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HISTOPATHOLOGY AND IMMUNOPATHOLOGY OF SKIN BIOPSY SPECIMENS IN MEDITERRANEAN SPOTTED FEVER

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Summary. - Histology of skin lesions and demonstration in them of *Rickettsia conorii* by direct immunofluorescence test (DIF) are presented in 13 patients with Mediterranean spotted fever (MSF). The lymphohistiocytic vasculitis which dominated the picture is not specific, however, it could be suggestive for the diagnosis of rickettsiosis. By DIF we demonstrated rickettsial coccobacillary forms in all the patients: in 12 macular lesions and in one „tache noire". The diagnosis was also confirmed by indirect immunofluorescence test in each case. DIF test was shown to be sensitive, specific and reliable in early diganosis of MSF.

Key words: Mediterranean spotted fever; histology; direct immunofluorescence

Introduction

Neither a histologic nor a clinical feature is specific for spotted fevers. However, both the clinical picture and histologic changes could be sufficiently characteristic; the diagnosis may be confirmed serologically and/or by identification of the agents in skin lesions. The coccobacillary forms of the rickettsiae could be identified by direct (DIF) or indirect immunofluorescent (IIF) staining of skin biopsies (Woodward *et al.*, 1976) which proved to be rapid and specific. Very recently, an immunoperoxidase (IP) technique has been shown to be more sensitive and easier to perform (Dumler *et al.*, 1990). By the latter methods rickettsiae can be discerned as early as two to four days after the onset of the disease (Woodward *et al.*, 1976; Raoult *et al.*, 1984; Dumler *et al.*, 1990).

Here we describe the histology of skin lesions as well as the demonstration in them of *Rickettsia conori* by direct immunofluorescent staining in 13 patients in which the diagnosis of Mediterranean spotted fever (MSF) was confirmed also by indirect IIF.

Materials and Methods

The lesion selected for biopsy was the macular material in 12 cases and the „tache noire" in 1 case. The diagnosis of rickettsiosis was suspected in 9 of 13 patients while in 4 cases other diseases were considered (in 2 an unknown infection, in 1 a medicamentous rash and in 1 a haemorrhagic syndrome). As controls we used skin biopsies from 13 patients with the diagnosis of dermatitis. Biopsy was done 5 to 13 days from the onset of the disease, in most cases prior to the therapy (7 cases) and in 6 cases 1–3 days since the beginning of the therapy (doxicycline). DIF test was performed according to the method proposed by Huang *et al.*, (1976) with slight modification made by Walker and Cain (1978). We have used rabbit antiserum to *Rickettsia conorii* from the Institute of Virology, Bratislava. Formalin fixed, paraffin embedded sections were cut at 4 μm and processed by the above mentioned method. The preparations were also stained with haematoxylin and eosin, by methods PAS, Giemsa, and Machiavello.

Results

Table 1 presents the histological findings in 13 patients with MSF. In all cases focal vasculitis (Fig. 1) of various intensity and distribution was found. In 6 cases these changes were located in the superficial dermis while in 7 cases they were extended throughout the whole dermis and in 1 case they reached the subcutaneous tissue as well. The number of inflammatory cells varied from single to very numerous; in more severe cases they formed larger foci of inflammation. The cells of the infiltrate were located predominantly around the small and medium-sized blood vessels and occasionally in their wall.

Table 1. Histopathological findings of 12 maculopapular (No. 1–12) eflorescences and 1 „tache noire" (No. 13) in 13 patients with Mediterranean spotted fever

Patient No.	Vasculitis	Inflammatory cells*	Thrombosis	Vasodil-station	Oedema	Haemorr-hagy
1.	+	L,H,Pmn,Eo	+	+	+	+
2.	+	L,H	-	+	+	-
3.	+	L,H,Pmn	-	+	+	-
4.	+	L,H,Pmn,Eo	+	+	+	+
5.	+	L,H,Pmn	-	+	+	-
6.	+	L,H	-	+	+	-
7.	+	L,H,Eo	-	+	+	+
8.	+	L,H,Pmn	-	+	+	-
9.	+	L,H,Pmn,Eo	+	+	+	+
10.	+	L,H,Pmn,Eo	-	+	+	-
11.	+	L,H,Pmn	-	+	+	+
12.	+	L,H,Pmn,Eo	-	+	+	+
13.	+	L,H,Pmn	-	+	+	+

