Acta virol. **36**: 19-24, 1992

SERUM ANTIBODIES IN RICKETTSIA PATIENTS AS DETERMINED BY IMMUNOBLOTTING TECHNIQUE

HANUKA, N., IGHBARIEH, J., MANOR, E.

Virology Unit, Faculty for Health Sciences, Ben-Gurion University of the Negev, 84105 Beer-Sheva, Israel

Received April 23, 1991

Summary. - The Western-blot technique (WB) was used to determine which polypeptides of Israeli spotted fever (ISF) isolates and other spotted fever group rickettssia (SFGR) reference isolates (G212, S484, A828) and two reference strains. *R. Rickettsii* (Sheila Smith strain) and *R. conorii* (Boutonneuse fever), were used as antigen sources for the WB. Immunoperoxidase assay (IPA) seropositive (titer>80) and seronegative (titer<) sera were examined with the separated polypeptides of the above strains. WB analysis of the rickettsial polypeptide-serum reactions showed that *R. conorii* and the three isolates of ISF reacted identically with the sera, except that in the three ISF strains a 175 kD protein was present. It was also observed that all of the IPA seropositive sera examined reacted with the following polypeptides: 18kD, 20kD, 22kD (28kD to 37 kD LPS group), while each seropositive and seronegative serum reacted differently with polypeptides 23kD, 42kD, 45kD, 46kD, 52kD, 55kD, 70kD, 82kD, 105kD, 125kD, 155kD and 175kD. Using this technique, no heat labile polypeptides (preelectrophoretic treatment: 100 °C for 2 min vs 37 °C for 20 min) were observed in SFGR strains used in this study. Our results indicate that the immunoblot technique shows no difference between *R. conorii* and ISF antigens except the existence of 175kD protein antigen in the latter.

Key words: Israeli spotted fever group rickettsiae; Western blotting; antigens

Introduction

The clinical manifestations of Israeli spotted fever (ISF) differ from those of Mediterranean spotted fever (MSF) in that the „tache noire" is almost invariably absent, whereas, in most well-studied sites, between 60 % and 100 % of the cases of MSF show this pathophysiology to a varied extent (Goldwasser *et al.*, 1974; Gross and Yakupsky, 1987).

Several approaches have been used to differentiate between numerous isolates (Goldwasser *et al.*, 1974; Philip *et al.*, 1978; Anacker *et al.*, 1987; Vitale *et al.*, 1989). Antigenic comparion of many strains has been achieved with acute phase antisera by indirect microimmunofluorescence (Philip *et al.*, 1978; Wang *et al.*, 1987). Although monoclonal antibodies have been used for antigenic comparison (Anacker *et al.*, 1987; Wang *et al.*, 1987; Vitale *et al.*, 1989), the use of polyclonal antibodies may be a more valid method for determination of similarities between strains with respect to immunodominant determinants.

In this study we compared the reaction of antibodies in human ISF seropositive sera to separated SFGR antigens. Three strains of ISF and two reference strains, *R. conorii* (RC) (Boutonneuse fever (BF)) and *R. rickettsii* (RR) (Rocky Mountain spotted fever), were examined in an attempt to identify the presence of antigens which could help to explain why the „tache noire" phenomenon is almost invariably absent in ISF patients.

Materials and Methods

Sera. Sera were obtained from patients suffering from, or convalescent from spotted fever group rickettsia (SFGR) infections. All exhibited rises in titres against SFGR by an immunoperoxidase assay (IPA), described previously (Hanuka *et al.*, 1988) and adapted for detection of antibodies to Rickettsia. As control sera we used: (A) sera from healthy people, and (B) sera from patients who had a febrile illness but were seronegative for SFGR by IPA. We also used rabbit antisera to SFGR (strain G212).

SDS polyacrylamide gel electrophoresis and Western blotting (WB). Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis and WB were performed essentially according to the methods described previously (Laemmli, 1970; Hanuka *et al.*, 1988). Briefly, Renografin purified rickettsial antigens were dissolved in sample buffer and separated by electrophoresis on a 12.5 % polyacrylamide slab gel. After transfer to a nitrocellulose membrane and incubation overnight with diluted sera, polypeptides reacting with serum antibodies were detected with horseradish peroxidase conjugated rabbit anti human IgG or horseradish peroxidase goat anti-rabbit antibodies (in the case of rabbit sera). Color development was with 3.3-Diaminobenzidine (Aldrich Chemical Co.).

