Rickettsia monacensis and Human Disease, Spain

Isabel Jado,* José A. Oteo,† Mikel Aldámiz,‡ Horacio Gil,* Raquel Escudero,* Valvanera Ibarra,† Joseba Portu,‡ Aranzazu Portillo,† María J. Lezaun,‡ Cristina García-Amil,* Isabel Rodríguez-Moreno,* and Pedro Anda*

We identified *Rickettsia monacensis* as a cause of acute tickborne rickettsiosis in 2 humans. Its pathogenic role was assessed by culture and detection of the organism in patients' blood samples. This finding increases the number of recognized human rickettsial pathogens and expands the known geographic distribution of Mediterranean spotted fever–like cases.

Tickborne rickettsioses are produced by spotted fever group (SFG) rickettsiae and cause an expanding spectrum of clinical signs. *Rickettsia conorii* is the etiologic agent of Mediterranean spotted fever (MSF) and is transmitted by *Rhipicephalus sanguineus*. *Rickettsia helvetica*, a widespread species, is carried by *Ixodes ricinus* (*1*). Recently, other SFG rickettsiae have been found in *I. ricinus* from Spain (*2*), Slovakia (*3*), and northeastern Italy (*4*), as well as in *I. nipponensis* from Japan (*5*). Subsequently, a new rickettsia species, *R. monacensis*, was isolated from *I. ricinus* from Germany (*6*) and detected in Hungary (*7*). The pathogenicity of this species is unknown. It constitutes a new rickettsial genotype and forms a separate cluster among the SFG rickettsiae (*3*), close to strain Cooleyi, which was isolated from *I. scapularis* in Texas (*8*). *I. ricinus* is well established in areas of northern Spain (*9*), where MSF-like cases are increasingly reported.

Our study aim was to identify the SFG rickettsial species involved in MSF-like rickettsioses in 2 patients in northern Spain. We report an association between *R. monacensis* and these rickettsioses.

The Study

Patient 1 was an 84-year-old man from La Rioja, who sought medical attention on June 19, 2003, 7 days after onset of fever (39.5ºC), general discomfort, headache, and joint pain. At the time of the physical examination, he had a nonpruritic, disseminated maculopapular rash, with no inoculation eschar, of the trunk and lower extremities, including palms and soles. Other than a slightly low platelet count (82,000/mm^3), examination findings were within normal limits. MSF was diagnosed, and serum and defibrinated blood samples were taken before a course of oral doxycycline (100 mg/12 h for 10 d) was initiated. Three days later, fever and rash were gone without sequelae. Additional serial serum samples were taken during weeks 4, 13, and 26 after onset and reserved for serologic analysis (Table).

Patient 2 was a 59-year-old woman from Basque Country, who sought medical attention on September 20, 2003, 4 days after onset of fever (38ºC), headache, and an erythematous rash, with no inoculation eschar, at the site of a tick bite. The patient reported a history of tick bites, most recently 1 week before symptom onset. Blood cell counts and other blood chemistry values were normal. MSF was diagnosed, and oral doxycycline (100 mg/12 h for 10 d) was prescribed. Serial serum samples were taken the day of the visit and weeks 4 and 6 after onset and were reserved for serologic analysis (Table). Defibrinated blood was also taken 2 days after treatment was initiated. The patient recovered without sequelae.

DNA was extracted with the QIAGEN Tissue kit (IZASA S.A., Barcelona, Spain), and an *ompA*-nested PCR was designed. The first set of primers (Rr190.70p and Rr190.602n) have been described (*10*). Those used for the nested amplification were designed in this study: NompA-F (5′-AGC GAT AAT GCT GAG TAG TAG-3′) and NompA-R (5′-TAT ATT TCC TAA ACC TGT ATA A-3′) nucleotide positions 150–170 and 576–555, respectively, were numbered according to Regnery et al. (*10*). Amplification conditions were as described, except annealing temperature was 40ºC for the second PCR and AmpliTaq Gold DNA Polymerase (Applied Biosystems, Branchburg, NJ, USA) was used. The specificity of the method was tested against DNA obtained from Vero cells and *Coxiella burnetii*, and fragments of the expected sizes (532 and 427 bp) were obtained from different rickettsia species (data not shown). The amplicons obtained from blood samples were run in 1% low-melt agarose gels (Pronadisa, Barcelona, Spain), and the bands of interest were excised, purified with QIAquick Gel Extraction kit (IZASA S.A), and sequenced as described (*9*).

