

Secondary Serological Response of Patients with Chronic Hepatosplenic Suppurative Brucellosis[∇]

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Chronic hepatosplenic suppurative brucellosis (CHSB) is a local reactivation of a previous brucellosis, coursing with an immunoglobulin G (IgG) and IgA secondary immunological response. The observation of two cases of CHSB with an apparent IgM response gave rise to a detailed serological study of three of our patients. We studied the first sample from all three patients and successive samples from two of them. In cases 1 and 2, we found samples with positive IgM lateral flow and IgM enzyme-linked immunosorbent assay results concomitantly with rheumatoid factor (RF); after absorption with anti-RF serum, these results were rendered negative. In patients 2 and 3 the diagnosis of brucellosis was delayed, because none of the test results were initially very significant. However, a clear seroconversion of IgG antibodies was observed in subsequent months; titers of the Brucellacapt and Coombs tests increased in similar ways, although Brucellacapt decreased more rapidly than Coombs, which persisted at high titers for years. In patient 3 a relapse was observed in the fourth year of follow-up, detected by Coombs and also by IgG lateral flow and counterimmunoelectrophoresis (CIEP), although not by the rose bengal, agglutination, or Brucellacapt tests. Serological changes in CHSB may sometimes be mild and are detected mainly by the Coombs test. Brucellacapt does not offer additional information, although IgG lateral flow and CIEP may be of some use. Careful surveillance of titer changes in the Coombs test is the best marker of infection activity. As the disease progresses, an intense IgG response may develop and RF sometimes appears, simulating an IgM response.

Chronic hepatosplenic suppurative brucellosis (CHSB) was first reported many years ago (23). Two recent series provided a current understanding of this rare focal form of the disease and emphasized that it is in fact a local reactivation of a previous episode of brucellosis (1, 5).

The diagnosis may be misleading because of the nonspecific clinical presentation of CHSB and the frequent negativity of blood and abscess pus cultures (1). Although modern PCR techniques have proved useful in identifying brucellar antigen in these pus cultures (6), in many cases the diagnosis is supported mainly by serological tests.

As CHSB is a reactivated disease, serological changes corresponding to a secondary immunological response are usually observed (1). Despite some controversial opinions (11), we previously demonstrated that the secondary response in patients with brucellosis relapse was always of anti-*Brucella* immunoglobulin G (IgG) and IgA, and not IgM, antibodies, as occurs with other thymus-dependent antigens (2, 12, 19, 25). In addition, this secondary serological response may be difficult to detect in some cases, depending on the point in the clinical course of the disease. Thus, the initial diagnosis of CHSB and the evaluation of its spontaneous or posttherapy outcome on

the basis of the serological profile of specific antibodies may prove confusing.

The observation of two of these CHSB cases with an apparent IgM serological response gave rise to a detailed study of the serological behavior of this unusual disease form in three of our patients. The concomitant use of classical and recently incorporated tests for quantifying anti-lipopolysaccharide (LPS) antibodies (the rose bengal [RB], agglutination [SAT], Coombs, and Brucellacapt tests) and of IgM and IgG lateral flow tests and counterimmunoelectrophoresis (CIEP) to detect anti-water-soluble cytosolic protein antibodies enabled us to identify some peculiar and interesting findings for this reactivated brucellosis. These findings may contribute to a better understanding of both the specific role of each serological test in the diagnosis of the disease and how to interpret the presence of antibodies with various levels of affinity.