* L - lymphocytes, H - histiocytes, Pmn - polymorphonuclears, Eo - eosinophils

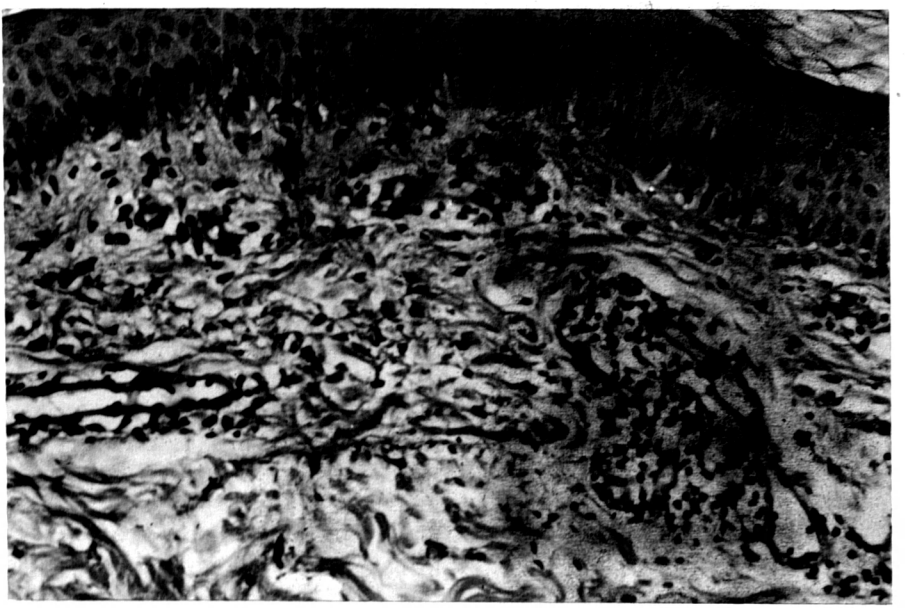

Fig. 1
Photomicrograph of focal lymphohistiocytic vasculitis from mild to moderate grade and oedema in the dermis of a patient with Mediterranean spotted fever
Haematoxyline-eosin stain. Magn. x 200.

Lymphocytes and histiocytes were seen in all foci; in 10 cases PMNs were also present and in 3 cases they were prominent. Rare eosinophils were observed in 5 cases accompanied mainly by PMNs. In addition to inflammatory reaction, vasculitis was always associated with swollen endothelial cells which showed rare mitoses. The swollen endothelial cells bulged into the lumen and in some cases appeared to obliterate it markedly. Vasodilatation and oedema of various intensity were found predominantly in the papillary part of dermis in almost all cases, mural thrombosis in 3 cases, haemorrhagic foci in 7 cases and acanthosis of epidermis in 12 cases. In the biopsy of "„tache noire", besides vasculitis necrosis of the epidermis and papillary dermis was seen with numerous PMNs. In case No. 9 a leucocytoclastic-type of vasculitis was found: disseminated karyorrhexis of PMNs plus fibrinoid necrosis of the walls of small blood vessels with accumulation of fibrinoid PAS positive material in their surroundings and with fibrin thrombi in some blood vessels. Karyorrhexis of a few isolated PMNs was found in 6 more cases. There was no correlation between the intensity of vasculitis in the skin biopsy and the time interval from the onset of the disease. No rickettsiae could be identified by Giemsa stain because of the presence of many granulas of mastocytes. By using the method of Machiavello, we found coccobacillary organisms only in one case with clinically suspected MSF.

Table 2. Results of direct immunofluorescent test (DIF) in skin biopsy specimens from 13 patients with Mediterranean spotted fever

Patient sex;age	Day of biopsy*	Day of therapy*	DIF results	IIF results
1, F, 70	5	5	+	+
2, M, 53	6	0	+	+
3, M, 20	6	3	+	+
4, M, 75	7	5	+	+
5, M, 35	7	6	+	+
6, F, 51	8	7	+	+
7, M, 31	8	0	+	+
8, F, 63	10	17	+	+
9, M, 53	10	0	+	+
10, F, 62	11	0	+	+
11, F, 44	11	9	+	+
12, F, 75	13	11	+	+
13, M, 47	8	0	+	+

* day from onset of the disease

Table 2 gives the sex and age of patient and the time at which biopsies were obtained, the onset of the therapy since the beginning of disease, and results of DIF and IIF tests. In all the cases the diagnosis was confirmed by both methods. Rickettsiae were seen as small (0.5–2.0 μm) coccobacillary organisms within the endothelial cells of small and medium-sized blood vessels, showing greenish immunofluorescence (Fig.2). They were present individually, in linear agglomerations and in clusters of 15 or more rickettsiae. Sometimes they were seen deeper in the wall of blood vessels and occasionally around it in the perivascular foci. The number of rickettsiae varied from case to case and from one visual field to another of the same preparation, regardless of the time that has passed from the onset of the disease (5 to 13 days) or of the antecendent therapy (1–3 days). No specimens from patients with dermatologic diseases other than MSF gave positive results by DIF or IIF tests.