A phylogenetically informative fragment of 446 bp of *gltA* was also sequenced from samples by nested PCR with primers designed for this study: GLTA1F (5′-GAC GGT GAT AAA GGA ATC TTG-3′) and GLTA1R (5′-CAT TTC TTT CCA TTG TGC CAT C-3′) for the first run, and GLTA2F (5′-CTA CGA ACT TAC CGC TAT TAG-3′) and GLTA2R (5′-GAC CAA AAC CCA TTA ACC TAA AC-3′) for the second; nucleotide positions 279–299,

*Centro Nacional de Microbiología, Majadahonda, Madrid, Spain; †Complejo San Millán-San Pedro de La Rioja, Logroño, Spain; and ‡Hospital de Txagorritxu, Vitoria, Spain

Table. Microimmunofluorescence titers obtained with different rickettsial antigens, 2 patients, northern Spain, 2003*

Patient	Week†	*Rickettsia conorii*	*R. monacensis*	*R. helvetica*	*R. akari*	*R. australis*
1	1	<1:40	<40	<40	<40	<40
	4	1,280	2,560	2,560	1,280	640
	13	1,280	1,280	1,280	1,280	1,280
	26	1,280	1,280	1,280	320	320
2	1	640	2,560	2,560	640	320
	4	320	1,280	1,280	320	160
	6	640	1,280	1,280	320	160

*A 1-fold decrease in titer is considered not significant.
†Week after symptom onset in which the samples were extracted.

1011–989, 566–586, and 1298–1277, respectively, were numbered according to Regnery et al. (*10*). PCR conditions included annealing temperatures of 65ºC and 50ºC for the first and second runs, respectively. The rest of the parameters were identical to those used above, and samples were subjected to 35 cycles of denaturing (20 s at 95ºC), annealing (30 s), and extension (2 min at 60ºC), with an initial denaturing cycle of 9 min at 95ºC.

Blood samples from each patient were cultured by using shell vial technique (*11*). Giménez stain and PCR, performed after 7 days of incubation, confirmed the growth of a *Rickettsia*-like organism (strain Rp-Sp1) from patient 1. The sequences of *ompA* and *gltA* of this isolate (GenBank accession nos. DQ157778 and DQ517498, respectively) were identical to those obtained from the blood samples of each patient and to that of *R. monacensis* (*6*) (GenBank accession nos. AF201329 and DQ100163). The sequences generated in this study were subjected to phylogenetic analyses as described (*9*) and belonged to the same clade as *R. monacensis* and other related strains that have been detected in *I. ricinus* (*3*,*4*,*12*) (Figure).

In-house microimmunofluorescence assay (IFA) ([*1*] and references therein) that used *R. monacensis, R. conorii, R. helvetica, R. akari,* and *R. australis* as antigens was performed in serial serum samples from each patient (Table). The isolate Rp-Sp1 from patient 1 could not be used as antigen because of poor adaptation of this isolate to culture in Vero cell monolayers; *R. monacensis* slides for IFA were obtained from the Department of Entomology, University of Minnesota, Minneapolis, MN, USA. Seroconversion against the 5 rickettsia species was observed from patient 1's second serum sample (day 30 after the onset). Patient 2's first serum sample also had high titers against the 5 antigens. Although the reactivity against the 5 rickettsial antigens was similar, the titers observed were slightly higher against *R. monacensis* and *R. helvetica*, which are phylogenetically closer to each other than to the other species tested. However, because the serologic results may only loosely implicate a given rickettsia species, isolation of *R. monacensis* from patient 1 and its detection by PCR for both patients confirm it as the etiologic agent.