Patient 1. Patient 1 was a 39-year-old man seen on 20 September 2000 in the Clínica Universitaria (Pamplona, Spain) for a left pleural effusion, diagnosed 1 month previously in another medical center. Computed tomography (CT) examination showed pleural collection (size, 6 by 8 cm) and a calcium density with hypodensity around 4 cm in the spleen. The patient's work involved cleaning up sheep stalls, and he referred to a previous episode of fever, asthenia, arthralgias, and weakness in 1990; however, suspected brucellosis could not be confirmed with serological tests at that time, and clinical findings disappeared in 6 months without any specific antibiotic therapy. Afterward, he lived for years in an area in which brucellosis was not endemic. In March 2000, he once again developed fever, arthralgias, and lumbar pain, and acute brucellosis was

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diagnosed; rifampin and doxycycline were given for 6 weeks, and he became well until a relapse in May 2000. A new therapeutic schedule of doxycycline and streptomycin was administered but with only partial improvement. In July 2000, clinical findings reappeared, along with intense left-side pain secondary to pleural effusion. Under prolonged doxycycline-rifampin therapy, he remained free of fever when evaluated in the Clinica Universitaria. At this time blood cultures were negative, but *Brucella melitensis* biogroup 3 was isolated from a pleural empyema. It is noteworthy that among the analytical results was the finding of 175 IU/ml of rheumatoid factor (RF), quantified by means of nephelometry (Beckman Image).

Patient 2. Patient 2 was a 67-year-old man with previous brucellosis, cured 40 years previously, but no further history of brucellosis-related complaints. He had not been exposed to any risk factor for brucellosis for years. He was admitted to the Hospital de Bellvitge (Barcelona, Spain) on 27 May 1999 because of high fever with chills and deteriorated general condition for 15 days. At admission, hypotension, peripheral hypoperfusion findings, scleral icterus, and painful hepatic enlargement of 3 to 4 cm were observed. The white blood cell count was 2,800/mm³, platelets were 49,000/mm³, aspartate aminotransferase/alanine aminotransferase was 3.9/3.9 μ kat/liter, and conjugated bilirubin/total bilirubin was 53/66 μ mol/liter. Blood cultures were negative. The RB test was negative, and SAT was positive at 1:40 and was considered a residual effect of previous brucellosis. Septicemia of an unknown source was diagnosed, and initial empirical combination treatment of amoxicillin-clavulanate plus gentamicin, as well as further doxycycline, was given. Over subsequent days, digestive bleeding appeared secondary to stress ulcer, and a gastrectomy was performed. An abdominal CT showed a calcium density in the liver without other pathological findings. Ten weeks after admission, fever reappeared, seroconversion was observed, and brucellosis was diagnosed (August 1999). Oral doxycycline at 100 mg/12 h plus intramuscular gentamicin at 240 mg/day for the first 15 days and then oral rifampin at 900 mg/day thereafter were given. In a new CT (19 August 1999), hypodensity next to the calcium image was apparent and a reactivation of latent brucellar liver granuloma was diagnosed, presumably related to the previous brucellosis. Since fever persisted with antibiotic therapy for 70 days, resection of the abscess was performed (October 1999); a pus culture was negative. The fever disappeared and the general condition was recovered, while antibiotic therapy was prolonged for 5 months. The outcome was good and the patient remained cured in subsequent controls for 4 years.

Patient 3. Patient 3 was a 49-year-old woman who reported a previous diagnosis of brucellosis more than 20 years earlier but with no other clinical problems throughout this period. She remained in an area in which brucellosis was not endemic and had not been exposed for any risk factor for brucellosis in recent years. She was admitted in May 1995 to the Hospital de Bellvitge (Barcelona, Spain) suffering from high fever, chills, sweating, arthralgias, and weakness for 1 week. At admission, hepatomegaly (3 cm) and splenomegaly (1 cm) were detected and there was right-side pain secondary to a pleural effusion. An abdominal CT showed a calcium density with surrounding hypodensity in the liver, pus being obtained through puncture aspiration. Blood cultures were negative, and no isolates were

