

Reactive arthritis with conjunctivitis, urethritis and diarrhea in a child: immunological study of potential bacterial trigger

Sirs,

Reactive arthritis (ReA) has been defined as a sterile synovitis developing 1-3 weeks after an infection in the genitourinary or gastrointestinal tract (1). The classical microbes in the gastrointestinal tract include *Yersinia*, *Salmonella* and *Shigella* (2). At the time of arthritis, stool cultures are negative and the background of ReA is usually confirmed by serological methods (2). However, there are no international standards for the tests, and techniques vary greatly (1, 2). In a case of child ReA, we identified the potential bacterial trigger by studying the antibody response to different components of three candidate bacteria.

A 12-year-old boy was admitted to the hospital complaining of fever, pain and swelling of the left knee. Days prior the admission, he had shown conjunctivitis, dysuria with pyuria and one month before he had had diarrhea. Apart from ESR (100 mm/h), CRP (48 mg/l), all the laboratory investigations were negative. Gram staining and culture of the synovial fluid (SF) were negative. A diagnosis of ReA was proposed. With the written consent of the patient and his parents (Declaration of Helsinki, 2000) serum and SF samples were remitted to the National University of San Luis.

Salmonella enteritidis, *Shigella flexneri* and *Yersinia enterocolitica* O:8 were used to prepare crude lysate (CL), outer membrane proteins (OMP), cytosolic fraction (CF), culture supernatant proteins (SN) and lipopolysaccharide (LPS) (3-5). *Yersinia* outer proteins (Yops) were prepared as described previously (6). IgA response in serum and SF were studied by ELISA and Western blot; the control group included 10 sera from unrelated healthy patients or 10 SF from patients with osteoarthritis. Bacterial DNA in SF was investigated by polymerase chain reaction (PCR) (7).

Higher IgA response (titer 1:100) was detected against SN and OMP of *Shigella* in SF and serum, respectively (Fig. 1A). The Yops Western blot was negative, and since this is a specific test for *Yersinia*-induced ReA diagnosis (8), this bacterium was excluded as a possible candidate. *Salmonella* OMP and SN Western blot were also negative. In contrast, Western blot for *Shigella* was positive in both serum and SF. Reaction against *Shigella* OMP 91, 63, 43, 36, 33, 28 and 23 kDa bands and a strong reaction to a 94 kDa protein band from *Shigella* SN were observed (Fig. 1B). It was not possible to find *Shigella* DNA in SF.

Except during epidemics, *Shigella* is not considered to be a frequent cause of enteric

