigens in hamsters.

Methods. Male hamsters of the Syria strain were intramuscularly injected into each of the hind paws with two doses of *Y. enterocolitica* LPS O:8 or O:5. The measurement of swelling using a plethysmometer, the analysis of histological changes by routine techniques and the kinetics of LPS-specific antibodies and autoantibodies evaluated by enzyme-linked immunosorbent assay (ELISA) were performed.

Results. LPS O:8 was demonstrated to be more arthritogenic than LPS O:5, inducing acute arthritis on day 3 post-injection as well as more significant and longer lasting joint swelling after a second dose. LPS O:8 caused severe histopathological changes in the joints. Important LPS O:8-specific IgG responses and antibodies against type I and II collagen were observed.

Conclusion. LPS O:8 administered alone has arthritogenic power and induces activation of autoreactive clones. This study supports the key role of LPS in the development of reactive arthritis.

KEY WORDS: Reactive arthritis, Lipopolysaccharide, Hamsters, Autoantibody.

Yersinia enterocolitica causes intestinal infection in humans and rodents. Reactive arthritis (ReA) is a known complication of certain gastrointestinal infections, such as those caused by *Y. enterocolitica.* It has been suggested that either microbial antigens or intact pathogens may be important for the pathogenesis of ReA, at least in the early phase of the disease [1]. The antibody response is directed mostly against lipopolysaccharide (LPS), an outer membrane structure common to all arthritis-inducing pathogens. LPS consists of three regions: lipid A, core oligosaccharide and O-antigen. Lipid A and core are highly conserved structurally and genetically within a given genus [2], but O-antigen varies considerably among bacterial species as regards sugar composition, structure, linkage between sugar residues and antigenicity [3]. The existence of *Yersinia* LPS in synovial samples of patients with *Yersinia*-triggered ReA has also been demonstrated [4].

LPS has been found to play a major role as an arthritis-triggering antigen in rats [5, 6], mice [7], rabbits [8] and hamsters [9, 10]. The choice of the hamster as an LPS-induced arthritis model in the present work was made taking into consideration that the specific features of the host as well as of the pathogen must coincide [11, 12]. Lim *et al.* proposed the hamster as an animal model for the study of *Borrelia burgdorferi*-induced Lyme arthritis since its propensity for the development of arthritis may lead to a better understanding of the immune mechanisms responsible for this disease [13]. In previous studies, hamsters have been used for the study of ReA induced by virulence plasmid-bearing *Y. enterocolitica* B1 O:5 Lis Xz and *Y. enterocolitica* 1821 O:8 [14, 15]. Animals were injected intramuscularly (i.m.) into their hind paws with 10^8–10^9 colony-forming units (c.f.u.)/animal. The development of arthritis was estimated by measuring inflammation and correlating with histological studies and serological assessment of the immune response. In these studies a constant immunoglobulin response against LPS was observed. This response reached a peak in the moment of development of chronic joint inflammation [14, 15]. In addition, no viable bacteria were detected in the joints of *Y. enterocolitica* O:8- or O:5-infected hamsters during the joint chronic inflammatory peak, but bacterial antigens were detected by immunofluorescence during this peak of arthritis [15].

The aim of the present work was to study O:8 and O:5 LPS arthritogenicity administered separately in hamsters. Histopathological changes, antibodies in serum and polyclonal activation were analysed.

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Materials and methods

Animals

Male hamsters of the Syria strain weighing between 60 and 120 g were purchased from Casa Izaguirre (Moron,

Buenos Aires, Argentina). They were kept at room temperature and given food and water *ad libitum.*

Bacteria

Yersinia enterocolitica strain 1821 serotype O:8 pYV (+) kindly provided by Dr Kapperud (Department of Bacteriology, Oslo, Norway), and virulent plasmid-bearing *Y. enterocolitica* B1 O:5 Lis Xz isolated from food samples [16] were used.