detected in the pus culture. Since the RB test was negative, brucellosis was initially ruled out and a probable diagnosis of liver pyogenic abscess was considered. Despite antibiotic therapy with amoxicillin-clavulanate and repeated percutaneous aspirations, clinical findings persisted for 4 weeks. In June 1995, a repeated RB test became slightly positive, and SAT and the Coombs test confirmed the diagnosis of reactivated previous brucellar liver granuloma. Given a doxycycline-streptomycin combination (45 days and 15 days, respectively), the patient became well. Successive abdominal CT performed over subsequent years showed progressive heterogeneous calcified images, with some hypodensity (6 by 3 cm) of difficult evaluation (January 2000). Two years later (January 2002), this image increased slightly in size (7 by 5.5 cm), but she remained symptom free until January 2004 (more than 8 years after initial therapy), when fever and upper-right abdominal pain once again appeared for 2 weeks, along with a smooth subcutaneous tumor in this area. CT examination showed a subcutaneous extension of liver abscess (February 2004) (Fig. 1). She underwent percutaneous aspiration of pus (the culture was again negative) and prolonged antibiotic therapy with doxycycline and rifampin for 5 months, before a surgical hepatic resection was performed in July 2004. The outcome was good for the following year.

MATERIALS AND METHODS

Serum samples, antigenic preparations, and serological tests. From patient 1, the serum sample obtained in the Clinica Universitaria was the only sample available for serological studies; from the other two patients, 10 and 12 successive samples were obtained during follow-up periods of 4 and 9 years, respectively. The RB test was performed with antigen produced at the Central Veterinary Laboratory (Weybridge, Surrey, United Kingdom). Positive sera were diluted in saline to give dilutions ranging from 1:1 to 1:512, and the RB test was repeated with these dilutions. SAT was performed in ordinary 96-well microtitration-type polystyrene plates by making twofold serial dilutions (i) in 50 μ l of phosphate-buffered saline (PBS), pH 7.2, with the addition of an equal volume to each 1:80 dilution of the milk ring test antigen (Central Veterinary Laboratory) (8, 12) and (ii) in 50 μ l of the dilution buffer at pH 5.0 used in the Brucellacapt (Vircell Company, Santa Fe, Granada, Spain) test, with the addition of an equal volume of the cell suspension included in the Brucellacapt kit. It should be noted that we never used the U-bottomed microtiter plates coated with anti-total human immunoglobulin included in the Brucellacapt kit. The Coombs test was performed by the method described by Otero et al. (17) using anti-IgG human immunoglobulins (Operon, Zaragoza) diluted 1:1,000 in PBS. The CIEP test, using water-soluble proteins, was performed as described previously (7). The cytosolic fraction was obtained from rough *B. melitensis* 115 by the method previously described (15). The indirect enzyme-linked immunosorbent assay (ELISA) for the detection of IgM antibodies and the immunochromatographic lateral flow assay for the detection of IgM and IgG (IgM flow and IgG flow) were performed by the methods described by Ariza et al. (2) and Smits et al. (22).

RESULTS

Table 1 shows the results obtained with the different serological tests when the first serum samples of the three patients were examined, while Tables 2 and 3 summarize the results of the evolution of serological data obtained with serum samples from patients 2 and 3, respectively.

Anti-IgM antibodies. In patient 1, the IgM lateral flow was 4+ and the IgM ELISA titer was 1:800 at the time of diagnosis. As it showed 175 IU/ml of RF, the serum was absorbed with an anti-RF serum (ELISA Sorbent; Vircell, Granada, Spain), after which the IgM lateral flow and IgM ELISA were negative. Similarly, when the presence of RF was studied in the serum