Rickettsial strains - antigens. Patients' isolates of ISF (G-212 and S-484) were kindly provided by Dr. R. A. Goldwasser, Biological Institute, Nes Ziona, Israel. A third patient isolate (A-828) was isolated and propagated in our laboratory. The SFG reference strains: *R. rickettsii*, Sheila Smith strain, and *R. conorii*, BF, Casablanca strain, were generously provided by Dr. C. J. Wisseman, J. of the University of Maryland Medical School. Rickettsiae were purified from infected cultures by Renografin density gradient centrifugation. Protein concentrations were determined by the Bio-Rad protein assay (Bio-Rad, Richmond, California).

Results and Discussion

Immunoblotting analysis of rickettsial polypeptides showed that the reactions of the three isolates of ISF were identical (Fig. 1). The most prominent difference between the local strains and *R. conorii* (BF) was the presence in the

former of a 175 kD polypeptide which was absent in the latter. This difference was equally apparent on Coomassie blue stained gels of the rickettsia (data not shown). These results are in agreement with those reported by Cwikel *et al.* (1990).

Fig. 1A shows the reaction of IPA seropositive sera with separated antigens of three strains of ISF, RC, and RR. The major polypeptide antigens of ISF recognized by the human sera have molecular masses of 18, 21, 23, 25, 57, 105, 125, 135, 155, and 175 kD, and there was also a dominant group of lipopolysaccharide (LPS) antigens with molecular masses between 28 and 42 kD. Polypeptides of 50, 70, and 90 kD reacted less consistently and varied in intensity from serum to serum. (These we term "minor" polypeptides).

Seronegative sera reacted strongly with the major polypeptides 57 kD, 125–135 kD, and 155–175 kD, and in some cases with a few minor polypeptides as well. Reaction of the seronegative sera with the LPS was not observed (Fig. 1B). Since both sera from the control groups and sera from SFGR patients reacted with the major proteins, these proteins cannot be used as diagnostic tools for SFG rickettsial infection. The 175 kD polypeptide which appeared in the three isolate strains of ISF, demonstrated a clear reaction with seropositive and seronegative sera. This band appeared only faintly when the sera were reacted with RR and was completely absent when the sera were tested on RC. No heat labile polypeptides [pre-electrophoresis antigen treatment: boiling (100 °C) for 2 min vs 20 min incubation at 37 °C] were observed by WB in SFGR strains used in this study.

LPS antigens were identified by their resistance to proteinase K digestion. In order to evaluate the molecular mass range of this group of reactive antigens, we resolved rickettsial antigens before and after proteinase K digestion and detected them by immunoblot using rabbit antisera against the G212 strain (Fig. 2). It was found that the LPS group ranged from 18 kD through 37 kD (for

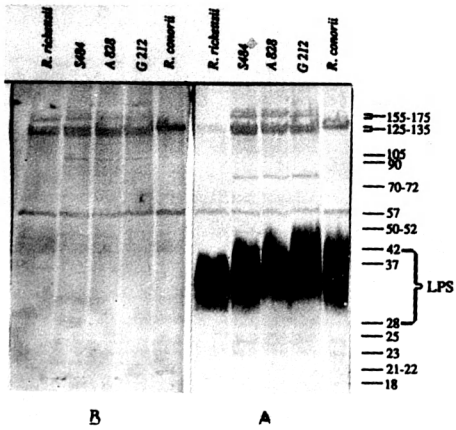

Fig. 1
Western blot reactivity against rickettsial antigens of: (A) serum from an ISF seropositive patient (IPA titre:1280), (B) serum from an ISF seronegative person (IPA titre <40)
Antigens: *R. conorii* (BF); three isolates ISF G212, A828, S484; *R. rickettsii* (SS).

