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KINETICS OF SPECIFIC ANTIBODIES IN MEDITERRANEAN SPOTTED FEVER DETERMINED BY WESTERN BLOTTING AND MICROIMMUNOFLUORESCENCE

L. BEATI[a,1], N. TEYSSEIRE[a], D. RAOULT[a,2]

[a]Centre National de Référence des Rickettsioses, CHU La Timone, 13385 Marseille, France

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Summary. - The kinetics of specific antibodies in human cases of Mediterranean Spotted Fever were studied by applying Western blot immunoassay and microimmunofluorescence test to 105 sera from 42 patients.

Key words: Rickettsia conorii; Mediterranean spotted fever; Western blot; microimmunofluorescence; kinetics of antibodies

Introduction

Mediterranean spotted fever (MSF) is a tick-transmitted rickettsiosis caused by *R. conorii* which is endemic in Southern Europe, Africa, and India. Microimmunofluorescence (MIF) is the most currently used test in serodiagnosis of rickettsial diseases (Newhouse *et al.*, 1976; Raoult *et al.*, 1985*a*). The Western blot assay (Wb) was proposed as a suitable alternative for the detection of antibodies (Ab) in regard of its sensibility and specificity (Walker *et al.*, 1985; Raoult and Dasch, 1989). We studied here the kinetics of specific Ab in human cases of MSF by applying Wb and MIF technique to 105 sera from 42 patients. We also compared the results obtained with these two serological methods.

Materials and Methods

Sera. A total of 105 sera from 42 patients suffering from MSF were obtained from the collection of the Centre National de Référence des Rickettsioses (Marseille). The selected patients had clear symptoms of MSF and their sera presented seroconversion toward *R. conorii* by MIF.

Antigen. R. conorii (Moroccan Strain - ATCC VR 141) was grown on Vero cells monolayers in 150 cm^2 tissue culture flasks. Heavily infected cells (5 days post-inoculation) were harvested with glass beads and pelleted by centrifugation at 10 000 rev/min for 15 min. The cells were broken up and the obtained rickettsial material was purified by differential centrifugations (Raoult and Dasch, 1989). Protein concentration was determined using the Lowry method and adjusted to 1 mg/ml.

Western blot. Rickettsial antigen was solubilized at room temperature in 50 % Laemmli solubilizer (Laemmli, 1970). The polyacrylamide gel electrophoresis of the antigen was carried out at 10

mA/gel in a Mini-Protean II cell (Bio-Rad) in an ice bath. 150 μl of solubilized antigen were put on a line on a 3.9 % stacking gel. The proteins were transferred to nitrocellulose papers (Towbin *et al.*, 1979) in a Transblot cell (Bio-Rad) at 50 Volts during one hour on ice. Nitrocellulose papers were blocked overnight in 5 % nonfat dry milk TBS. After washing in TBS, the papers were cut in 20 vertical strips. Each human serum was diluted at 1/200 in 3 % nonfat dry milk TBS, applied on three strips and left for 12 hr on a rocker. Unfixed antibodies were removed by three 10 min washes in TBS. For each serum, goat peroxidase conjugated anti-human (anti-α at 1/100 in 3 % nonfat dry milk TBS, anti-μ at 1/100 or anti-γ at 1/400) immunoglobulins (Pasteur) were incubated with strips for one hour. After washing, the bound enzyme was detected by reaction with 0.015 % 4-chloro-1-naphtol, 0.015 % hydrogen peroxide in 16.7 % methanol in TBS. The strips with their revealed bands were then washed in water and dried between filter papers (Raoult and Dasch, 1989).

MIF. The MIF test was performed following the usual method (Raoult *et al.*, 1985*a*). Heavily infected cells were applied by pen point on microscope slides, air dried and fixed with acetone. They were overlaid with serum dilutions for 30 min at 37 °C in a humid chamber. A previous adsorption of the IgG (Behring) has to be done in sera used to detect IgM and IgA Ab. The slides were then treated with specific fluorescein isothiocyanate conjugated goat anti-human anti-α, -μ and -γ specific conjugates (Pasteurs). Positive titres are $\geqq$1/128 for IgG and $\geqq$1/64 for IgM and IgA (Raoult *et al.*, 1985*b*).

Results

Microimmunofluorescence

On the 5th day after the onset of disease (first day with fever), 30 % of the sera were positive with anti-γ conjugates, 25 % with anti-μ and 0 % with anti-α. IgA antibodies were only detected after a 5 days delay. The kinetics of IgG and IgM antibodies are similar. The percentage of the positive sera reached 55 % (IgM) and 75 % (IgG) on the 10th day, 85 % after the 15th day (IgG and IgM) and a plateau of 100 % after the 35th day (IgG, IgM, and IgA). Unlike the IgG and the IgM antibodies which persisted after the 36th day, the percentage of IgA positive sera fell to the 30 %.