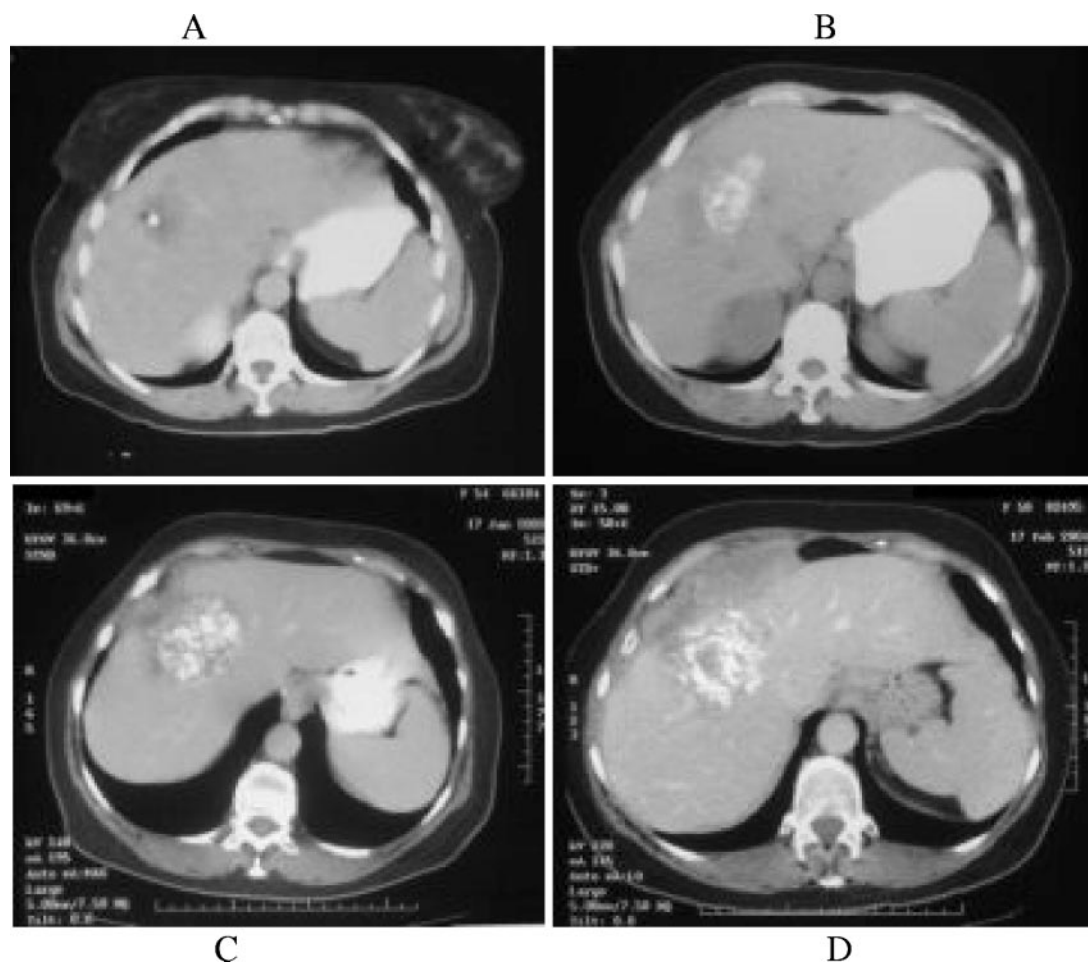


FIG. 1. (A) CT of the liver, showing a calcium density and slight surrounding hypodensity 1 year after therapy. (B) Abdominal CT 4 years after therapy, with progressive heterogeneous calcified images and some hypodensity (6 by 3 cm) of difficult evaluation. (C) Increase in the size of this image (7 by 5.5 cm) 6 years after therapy. (D) CT examination 8 years after therapy, showing a subcutaneous extension of the liver abscess.

samples from patient 2 by using latex particles coated with human gamma globulin (Biokit, Barcelona, Spain), all samples with positive IgM flow results agglutinated them but were negative after absorption with anti-RF serum (ELISA Sorbent; Vircell, Granada, Spain) (Table 2). Finally, all serum samples from patient 3 were RF and IgM lateral flow negative (Table 3).