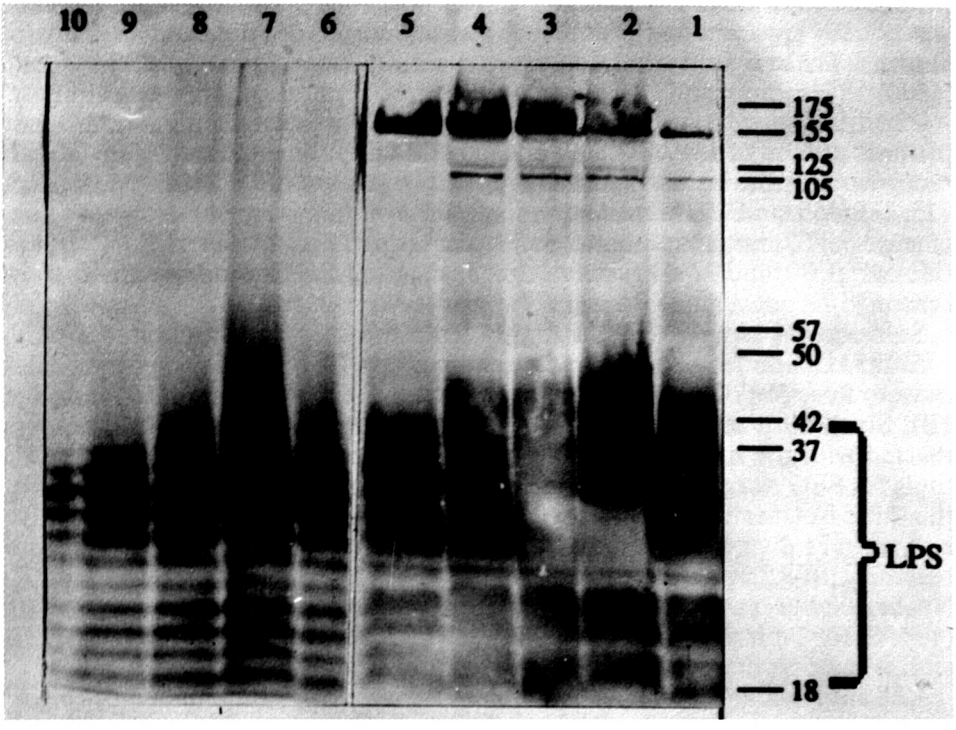

Fig. 2

Immunoblot reactivity of rickettsial antigens with rabbit antiserum to strain G212 Lanes 1 through 5, mock treated with proteinase K; lanes 6 through 10, treated with proteinase K. Lanes 1 and 6: *R. conorii*; lanes 2 and 7; G212 ISF strain; lanes 3 and 8: A828 ISF strain; lanes 4 and 9; S484 ISF strain; lanes 5 and 10: *R. rickettsii.*

RR) and from 18 kD to 42 kD (for the other strains). In other words, LPS bands could also be detected among the low molecular weight antigens.

LPS is a major immunogen for humans. It is known to be a group antigen of typhus and spotted fever group rickettsiae, and it also contains determinants which cross-react between the two groups and with *Proteus* strains OX2 and OX19 and *Legionella bozmanii* (Lenz *et al.*, 1988; Raoult and Dasch, 1989*a*). The possibility that antigens in the ladder-like pattern of the LPS (28–42 kD) include one or more proteins cannot be ruled out, and will require further investigation.

Our results are in agreement with those of other researchers (Walker *et al.*, 1985; Anacker *et al.*, 1985; Feng *et al.*, 1986; Feng *et al.*, 1987; Anacker *et al.*, 1987; Lenz *et al.*, 1988; Raoult and Dasch, 1989b), who compared immunoreactive polypeptides of SFGR and reported that antibodies reactive with the major

proteins are stimulated by infection of humans, rabbits, guinea pigs, and mice, and cross-react with the antigens of closely related, heterologous SFG rickettsial species. In all cases, the higher molecular weight antigens were considered to be more important rickettsial antigens for protective immunity.

In this study we detected the same high molecular weight polypeptides for ISF isolates strains as for other SFGR strains. It would be reasonable to assume that these proteins can stimulate a protective antibody reaction; thus, the two immunoprotective surface proteins and the LPS seem to be the immunodominant surface antigens for many SFGR.

Our results indicate that WB does not show significant differences between RC and ISF isolates' antigens, except in the antibodies' reactivity with the 175 kD in the ISF isolates. It may be that gradient gel or two dimension gel electrophoresis could furnish more information about this protein. Such studies are now underway. Determination of the possible relevance of the 175 kD protein to the lack of Tache Noire development in ISF will require further investigation.