Cultures

The bacteria were cultured at room temperature in tryptic soy broth (TSB) on an orbital shaker (3 Hz frequency) for 18 h. Bacterial cells were collected by centrifugation and washed with 0.9% NaCl. Counts of viable cells were performed on tryptic soy agar (TSA).

LPS

LPS was obtained by extraction with hot phenol–water from *Y. enterocolitica* O:8 and O:5 using the method of Westphal *et al.* [17]. LPS contained less than 2% protein, as determined by Lowry's procedure [18], and 3–4% 2-keto-3-deoxyoctulosonic acid [19] and showed a ladder-like appearance in silver-stained polyacrylamide gels [20, 21].

Animal injection

Five hamsters were injected i.m. into each of the hind paws with a 0.2 ml single dose of 300 μg of LPS O:8 or O:5. A second dose of LPS O:8 was administered on day 160. Five control animals received equal doses of 0.9% NaCl. All the experiments were repeated at least twice.

Assessment of arthritis

Hind paw inflammation was measured with a plethysmometer (Ugo Basile-7150). The mean value from two hind paws was used as an index of the severity of the swelling from arthritis. The values were compared with those from non-injected animals on different days post-injection (p.i.).

Analysis of histological changes

LPS-injected and control hamsters were killed on day 3 p.i. One of the hind paws was amputated, fixed, decalcified and processed according to routine histological techniques. Sections were stained with haematoxylin–eosin.

Antibody response by enzyme-linked immunosorbent assay (ELISA)

Polystyrene plates were coated overnight at 4°C with either *Y. enterocolitica* O:8 or O:5 LPS. The hamster sera obtained on different days p.i. were diluted and incubated for 1 h at 37°C. Hamster IgG was detected with rabbit anti-hamster IgG followed by incubation with peroxidase-conjugated goat anti-rabbit immunoglobulins, and 1,2-*o*-phenylenediamine was used as the chromogen. Absorbance was determined in an ELISA photometer (Bio-Rad, Hercules, CA, USA).

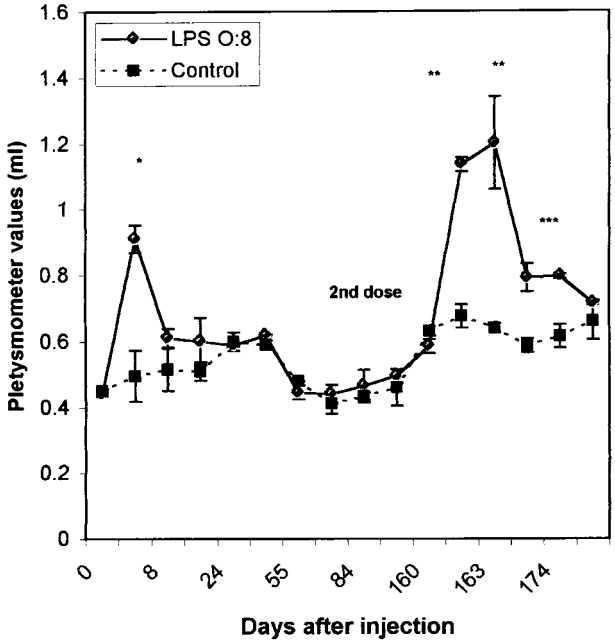

FIG. 1. Inflammation response (plethysmometer values) in hind paws. An acute inflammatory peak (day 3) was observed in hamsters injected with 300 μg of lipopolysaccharide (LPS). The second dose induced a more significant inflammatory peak. The values represent the mean of five hamsters ($*P < 0.05$, $**P < 0.001$, $***P < 0.005$).