Anti-LPS IgG antibodies. For patient 1, all tests detecting anti-LPS antibodies were clearly positive, making the initial diagnosis easy. The diagnosis of patient 2 was delayed because the initial titers of SAT and Coombs IgG at admission were interpreted as a residual sign of the previous brucellosis (Table

1). Likewise, the titers obtained in the SAT at pH 5.0 (Brucelacapt) and IgG lateral flow were insignificant or negative. However, a serological study of the serum sample taken after 3 months demonstrated clear seroconversion and that the antibiotic administration was unable to avoid the increment of titers. Moreover, a progressive diminution of titers was observed after surgical resection of the abscess (Table 2). Figure 2 shows the evolution of titers of SAT and Coombs IgG performed at pH 7.2 and 5.0. The results show that before the surgical resection, the titers of the four tests increased progressively and the titers of the SAT (pH 5.0) and Coombs IgG (pH 7.2) were practically the same. However, after surgical resec-

TABLE 1. Results of serological tests at admission^a

Patient	RF (IU/ml)	Titer obtained with indicated test				CIEP result (no. of bands/titer)	Lateral flow ^b	
		RB	SAT		Coombs IgG		IgM	IgG
			pH 7.2	pH 5.0				
1	175	32	1,280	40,960	40,960	6/128	4+*	4+
2	Neg	Neg	40	160	1,280	Neg	Neg	1±
3	ND	Neg	Neg	ND	ND	ND	ND	ND

^a Abbreviations: Neg, negative; ND, not done.

^b 1+, weak staining; 2+, moderate staining; 3+, strong staining; 4+, very strong staining; *, IgM was initially 4+ but became negative after absorption of RF.

TABLE 2. Serological results over time from patient 2^a

Time of serum sample	RF (IU/ml)	Titer obtained with indicated test					CIEP result (no. of bands/titer)	Lateral flow ^c	
		RB	SAT ^b		Coombs IgG			IgG	IgM
			pH 7.2	pH 5.0	pH 7.2	pH 5.0			
25 May 1999	Neg	Neg	40	160	1,280	2,560	Neg	1±	Neg
7 August 1999	132	4	160	2,560	2,560	5,120	3/8	2+	2+
Antibiotic therapy									
19 August 1999	2+	8	320	5,120	10,240	10,240	4/32	2+	2+
10 September 1999	2+	16	640	40,960	40,960	40,960	5/64	3+	3+
18 October 1999	2+	16	1,280	81,920	81,920	81,920	5/64	3+	3+
Liver resection (2 November 1999)									
6 mo	Neg	8	320	5,120	20,480	40,960	2/16	ND	ND
1 yr	Neg	4	80	640	10,240	10,240	2/8	1+	Neg
2 yr	Neg	4	80	320	5,120	10,240	2/4	ND	ND
3 yr	Neg	2	40	320	5,120	10,240	1/1	Neg	Neg
4 yr	Neg	2	40	320	5,120	10,240	1/1	ND	ND

^a Abbreviations: Neg, negative; ND, not done.

^b The SAT at pH 7.2 was done with milk ring test antigen (PBS); the SAT at pH 5.0 was done with Brucellapt antigen (Vircell).

^c 1+, weak staining; 2+, moderate staining; 3+, strong staining.

tion the titers of the SAT (pH 5.0) (Brucellapt) descended more rapidly than the Coombs IgG (pH 7.2) titers. The persistence of Coombs (pH 7.2) titers of 1:5,120 for 4 years after admission should be emphasized.

For patient 3, the RB test was negative at admission and, thus, other serological tests were not performed. Due to the persistence of clinical findings under amoxicillin-clavulanate therapy, serological tests were repeated a month later and seroconversion was detected (RB, positive; SAT, 1:80; Coombs, 1:640); the patient was then treated with a combination of doxycycline-streptomycin. The results of serological tests carried out with the serum sample obtained 3 months after therapy showed anti-LPS and anti-cytosolic protein IgG antibodies, although the IgM lateral flow was negative. The results of these serological tests showed that after 4 years, the patient had a new antigenic stimulus, even though he did not show clinical symptoms or signs; in fact, 4 years later (26 April 2000), when the patient was free of brucellosis-related symp-

toms, a significant increase in anti-LPS IgG antibodies was detected by the Coombs test and the IgG lateral flow assay. However, no serological changes were shown by the RB, SAT (pH 7.2), or SAT (pH 5.0) (Brucellapt) tests, and those appearing in the abdominal CT were unclear (Fig. 1B). Eight years later (18 February 2004), a clinical relapse of liver sup-puration was demonstrated, while titers of the RB, SAT (pH 7.2), and SAT (pH 5.0) (Brucellapt) tests continued to show a progressive decrease; only the Coombs test maintained a notoriously high titer, 1:2,560 (Table 3).