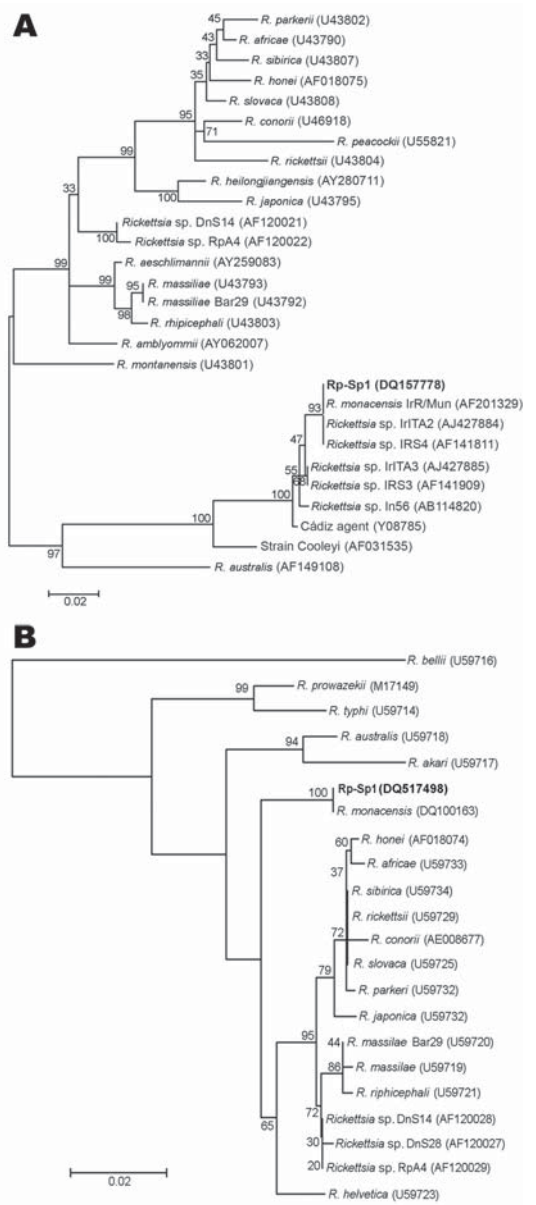

Figure. Neighbor-joining phylogenetic analysis based on *ompA* (panel A) and *gltA* (panel B). Mega 3 software (www.megasoftware.net) was used for the calculation of pairwise distances. Numbers near each node represent the bootstrap values. The isolate from patient 1 is shown in **boldface**. GenBank accession no. for each sequence is in parentheses.

Conclusions

We describe a new, to our knowledge, rickettsia species that caused human disease. *R. monacensis* was the etiologic agent of MSF-like illness in northern Spain. Strain Rp-Sp1 was obtained from 1 patient. Because the sequences of *ompA* and *gltA* were identical to this rickettsia species and also amplified from blood samples of each patient studied, we conclude that this rickettsia is responsible for the symptoms observed in these patients. Therefore, *R. monacensis* joins the list of autochthonous rickettsia species (*R. conorii* [*13*], *R. slovaca* [*14*], *R. typhi* [*15*]) confirmed as human pathogens in Spain.

We were not able to study the vectors involved; however, each patient contracted the disease in areas where *I. ricinus* is the most prevalent tick species (*9*), and strains close to *R. monacensis* have been recently detected in *I. ricinus* in Spain (*2*,*12*). Thus, *I. ricinus* may eventually be shown to be the vector. Studies of *R. monacensis* incidence in autochthonous *I. ricinus* specimens are in progress to evaluate the risk of its transmission to humans.

Acknowledgments

We thank Ulrike Munderloh for providing *R. monacensis* slides for IFA.

Grant support was provided by Fondo de Investigación Sanitaria "Red Temática de Investigación Cooperativa EBATRAG (G03/057)."