Discussion

Immunofluorescence method for identification of *Rickettsia rickettsii* in bioptic skin specimens of patients with Rocky Mountain Spotted Fever (RMSF) was first described by Woodward *et al.* (1976). Until now in a number of reports rickettsiae were identified in frozen and paraffin embedded sections from skin bioptic material by DIF and IIF in RMSF (Woodvard *et al.*, 1976;

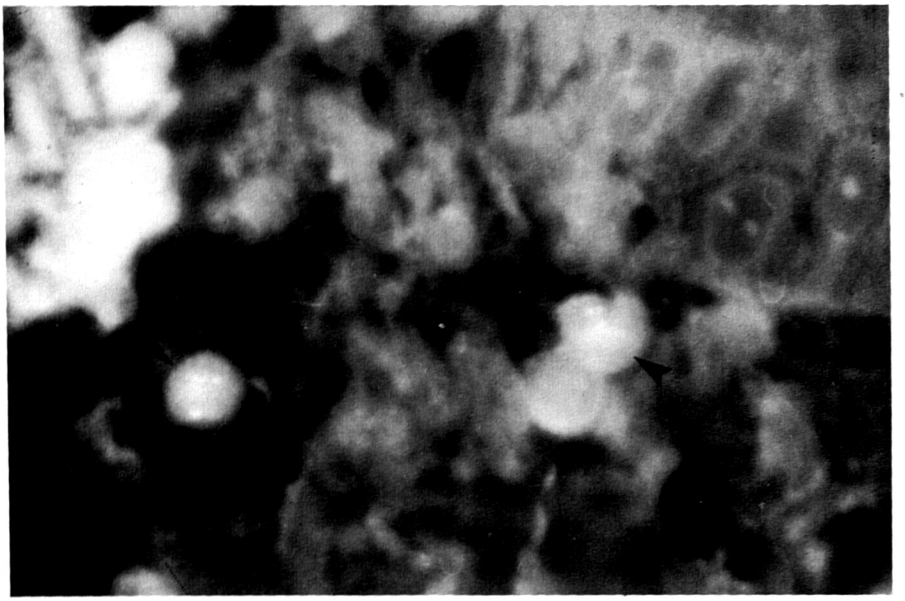

Fig. 2
Immunofluorescent demonstration of *Rickettsia conorii* in the swollen endothelial cells of small blood vessels in the dermis from a patient with Mediterranean spotted fever (arrows) DIF with FITC conjugated rabbit anti-globulin to *Rickettsia conorii.* Magn. x 1250.

Walker *et al.,* 1978; Walker and Cain, 1978), MSF (Motenigro *et al.,* 1983; Raoult *et al.,* 1984; Raoult *et al.,* 1986; Walker *et al.,* 1988), and murine typhus (Walker *et al.,* 1989). In all these reports it was emphasized that IF method was rapid, sufficiently sensitive and specific. Our results support this view. By DIF we identified *R. conorii* in all the 13 patients in which this diagnosis was also confirmed by IIF test. In all cases the diagnosis was made significantly earlier by DIF (5–13 days from the onset of the disease) than by the IIF method. Rickettsiae were easily identified by their greenish fluorescence, coccobacillary forms and their localization within endothelial cells. They varied in number from case to case regardless of the time that passed from the onset of the disease or the antecendent therapy. Sometimes it was difficult to discern the precise localization of rickettsiae, particularly when they were located deeper in the walls of blood vessels or in perivascular inflammtory infiltrates. In these cases the question was whether rickettsiae were located in the hyperplastic endothelium or in the smooth muscle cells of the vessels walls, or perhaps in surrounding histiocytes. This dilemma could be overcome by the IP method in which all the histological structures are clearly visible. The negative results of DIF test in all control patients point to the specificity of this method. Some

structures of dermis which gave fluorescence, like the chromolipid granulas in the cytoplasm of sweat and apocrine glands or the degenerative elastic fibers, were easily differentiated by their localization and larger size, by their presence in the unstained preparations as well as in those stained by haematoxylin-eosin.

The histologic picture of skin lesions stained by standard methods was not specific for the rickettsial diseasse. It was characterized by focal lymphohistiocytic vasculitis and swollen hyperplastic endothelial cells. In our material vasculitis was present in all skin lesions. It was of various intensity and distribution and always associated with vasodilatation and oedema of papillary dermis. Swollen endothel sometimes significantly reduced the lumen of blood vessels. In addition to these basic histologic changes PMNs were often seen in inflammatory infiltrates, while eosinophils were rarely found and thrombosis of small vessels occurred only occasionally. In the case of "tache noire" in addition to vasculitis, necrosis of epidermis and papillary dermis was prominent. In one case the histologic changes had the characteristics of leukocytoclastic-type of vasculitis.

We conclude that although the above described histologic changes were not in themselves specific for the rickettsiosis, but they were characteristic and suggestive enough for this diagnosis. This was the case in 4 of our patients who at first were not suspected to have MSF. The classic staining methods (Giemsa and Machiavello) were not adequate. By contrast, the DIF method proved to be very sensitive and specific for identification of rickettsiae in the skin lesions of MSF and very suitable in early diagnosis of the disease before the results of serological tests could be obtained.

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