Anti-cytosolic protein antibodies (CIEP). In CIEP with water-soluble cytosolic proteins, the three patients developed precipitation lines. When CIEP was carried out with a whole serum sample from patient 1, the development of six precipitation lines was shown, with a titer up to 1:128. For patient 2, CIEP became positive after 3 months. As with the titers of serological tests detecting anti-LPS antibodies, the number of precipitation lines and the CIEP titer increased progressively before descending

TABLE 3. Serological results over time from patient 3^a

Time of serum sample	RF	Titer obtained with indicated test					CIEP result (no. of bands/titer)	Lateral flow ^c	
		RB	SAT ^b		Coombs IgG			IgM	IgG
			pH 7.2	pH 5.0	pH 7.2	pH 5.0			
27 May 1995	ND	Neg	ND	ND	ND	ND	ND	ND	ND
26 June 1995	ND	2	80	ND	640	ND	ND	ND	ND
Specific antibiotic therapy (45 days)									
3 mo	Neg	16	160	320	2,560	2,560	2/8	Neg	3+–4+
6 mo	Neg	16	80	160	2,560	2,560	2/4	Neg	2+
1 yr	Neg	8	80	160	1,280	1,280	2/2	Neg	2+
2 yr	Neg	4	20	80	1,280	1,280	2/2	Neg	1+
4 yr	Neg	2	40	80	5,120	5,120	2/4	Neg	3+–4+
6 yr	Neg	Neg	20	40	2,560	5,120	2/2	Neg	2+
8 yr	Neg	Neg	20	40	2,560	2,560	2/2	Neg	1+–2+
February 2004 (clinical relapse; antibiotic therapy, 6 mo)									
April 2004 (therapy, 2 mo)	Neg	Neg	20	40	2,560	1,280	2/4	Neg	1+–2+
July 2004 (liver resection)									
November 2004	Neg	Neg	20	40	1,280	1,280	2/4	Neg	1+–2+

^a Abbreviations: Neg, negative; ND, not done.

^b The SAT at pH 7.2 was done with milk ring test antigen (PBS); the SAT at pH 5.0 was done with Brucellapt antigen (Vircell).

^c 1+, weak staining; 2+, moderate staining; 3+, strong staining; 4+, very strong staining.

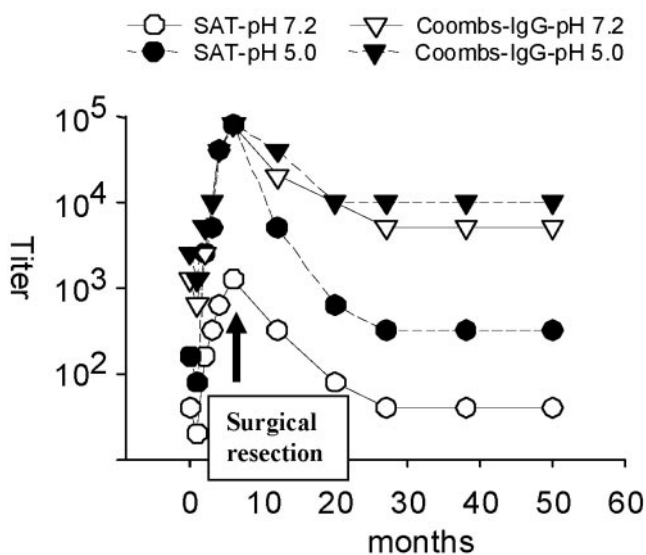


FIG. 2. SAT (at pH 7.2 and pH 5.0 [Brucellacapt]) and Coombs (at pH 7.2 and pH 5.0) titers over time from patient 2. Note the rapid decrease in Brucellacapt titers in contrast to the persistence of those of the Coombs test.

after surgical resection. For patient 3, the results of CIEP carried out with the serum corresponding to 3 months were positive (two precipitation lines and a titer of 1:4); moreover, it should be noted that CIEP remained positive 8 years later.