Detection of polyclonal activation

The technique was performed as described by Falcào *et al.* [22]. Rabbit brain myelin basic protein (MBP), calf skin collagen type I, bovine tracheal cartilage collagen type II and rabbit muscle myosin (Sigma, St Louis, MO, USA) were used as antigens. Autoantibodies were detected by ELISA as follows. Polystyrene microtitre plates were sensitized with 100 μl of the autoantigens diluted with 0.06 M sodium carbonate–bicarbonate buffer, pH 9.6 (myosin 20 μg/ml, myelin 20 μg/ml, collagen type I 20 μg/ml, and collagen type II 20 μg/ml). The plates were then incubated for 18 h at 4°C and washed three times with phosphate-buffered physiological saline containing 0.05% Tween 20 (PBS/T). After washing, 100 μl of a serum sample diluted 1/100 in PBS/T containing 1% bovine serum albumin (PBS/T/BSA) was added and incubated for 2 h at 37°C. The plates were washed and 100 μl of a peroxidase-conjugated rabbit anti-mouse immunoglobulin diluted 1/1000 in PBS/T/BSA was added. After 1 h of incubation at 37°C and another washing, 100 μl of 1 mg/ml *o*-phenylene diamine in 0.1 M citrate phosphate buffer, pH 5.0, containing 0.03% hydrogen peroxide was added and the plates were incubated at 35°C for 10 min. Finally, the reaction was stopped with 100 μl of 3 N hydrochloric acid. Absorbance was read with an ELISA photometer (Bio-Rad) at 450 nm. All serum samples were assayed in duplicate and the results were expressed as the mean value.