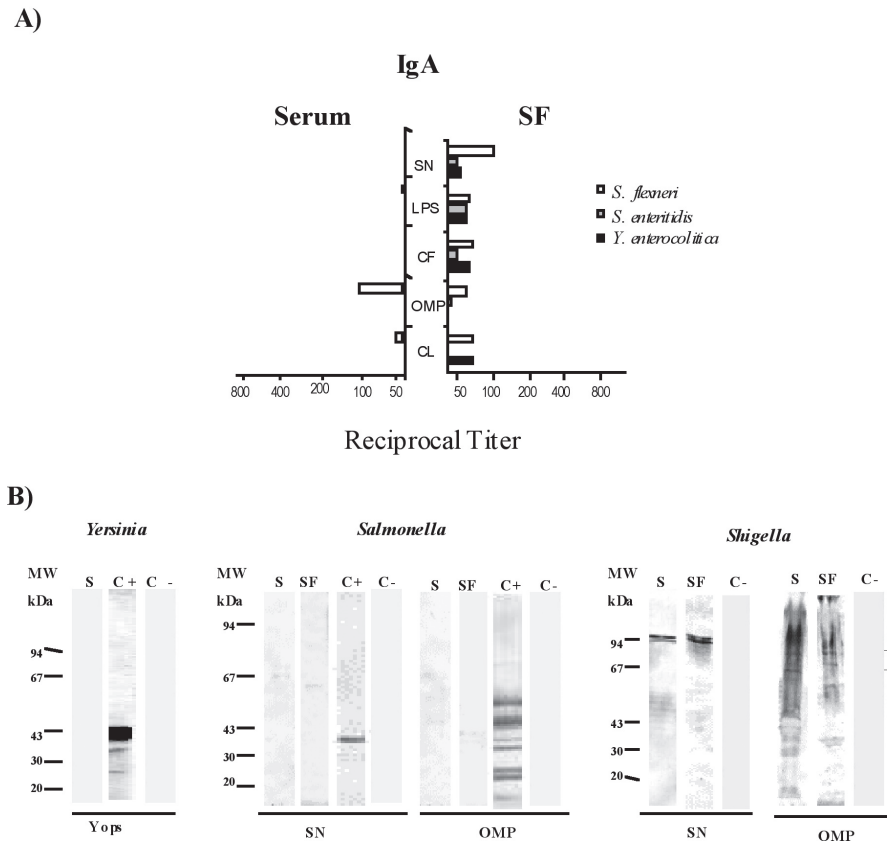


Fig. 1. (A): Levels of IgA by ELISA in serum (S) and synovial fluid (SF). The response is represented as the reciprocal titer to each antigen, which was determined as the last dilution with optical density (OD) over the cut-off value (OD mean + 2 SD of the control group). The control group included 10 sera from unrelated healthy patients or 10 SF from patients with osteoarthritis. CL: crude lysate, OMP: outer membrane proteins, CF: cytosolic fraction, LPS: lipopolysaccharide, SN: supernatant proteins. (B): IgA response by Western blot in S and SF. *Yersinia* outer proteins (Yops), *Salmonella* and *Shigella* SN and OMP were used as antigens. MW: Molecular weight marker, C+: positive control sera for *Yersinia* or *Salmonella*. C-: negative control from healthy sera or osteoarthritis SF.

ReA (9). However, this might be due to lack of reliable antibody tests, which makes diagnosis difficult. Therefore, the confirmation of *Shigella* infection depends on a positive stool culture (2). Although our patient did not consult during his diarrhea episode and stool cultures were not performed, our immunological study suggested *Shigella* as the cause of the ReA, since only *Shigella* OMP and SN Western blot were positive. The OMP bands of 63, 43, 36 and 33 kDa might correspond to Ggt, Tol B, AnsB and OmpA of 61.8, 46, 36.8 and 35 kDa, respectively (10). Interestingly, a strong reaction to a 94 kDa protein from SN was observed. There is no evidence in the literature of immunogenic secreted *Shigella* proteins with similar molecular weight. The association of these proteins with *Shigella* arthritogenicity could be identified in further studies.

As far we know, this is the first report of differentiation of *Shigella* as the cause of ReA by antibody response to different bacterial antigens. Thus, our findings suggest that *Shigella* OMP and SN, as Yops for *Yersinia*, are the differential *Shigella* antigens. This work could contribute to develop a specific diagnostic method for *Shigella* induced-ReA.

M.G. LACOSTE, Lic
D.E. CARGNELUTTI, Lic
H. TAMASHIRO, MD
M.S. DI GENARO, PhD

Immunology, Laboratory of Microbiology, Chemistry, Biochemistry and Pharmacy Faculty, National University of San Luis, San Luis, Argentina.

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Please address correspondence to: María S. Di Genaro, Immunology, Laboratory of Microbiology, Chemistry, Biochemistry and Pharmacy Faculty, National University of San Luis, San Luis, Chacabuco y Pedernera, 5700, San Luis, Argentina.

E-mail: sdigena@unsl.edu.ar.

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Severe hypertriglyceridemia in a rheumatoid arthritis patient treated with leflunomide

Sirs,

Leflunomide (LEF) is a well-established treatment for rheumatoid arthritis (RA). We describe an interesting case of a patient exhibiting severe hypertriglyceridemia, attributed to LEF.