DISCUSSION

Detailed serological study of our patients has once again demonstrated that reactivation of CHSB is associated mainly with a secondary IgG serological response. These results confirm our previous findings regarding secondary serological changes in brucellosis relapse (2, 12, 19), which did not show an increase in IgM antibodies, as in the case of other thymus-dependent antigen responses (25).

The IgM lateral flow assay has been reported to be a very specific and sensitive test for the diagnosis of human brucellosis (22). It is known that IgM antibodies decrease after the first few months and therefore are not observed in some patients with prolonged disease (2, 9). However, their specificity is currently considered to be very high, close to 100%, and no identified false-positive results have been described. Thus, the finding of positive IgM lateral flow and IgM ELISA titers with two of our patients was surprising and suggested the emergence of a secondary response with an increase in IgM antibodies; it was this finding that motivated us to reexamine our data.

Patient 1 had 175 IU/ml of RF. Nowadays, RF is recognized as presenting one of the most serious problems in IgM testing (13), provided that the RFs are anti-IgG IgM antibodies. Several authors have reported the presence of RF in patients with brucellosis (3, 10, 14), but the potential interference of RF in diagnostic tests based on the detection of IgM antibodies to *Brucella* has not been evaluated. The concomitant observation of positive RF and anti-IgM tests led us to suspect a relationship between these two findings. This hypothesis was con-

firmed, given that all positive IgM lateral flow and IgM ELISA samples were rendered negative when studies were repeated after absorption of the samples with anti-RF serum. The presence of false-positive results may be of relevance for some patients with CHSB who report an acute clinical presentation (1), where an apparent IgM response may make it difficult to diagnose a reactivation of previous brucellosis. Thus, the elimination of RF by absorption should be performed in a routine way before testing of sera for the presence of IgM-type antibodies.

Moreover, the frequency of positive RF in patients with brucellosis seems to be low overall but prevails in patients with focal and prolonged disease, in whom an intensive antigenic stimulation could be assumed. Thus, in a large series of patients, Mousa et al. (14) reported positive RF in 8.8% of 169 patients with osteoarticular disease but in only 0.2% of 283 patients without these complications. These results suggest that if IgM antibodies are found in patients with supposed brucellosis relapse or reactivation, or in patients with focal or prolonged disease, the possibility of a positive RF as the cause of false-positive results should be ruled out.

Patients with CHSB often have negative pus cultures and usually show negative blood cultures, despite some of them presenting with acute clinical symptoms and significant systemic repercussions (1). This surprising observation may illustrate the nature of this clinical form of brucellosis, which is in fact a local reactivation of a previous calcified granuloma. This is characterized by the presence of a low local bacterial inoculum, which probably produces this general repercussion through late hypersensitivity mechanisms. In fact, the systematic presence of small quantities of microorganisms has been demonstrated by Colmenero et al. using PCR techniques with pus and calcified granuloma samples, the results showing negative cultures (5, 6). Centrifugation-shell vial culture may also be a successful method for detecting bacteria in these cases (20). In this context, diagnosis must be based on serological results if PCR techniques or shell vial cultures are not available.

The secondary serological response detected by classical anti-LPS tests, such as the RB test, SAT, and the Coombs test, may provide confusing results at the time of clinical diagnosis for some patients. This occurred in two of our cases and led to a delay in the diagnosis of brucellosis. It should be noted that for one of these cases (patient 2), in which we were able to extend the study of this initial sample using various classical or modern serological tests, such as Brucellacapt, IgG lateral flow, and CIEP, no additional contribution to the diagnosis was obtained.