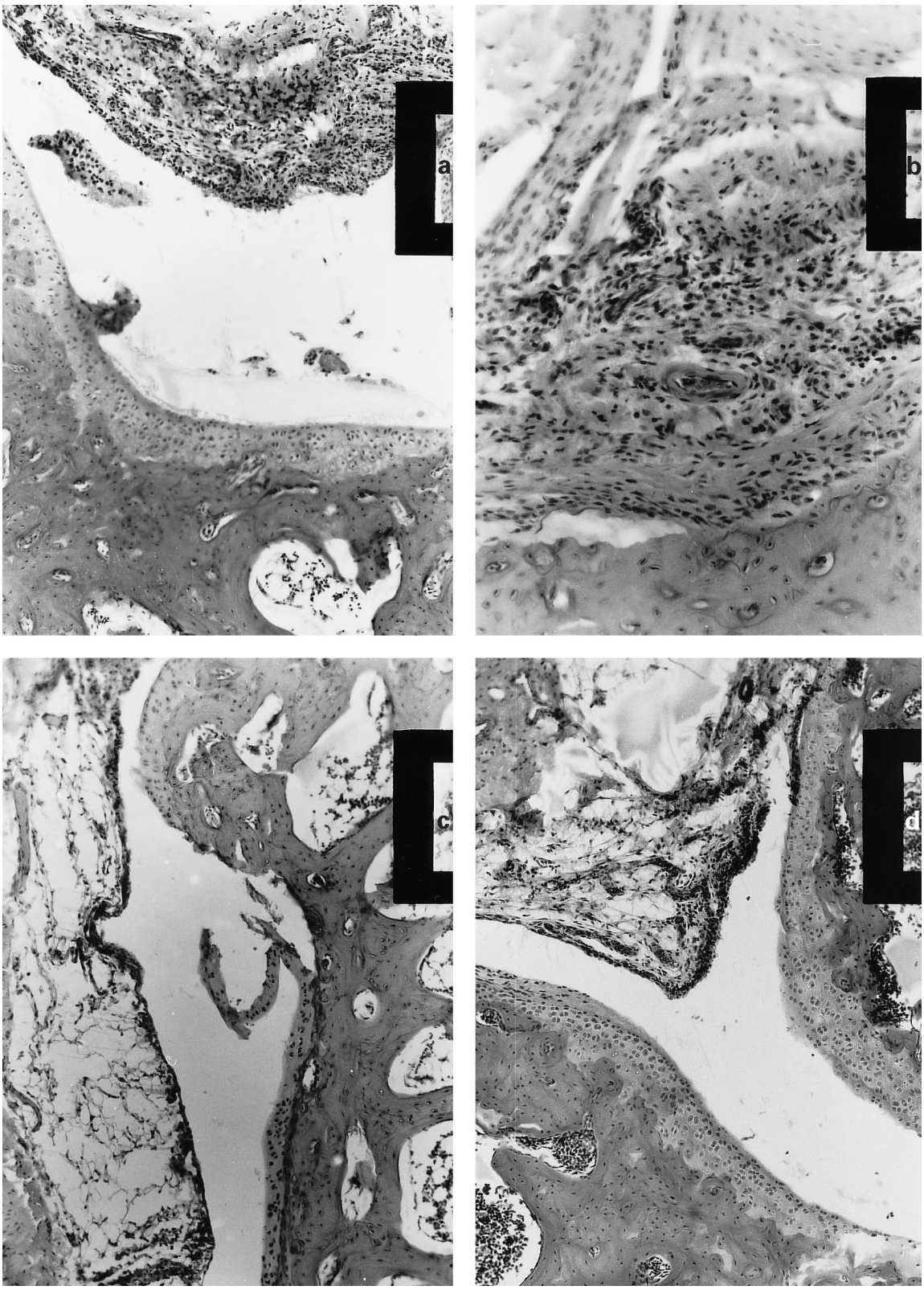

FIG. 2. Histopathology of arthritic joints. (a) Mononuclear infiltration with destruction of synovia in hamsters receiving lipopolysaccharide (LPS) O:8. (b) The same infiltration was observed in the surrounding tissue. (c) Slightly reduced synovial lining cell layer and increased nuclear pycnosis in hamsters receiving LPS O:5. (d) Joint section of negative control animals (haematoxylin–eosin stained). The original magnification was $\times 100$ for (a), (c) and (d), and $\times 200$ for (b).

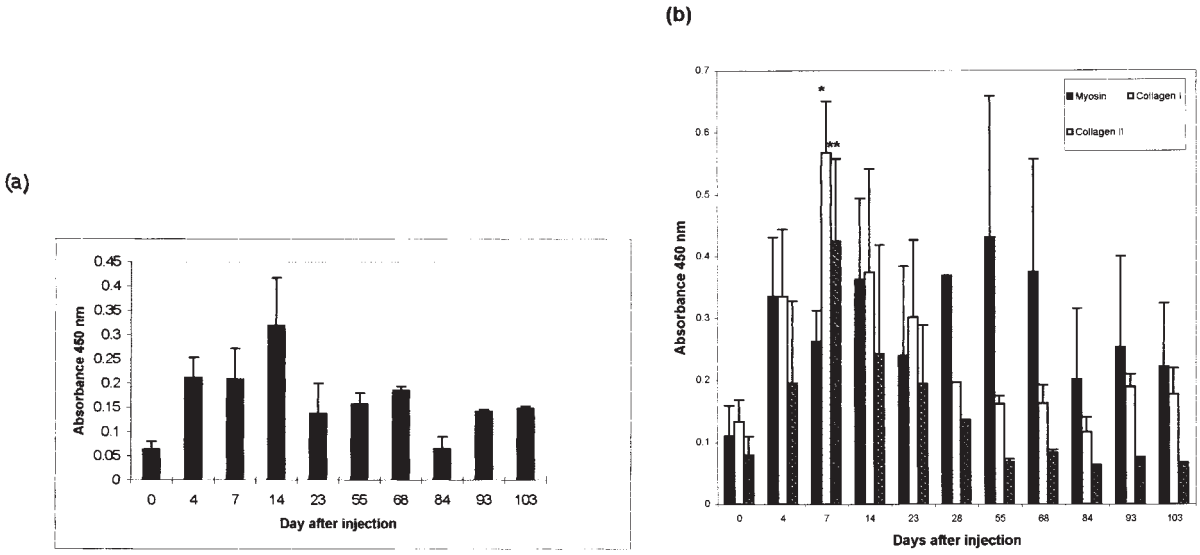

FIG. 3. Kinetics of antibody response in hamsters receiving lipopolysaccharide (LPS) O:8. (a) A significant response of LPS-specific IgG was observed on day 14, and (b) IgG against collagen I and II on day 7. The response was compared with negative control serum (day 0) (*$P < 0.01$, **$P < 0.001$).