Acknowledgements. Dedicated to the memory of Israel Sarov, an exemplary scientist and teacher. This study was supported by NIH-NIAID Contract No. AI 126688. Thanks are due to Dr. Maureen Friedman for her assistance in writing this manuscript.

References

Anacker, R. L., List, R. H., Mann, R. E., Hayes, S.F., and Thomas, L.A. (1985): Characterization of monoclonal antibodies protecting mice against *Rickettsia rickettsii. J. infect. Dis.* **151**, 1052-1060.

Anacker, R. L., McDonald, R.A., List, R. H., and Mann, R. E. (1987): Neutralizing activity of monoclonal antibodies to heat-sensitive and heat-resistant epitopes of *Rickettsia rickettsii* surface proteins. *Infect. Immun.* **55**, 825-827.

Anacker, R. L., Mann, R. E., and Gonzales, C. G. (1987): Reactivity of monoclonal antibodies to *Rickettsia rickettsii* with spotted fever and typhus group rickettsia. *J. clin. Microbiol.* **25**, 167-171.

Cwikel, B., Ighbarieh, J., and Sarov, I. (1990): Antigen polypeptides of Israeli spotted fever isolates compared with other spotted fever group ricckettsiae, pp. 381-388. In *Rickettsiology: Current Issues and Perspectives,* Vol. 590, Annals of the N. Y. Academy of Sci.

Feng, H. M., Kirkman, G., and Walker, D. H. (1986): Radioimmunoprecipitation of (^{35}S) methionine-radiolabelled proteins of *Rickettsia conorii* and *Rickettsia rickettsii. J. infect. Dis.* **154**, 717-721.

Feng, H. M., Walker, D. H., and Wang, J. G. (1987): Analysis of T-cell-dependent and independent antigens of *Rickettsia conorii* with monoclonal antibodies. *Infect. Immun.* **55**, 7-15.

Goldwasser, R. A., Steiman, Y., Klingberg, T. A., Schwartz, L. M., and Klingerg, M. A. (1974): The isolation of strains of rickettsiae of the spotted fever group in Israel and their differentiation from other members of the group by immunoprecipitation. *Scand. J. infect. Dis.* **6**, 53-62.

Gross, E., and Yagupsky, P. (1987): Israeli rickttsial spotted fever in children. *Acta Trop. Med. (Basel),* **44**, 91-96.

Hanuka, N., Glasner, M., and Sarov, I. (1988): Detection of IgG and IgA antibodies to *Chlamydia trachomatis* in sera of patients with chlamydial infections. Use of immunoblotting and immunoperoxidase assays. *Sex. Trans. Dis.* **151**, 93-99.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London),* **227**, 680-685.

Lenz, K., Li, B., and Walker, D. H. (1988): Protective monoclonal antibodies recognize heat-labile epitopes on surface proteins of spotted fever group rickettsiae. *Infect. Immun.* **56**, 2587-2593.

Philip, R. N., Casper, E. A., Burgdorfer, W., Gerloff, R. K., Hughes, L. E., and Bell, E. J. (1978): Serological typing of rickettsiae of the spotted fever group by microimmunofluorescence. *J. Immunol.* **12**, 1961-1968.

Raoult, D., and Dasch, G. A. (1989*a*): The line plot in an immunoassay for monoclonal and other antibodies. *J. immunol. Methods* **125**, 57-65.

Raoult, D., and Dasch, G. A. (1989*b*): Line blot and Western blot immunoassays for diagnosis of Mediterranean spotted fever. *J. clin. Microbiol.* **27**, 2073-2079.

Vitale, G., DiStefano, R., Damini, G., and Mansueto, S. (1989): Characterization of sicilian strains of spotted fever group rickettsiae by monoclonal antibodies. *J. clin. Microbiol.* **27**, 1081-1085.

Walker, D. H., Hanff, P., and Hegarty, B. (1985): Analysis of protein antigens of *Rickettsia rickettsii* and *Rickettsia conorii* by Western immunoblotting, p. 92. In *Rickettsiae and Rickettsial Diseases.* Publishing House of the Slovak Academy of Sciences, Bratislava, Czechoslovakia.

Wang, J., Walker, D. H., Li, H., Lenz, B., and Jerrells, T. R. (1987): Barbash strain spotted fever group rickettsia A strain of *Rickettsia conorii* and difference from *Rickettsia sibirica. Acta virol.* **31**, 489-498.