Dr Jado is a microbiologist at the "Unidad de Alerta y Emergencias" and Laboratorio de Espiroquetas y Patógenos Especiales, Centro Nacional de Microbiología, Instituto de Salud Carlos. Her research interest is bacterial zoonoses, specifically tickborne pathogens.

References

1. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev. 2005;18:719–56.
2. Márquez FJ, Muniain MA, Soriguer RC, Izquierdo G, Rodríguez-Bano J, Borobio MV. Genotypic identification of an undescribed spotted fever group rickettsia in *Ixodes ricinus* from southwestern Spain. Am J Trop Med Hyg. 1998;58:570–7.
3. Sekeyova Z, Fournier PE, Rehacek J, Raoult D. Characterization of a new spotted fever group rickettsia detected in *Ixodes ricinus* (Acari: Ixodidae) collected in Slovakia. J Med Entomol. 2000;37:707–13.
4. Beninati T, Lo N, Noda H, Esposito F, Rizzoli A, Favia G, et al. First detection of spotted fever group rickettsiae in *Ixodes ricinus* from Italy. Emerg Infect Dis. 2002;8:983–6.
5. Ishikura M, Ando S, Shinagawa Y, Matsuura K, Hasegawa S, Nakayama T, et al. Phylogenetic analysis of spotted fever group rickettsiae based on *gltA*, 17-kDa, and *rOmpA* genes amplified by nested PCR from ticks in Japan. Microbiol Immunol. 2003;47:823–32.
6. Simser JA, Palmer AT, Fingerle V, Wilske B, Kurtti TJ, Munderloh UG. *Rickettsia monacensis* sp. nov., a spotted fever group rickettsia, from ticks (*Ixodes ricinus*) collected in a European city park. Appl Environ Microbiol. 2002;68:4559–66.
7. Sreter-Lancz Z, Sreter T, Szell Z, Egyed L. Molecular evidence of *Rickettsia helvetica* and *R. monacensis* infections in *Ixodes ricinus* from Hungary. Ann Trop Med Parasitol. 2005;99:325–30.
8. Billings AN, Teltow GJ, Weaver SC, Walker DH. Molecular characterization of a novel *Rickettsia* species from *Ixodes scapularis* in Texas. Emerg Infect Dis. 1998;4:305–9.
9. Escudero R, Barral M, Pérez A, Vitutia MM, García-Pérez AL, Jiménez S, et al. Molecular and pathogenic characterizacion of *Borrelia burgdorferi* sensu lato isolates from Spain. J Clin Microbiol. 2000;38:4026–33.
10. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol. 1991;173:1576–89.
11. La Scola B, Raoult D. Diagnosis of Mediterranean spotted fever by cultivation of *Rickettsia conorii* from blood and skin samples using the centrifugation-shell vial technique and by detection of *R. conorii* in circulating endothelial cells: a 6-year follow-up. J Clin Microbiol. 1996;34:2722–7.
12. Fernández-Soto P, Pérez-Sánchez R, Encinas-Grandes A, Sanz RA. Detection and identification of *Rickettsia helvetica* and *Rickettsia* sp. IRS3/IRS4 in *Ixodes ricinus* ticks found on humans in Spain. Eur J Clin Microbiol Infect Dis. 2004;23:648–9.
13. Bernabeu-Wittel M, Segura-Porta F. Rickettsiosis. Enferm Infecc Microbiol Clin. 2005;23:163–72.
14. Oteo JA, Ibarra V, Blanco JR, Martínez de Artola V, Márquez FJ, Portillo A, et al. *Dermacentor*-borne necrosis erythema and lymphadenopathy: clinical and epidemiological features of a new tickborne disease. Clin Microbiol Infect. 2004;10:327–31.
15. Hernández-Cabrera M, Ángel-Moreno A, Santana E, Bolaños M, Frances A, Martín-Sánchez MS, et al. Murine typhus with renal involvement in Canary Islands, Spain. Emerg Infect Dis. 2004;10:740–3.

Address for correspondence: Pedro Anda, Laboratorio de Espiroquetas y Patógenos Especiales, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220-Majadahonda, Madrid, Spain; email: panda@isciii.es