Statistics

Data were subjected to analysis of variance followed by Tukey comparison. Student's *t*-test was used for inhomogeneous variance. Values of $P < 0.05$ were considered statistically significant.

Results

Development of arthritis in hamsters

As shown in Fig. 1, LPS O:8 induced marked swelling of the hamsters' joints, with an acute inflammatory peak on day 3 ($P < 0.05$). A second administration of LPS O:8 induced a new inflammatory peak, which was more significant and longer lasting [$P < 0.001$ on days 2 and 3 post-second injection (p.s.i.), and $P < 0.005$ on day 8 p.s.i.].

Histological changes

The histological analysis showed significant histopathological changes in the joints of LPS O:8-injected hamsters (Fig. 2a). In these animals, infiltration of polymorphonuclear leucocytes and mononuclear cells, predominantly lymphocytes, was observed. Dilation of the joint cavity, luminal disorganization and desquamation of the synovial membrane, decrease in synoviocyte number and lack of underlying adipocytes were also observed. Also, there was marked mononuclear infiltration of surrounding connecting tissue (Fig. 2b). The joints of LPS O:5-injected hamsters showed a slightly reduced synovial lining cell layer, increased nuclear pycnosis, and less marked mononuclear infiltration (Fig. 2c). The cartilaginous surface and bone histoarchitecture were not altered by LPS O:8 or LPS O:5. No histological changes were observed in control hamster joints (Fig. 2d).

Kinetics of serum antibodies

As shown in Fig. 3, a significant serum LPS O:8-specific IgG was detected on day 14 p.i. ($P < 0.01$). Also, antibodies against collagen I and II were significant on day 7 p.i. ($P < 0.001$ and $P < 0.01$, respectively).

Discussion

Yersinia enterocolitica O:8, although less frequent than serotype O:3, has been described as arthritogenic in humans [11]. However, we have not found reports linking serotype O:5 with human arthritis. Both serotypes have been employed in animal models of *Yersinia*-triggered arthritis [11, 14, 15]. LPS has been postulated, among others, as one of the possible arthritogenic factors [11] and, in fact, it has been detected in the synovial fluid cells of patients with ReA [23].

Bacterial LPS is a potent toxin released from Gram-negative bacterial cell walls, and it is notorious for the initiation of inflammation [24]. Earlier studies by our group have demonstrated the existence of *Yersinia* LPS in the joints of hamsters with *Yersinia*-triggered arthritis. In the present study, LPS alone was administered in hamsters and induced the swelling of joints as well as histological changes. Wuorela *et al.* [4] have suggested that LPS released from bacteria ends up in corresponding compartments of the cell as isolated LPS. The LPS-containing phagocytes are a constant source of membrane-active LPS in their microenvironment, such as in the joints of arthritic patients.

Low bactericidal capacity of the host and appropriate virulence potential of the pathogen have been suggested as a prerequisite for arthritis development [11]. Significant joint inflammation and marked histological changes were observed in joints of LPS O:8-injected

hamsters. At the same concentration, LPS O:8 was more arthritogenic than LPS O:5, when both were administered alone. It is therefore the variation in antigenicity that could be the cause of the different arthritogenic power observed between LPS O:8 and LPS O:5 in the present study.