Western blot

The specific humoral response against *Rickettsia conorii* consisted of antibodies against the lipopolysaccharide (LPS), the 115 kD and sometimes against the 135 kD antigen (Fig. 1). The antibodies against LPS bands were localized on the lower molecular weight range (<60 kD). On the 5th day after the onset of the disease seroconversion occurred in 25 % of the cases with IgM and 45 % with IgG. On the 10th day, 80 % of the tested sera were IgM and IgG positive. All sera were positive before the 35th day. For each Ab class (IgG, IgM, and IgA), the reaction to LPS antigen was the more precocious and was closely followed by response to the 115 kD protein. The percentage of IgA and IgM positive sera decreased after the 35th day. Each sera reacted against the LPS and the 115 kD antigens, whereas Ab against the 135 kD antigen were detected in at most 60 % of the tested sera with anti-γ conjugates and in only 15–20 % of the sera with anti-α and anti-μ conjugates. After the 35th day, the Ab against the 135 kD antigen decreased in each Ab class.

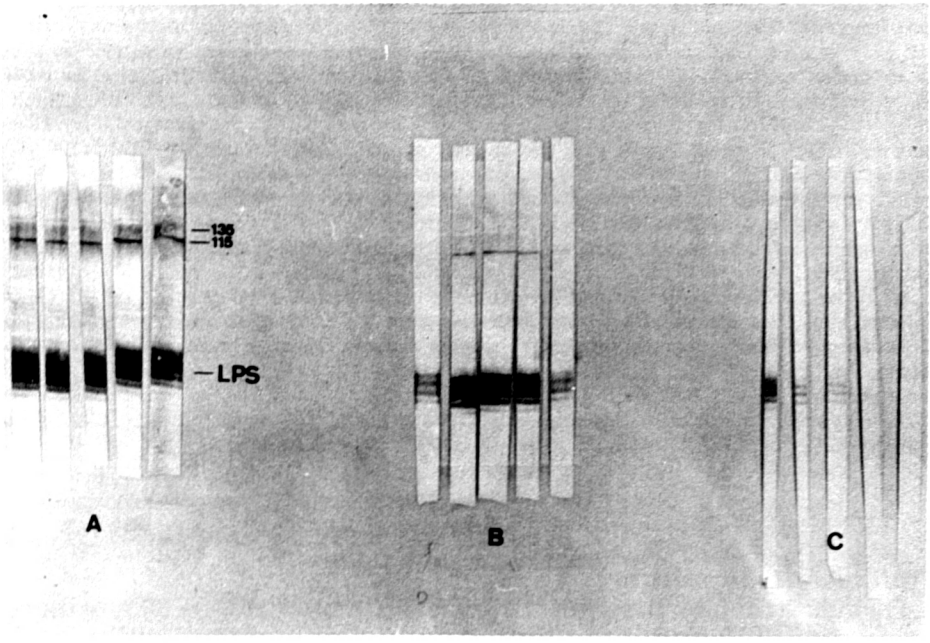

Fig. 1
Western blot reations against *R. conorii* antigens of a sequence of 5 sera from the same patient (A) IgG reaction. (B) IgM reaction. (C) IgA reaction. a: 1st serum (13th day); b: 2nd serum (21st day); c: 3rd serum (27 day); d: 4th serum (50 th day); e: 5th serum (70th day).

Fig. 1 shows the sequence of 5 sera from the same patient. IgG antibodies reacted against the LPS, the 115 kD and the 135 kD antigen since the 13th day and persisted agter the 70th day. On the 13th day IgM reacted only with the LPS, the reactions against the 115 kD and the 135 kD antigens appeared after the 21st day and the band corresponding to the 135 kD protein disappeared after the 27th day. In this case, the IgA response was directed only toward the LPS and decreased after the 27th day.

Discussion

With the MIF test, we detected 30 % of the positive sera on the 5th day, 75 % on the 10th day (Ig), and 100 % after the 35th day. The previous described kinetics of Ab in human cases of MSF (Mansueto *et al.*, 1983; Mansueto *et al.*, 1985; Raoult *et al.*, 1986) were obtained using the MIF test, the indirect haemagglutination test and the latex agglutination (Raoult *et al.*, 1986). Even if the signifi-

cant level of positivity was sometimes (Mansueto *et al.,* 1985) lower (1/40) and the detection of positive sera was performed with a different technique (Bio Mérieux-Kit), the kinetics we observed in our study confirmed nevertheless the results obtained by the other authors.

Using the Wb assay, the major immunogenic antigens for humans during MSF are the LPS (group-specific antigen) and the 115 kD protein which is, like the 135 kD protein, a species-specific antigen (Anacker *et al.,* 1987; Li *et al.,* 1988). In fact, each sera and each antibody class reacted with the LPS and the 115 kD antigen. Unlike the previous described kinetics (Mansueto *et al.,* 1985), the number of IgM positive sera decreased after the 25th day. That can be explained by the relative small sample of sera collected in the late stages of the disease. On the other hand, the decrease in the number of IgA positive sera seems to be characteristic in MSF after the 20th day (Mansueto *et al.,* 1983). According to our results, seroconversion occurs at the latest on the 35th day, sera are never IgA positive after the 40th day and the 135 kD antigen is never detected before the 5th day.

The specificity and sensitivity of the Wb and the MIF are considered to be similar (Raoult and Dasch, 1989). In our study, in the first 10 days after the onset of the disease, the seroconversion occurred only 1 or 2 days earlier with Wb than with MIF, so that we can confirm that the sensitivity of these two methods is very similar. After the 10th day, the evolution patterns of Wb reactions exhibited an obvious parallelism with the MIF assay. In conclusion, this study tend to confirm that Wb can provide a very sensitive method in serodiagnosis of MSF, especially in the early stages.

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