A 54-year-old male, with a diagnosis of RA for 11 years, was treated with methotrexate (MTX) from 1996 to 2005, and since 2002 in combination with infliximab. For several years he was also on enalapril, hydrochlorothiazide, carvedilol, digoxin and acenocoumarol because of hypertension and chronic atrial fibrillation. On June 2005, MTX was switched to LEF due to RA exacerbation (DAS28 = 6.5). From that time, triglyceride (TG) levels started to rise (Table I), despite specific instructions for dietary and lifestyle modifications. Additionally, at several time points, our patient denied the addition of a lipid lowering drug, due to polypharmacy. On March 2006, TG were 1200 mg/dl and he complained of diarrhea, bloating, insomnia and dizziness, symptoms that although not specific enough, could be attributed to LEF. As a result, a diagnosis of LEF induced hypertriglyceridemia was suggested, LEF was immediately discontinued and a wash out procedure was performed (cholestyramine 8 gr, three times daily for 11 days). On the follow up visits, TG started to decline gradually, reaching normal values 8 months later (Table I).

Cholestyramine, a bile acid sequestrant (BAS), serves as an antidote in LEF toxicity presumably by interrupting the excessive

Table I.

	5/05	6/05	7/05	12/05	3/06	5/06	9/06	11/06
Glu	96	98	117	106	104	101	123	99
Ur	25	46	28	27	40	34	27	25
Cr	0.7	1.0	0.8	0.8	0.9	0.8	0.8	0.7
UA	8.8	9.0	6.5	5.5	6.8	8.7	6.9	5.3
TC	222	222	240	255	339	249	187	146
LDL	154	153	153	126	84	132	111	94
HDL	31	27	30	30	13	28	29	29
TG	187	212	283	496	1210	445	234	115
CRP	1.61	2.42	1.53	0.43	0.31	0.5	0.31	1.54

Glu: Glucose (70-110 mg/dl); Ur: Urea (15-55 mg/dl); Cr: Creatinine (0.6-1.2 mg/dl); UA: Uric acid (3.0-8.2 mg/dl); TC: Total Cholesterol (150-220 mg/dl); LDL: Low Density Lipoprotein (<130 mg/dl); HDL: High Density Lipoprotein (<40 mg/dl); TG: Triglycerides (<150 mg/dl); CRP: C- Reactive Protein (0.08-0.8 mg/dl).

enterohepatic circulation of LEF. It is also known as a lipid lowering drug. Thus someone could argue that the decline of TG in our patient is principally through the hypolipidemic action of cholestyramine than lowering of LEF levels. However, it is known that BAS, even though they are able to decrease LDL cholesterol levels, they also tend to increase VLDL production and therefore raising TG levels (1). Additionally, our patient was also receiving specific antihypertensive drugs (thiazide diuretics and beta blockers) that can also raise TG levels. Therefore, one could support the view that the LEF induced hypertriglyceridemia was indirect, by increasing the levels of those antihypertensive drugs. However, hydrochlorothiazide is able to increase TG levels but it also increases uric acid levels. On the contrary, our patient had a decline in uric acid levels after LEF initiation, probably due to the ability of LEF to reduce serum uric acid concentrations by increasing urate renal excretion (2, 3). Additionally, although beta-blockers in general increase serum TG levels, carvedilol decreases TG levels or has no effect at all (4, 5). Finally, the strongest evidence that our patient hypertriglyceridemia should be attributed to LEF is that upon discontinuation of LEF, TG levels started to decline and reached normal values 8 months later. To the best of our knowledge, there is only one case report in the literature describing a case of increased TG levels, attributed to LEF (6). Concerns about LEF discontinuation rates are currently rising (9, 10) and although several clinical studies have been published examining the efficacy and safety of LEF in various diseases, none of them had shown any side effect on triglycerides levels. In two of them (7, 8), 1 patient in each study developed hypercholesterolemia, i.e., an increase in total cholesterol levels. As severe hypertriglyceridemia is a well-described cause of pancreatitis, a disease with significant mortality, we recommend lipid profile examination at least every 3 months, for the first year after the initiation of LEF.

E.K. SPANAKIS¹*
G.A. KATSIKAS²*
P.I. SIDIROPOULOS²
H.D. KRITIKOS²

*These authors contributed equally to the project.

¹Department of Internal Medicine, and

²Division of Rheumatology, Clinical Immunology and Allergy, University of Crete University Hospital of Heraklion, Greece.

Please address correspondence to:

Elias Spanakis / Georgios Katsikas,
1 Voutes street, 71110 Heraklion,
P.O. Box 1352, Crete, Greece.

E-mail: ispanaki@bidmc.harvard.edu,
ykatsikas@hotmail.com

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