Only acute inflammation was observed after the first dose of LPS O:8, indicating that it was probably removed by monocytes and macrophages [25, 26]. However, a second dose of LPS O:8 induced more significant and longer lasting joint inflammation. Several reports have suggested that the O-polysaccharide chain of LPS is also partially degraded by these cells [27, 28], and that this portion of LPS is retained in highly mobile cells. Phagocytes could transport LPS from the site of primary infection to the other parts of the body, such as the swollen joints of arthritic patients [4]. Partially degraded LPS from the first dose probably causes the more important inflammation after the second dose. In previous studies, a chronic inflammatory peak was observed when whole bacteria were administered [14, 15], suggesting that live bacteria probably persist somewhere in the body, continuously providing antigenic material into the blood circulation and stimulating the persistent immune reactions seen in the patients [29]. Granfors *et al.* [23] observed that 4 yr after the onset of *Y. enterocolitica* O:3-induced ReA, LPS was present in peripheral blood cells in most of their patients.

Our results indicate that, unlike LPS O:5, LPS O:8 causes a transient response of specific antibodies, and activation of autoreactive clones, with production of autoantibodies against collagen I and II. These are the most abundant collagens in the joints [30]. In a study of autoantibodies in mice infected with *Y. enterocolitica* O:3, Falcào *et al.* [22] observed responses against myosin, myelin and (trinitrophenyl) TNP-BSA, while no response was obtained against the collagens. These different results might reveal that the activation of different autoreactive clones depends on the host and the serovar employed in the experimental infection. In the present work, serum antibodies decayed with time, probably due to the fact that they deposited in the joint collagens or because the degraded LPS stopped inducing polyclonal activation.

In conclusion, we have demonstrated in hamster the different arthritogenic responses to LPS from different serovars of *Y. enterocolitica*. LPS O:8 administered alone has arthritogenic power and induces activation of autoreactive clones. These results support the key role of LPS in the development of ReA.

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References

1. Wuorela M, Granfors K. Infectious agents as triggers of reactive arthritis. Am J Med Sci 1998;316:264–70.
2. Jansson PE, Lindberg AA, Wolling R. Structural studies on the hexose region of core lipopolysaccharide from Enterobacteriaceae. Eur J Biochem 1981;115:571–7.
3. Zhang L, Radziejewska-Lebrecht J, Krajewska-Pietrasik D, Toivanen P, Skurnik M. Molecular and chemical characterization of the lipopolysaccharide O antigen and its role in the virulence of *Yersinia enterocolitica* serotype O:8. Mol Microbiol 1997;23:63–76.
4. Wuorela M, Jalkamem S, Toivanen P, Granfors K. *Yersinia* lipopolysaccharide is modified by human monocytes. Infect Immun 1993;61:5261–70.
5. Noyori K, Okamoto R, Takagi T, Hyodo A, Suzuki K, Koshino T. Experimental induction of arthritis in rats immunized with *Escherichia coli* O:14 lipopolysaccharide. J Rheumatol 1994;21:484–8.
6. Suzuki K. Induction of arthritis in the ankles of rat immunized by intraperitoneally-injected lipopolysaccharide (LPS) or lipid A. Nippon Seikeigeka Gakkai Zasshi 1994;68:272–82.
7. Terato K, Ye XJ, Miyahara H, Cremer MA, Griffiths MM. Induction of chronic autoimmune arthritis in DBA/1 mice by oral administration of type II collagen and *Escherichia coli* lipopolysaccharide. Br J Rheumatol 1996;35:828–38.
8. Idogawa H, Imamura A, Matsuo K, Yoshitake K, Umemura T, Ohashi M. A monoarthritis model in rabbits induced by repeated intra-articular injections of lipopolysaccharide. Int J Exp Pathol 1998;79:93–104.
9. Otterness IG, Bliven ML, Milici AJ, Poole AR. Comparison of mobility changes with histological and biochemical changes during lipopolysaccharide-induced arthritis in the hamster. Am J Pathol 1994;144:1098–108.
10. Gruys E, Tooten PC, Cathcart ES. Polyarthritis and periostitis induced by *Escherichia coli*. Lipopolysaccharide injection in young male hamsters. J Rheumatol 1998;25:748–52.
11. Gaede KI, Heesemann J. Arthritogenicity of genetically manipulated *Yersinia enterocolitica* serotype O8 for Lewis rats. Infect Immun 1995;63:714–9.
12. Brown CR, Reiner SL. Genetic control of experimental Lyme arthritis in the absence of specific immunity. Infect Immun 1999;67:1967–73.
13. Lim LCL, England DM, DuChateau BK *et al.* Development of destructive arthritis in vaccinated hamsters challenged with *Borrelia burgdorferi.* Infect Immunol 1994;62:2825–33.
14. Di Genaro MS, Escudero ME, Velázquez LC *et al.* Humoral immune response in *Yersinia enterocolitica* O:5 induced arthritis in hamsters. Microbiol Immunol 1997;41:615–20.
15. Di Genaro MS, Escudero ME, Muñoz E *et al.* Arthritogenicity of *Yersinia enterocolitica* O:8 in hamsters: analysis of immune response. Folia Microbiol (Praha) 1998;43:690–6.
16. Velázquez LC, Escudero ME, Guzmán AMS. Prevalence of *Yersinia enterocolitica* in hake (*Merluccius hubbsi*) fillets. J Food Protect 1996;59:781–3.
17. Westphal O, Lüderitz O, Bister F. Über die Extraction von Backterien mit Phenol/Wasser. Z Naturforsch B 1952;7:148–55.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein

measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
19. Karkanis YD, Zeltner JY, Jackson JJ, Carlo DJ. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of Gram-negative bacteria. Anal Biochem 1977;85:595–601.
20. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;277:680–5.
21. Tsai CM, Frasch CE. A sensitive silver strain for detecting lipopolysaccharides in polyacrylamide gels. Anal Biochem 1982;119:115–9.
22. Falcào DP, Madeiros BMM, Moraes Costa A, Araujo PMF. Presence of autoantibodies in the serum of mice infected with *Yersinia enterocolitica* O:3. Contrib Microbiol Immunol 1995;13:195–8.
23. Granfors K, Merilahti-Palo R, Luukkainen R *et al.* Persistence of *Yersinia* antigens in peripheral blood cells from patients with *Yersinia enterocolitica* O:3 infection with or without reactive arthritis. Arthritis Rheum 1998;41:855–62.
24. Yang Z, Carter CD, Miller MS, Bochsler PN. CD14 and tissue factor expression by bacterial lipopolysaccharide-stimulated bovine alveolar macrophages *in vitro.* Infect Immun 1995;63:51–6.
25. Munford RS, Hall CL. Detoxification of bacterial lipopolysaccharides (endotoxins) by human neutrophil enzyme. Science 1986;234:203–5.
26. Peterson AA, Munford RS. Dephosphorylation of the lipid A moiety of *Escherichia coli* lipopolysaccharide by mouse macrophages. Infect Immun 1987;55:974–8.
27. Fox ES, Thomas P, Broitman SA. Clearance of gut-derived endotoxins by the liver. Gastroenterology 1989;96:456–61.
28. Freudenberg MA, Kleine B, Galanos C. The fate of lipopolysaccharide in rats: evidence for chemical alteration in the molecule. Rev Infect Dis 1984;6:483–7.
29. Granfors K, Jalkanen S, von Essen R *et al. Yersinia* antigens in synovial-fluid cells from patients with reactive arthritis. N Engl J Med 1989;320:216–21.
30. Schulze-Koops H, Burkhardt H, Heesemann J, von der Mark K, Emmerich F. Characterization of the binding region for *Yersinia enterocolitica* adhesin Yad A on types I and II collagen. Am Coll Rheumatol 1995;38:1283–9.