Some specific issues regarding the comparison of the Brucellacapt test with SAT and Coombs test results should be considered. The Brucellacapt test has recently been developed by the Vircell Company (Santa Fe, Granada, Spain) as an immunocapture-agglutination test; it uses dilution buffer at pH 5.0, a cell suspension of *Brucella* in buffer, also at pH 5.0, and U-bottomed microtiter plates coated with anti-total human immunoglobulin, included in the Brucellacapt kit. Reported results have been very promising and are closely related to those of the Coombs test; however, it has been suggested that they may become a better marker of disease activity (4, 16).

In the present study we performed SAT and the Coombs test at pH 5.0 with an antigenic suspension of Brucellacapt but

using ordinary microtitration-type polystyrene plates, because it has been demonstrated in our laboratory that results of the Brucellacapt test with the U-bottomed microtiter plates coated with anti-total human immunoglobulin included in the Brucellacapt kit were identical to those obtained with ordinary microtitration-type polystyrene plates (J. L. del Pozo, M. Rubio, and R. Díaz, Abstr. X Congr. Nacional Soc. Española Enfermedades Infecc. Microbiol. Clin. [SEIMC], abstr. 599, 2002).

For patient 2 (Fig. 2), the titers of Brucellacapt (SAT at pH 5.0) and Coombs (pH 7.2) were similar in the third, fourth, and fifth samples obtained during the 2 months of antibiotic therapy but prior to liver resection; in contrast, the subsequent titer profile decreased more rapidly with Brucellacapt (SAT at pH 5.0) than with the Coombs (pH 7.2) test. The persistence of high Coombs (pH 7.2) and Coombs (pH 5.0) titers (1:5,120) 4 years later, despite the patient being cured, should be noted; this may be a residual effect following an intense and prolonged secondary immunological stimulation. Although the titers of the Coombs test performed at pH 5.0 and at pH 7.2 were identical, the titers of SAT at pH 5.0, performed with the antigenic suspension included in the Brucellacapt kit, were several times higher than those of SAT (pH 7.2) performed with ring milk test antigen.

There are two possible explanations for this fact. It is known that pH modifies antibody affinity (18), and it has been demonstrated that the agglutinating ability of IgG and IgA antibodies increases at acidic pH (21). The rapid fall of SAT (pH 5.0) titers obtained with the Brucellacapt antigen, with respect to the decrease in antigenic stimulation following antibiotic therapy, suggests that the agglutinating activity is due to high-affinity antibodies. However, it has also been demonstrated that LPS of *Brucella* contains acyl chains that are not present in other gram-negative bacteria, with the exception of *Ochrobactrum* spp., resulting in an increase in its hydrophobic characteristics (24) and in a conformation that may hide immunodominant epitopes of the O chain. Therefore, the hypothesis that acidic pH, increasing the ionic forces, may carry with it a decrease in the hydrophobic characteristics of LPS and then a separation of the chains and an exposition of unrecognizable epitopes at neutral pH cannot be ruled out.

Analysis of the serological profile of patient 3 showed that evaluation during late clinical follow-ups of patients after therapy may be misleading. This patient, undergoing percutaneous abscess drainage and antibiotic therapy, suffered from a clinical relapse 8 years later. However, in addition to radiological findings, serological changes indicative of further clinical relapse were detected 4 years earlier by the Coombs test, though no such changes were observed in the RB test, SAT at pH 7.2, or SAT at pH 5.0 (Brucellacapt). It should be noted that this relapse was also detected by the IgG lateral flow assay and CIEP, along with the changes in the Coombs test.

The results of our study indicate that in some patients with CHSB, serological changes occurring due to the reactivation of the infection, either at the beginning of the disease or subsequently, during follow-up, may be mild and include low-affinity antibodies or small quantities of high-affinity antibodies, detected mainly by the Coombs test. Brucellacapt and other anti-LPS tests do not offer any additional information in this context, but the IgG lateral flow assay and CIEP may be of some use. Since high Coombs test titers may persist for a long time

in cured patients, careful surveillance of titer changes in this test in paired samples would seem to be the best marker of infection activity. As the disease progresses, an intense IgG secondary response may develop and RF sometimes appears, simulating an IgM response.

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