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T lymphocyte subsets in Mediterranean spotted fever[1]

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Summary

Several studies have previously suggested the possible role of a T lymphocyte suppressor population in infections by species of the genus *Rickettsia*. In 15 patients with Mediterranean spotted fever (MSF), we quantified, during the acute and convalescent phases of the disease, the peripheral blood lymphocyte populations using monoclonal antibodies that recognize $CD3^+$, $CD4^+$, $CD8^+$, $CD38^+$ and $CD20^+$ cells. In three cases a reversal in the normal ratio of T lymphocyte helper-inducer/suppressor-cytotoxic subsets was detected lasting, in two of them, up to the fifth week of the disease. This disturbance was always weak and lacked clinical significance.

Key words: *Rickettsia conorii;* Rickettsial diseases; Mediterranean spotted fever; Boutonneuse fever; lymphocyte populations.

Introduction

Mediterranean spotted fever (MSF) ("boutonneuse fever") is an acute infectious disease caused by *Rickettsia conorii*, a member of the spotted fever group (SFG) of rickettsiae. It occurs over a wide geographic distribution and is well documented in zones of Southern Europe, Africa, India and the Middle East (Bernard et al., 1963; Gear et al., 1983; Gutman et al., 1973; Raoult et al., 1985; Weyer, 1978). During the last few years, an increase in the incidence of MSF has been recognized along the Mediterranean basin (Ruiz-Beltrán et al., 1985; Scaffidi, 1982; Segura and Font, 1982). Moreover, variable rates (as high as 26.3%) of human sera with antibodies against *R. conorii* have been detected

[1] A preliminary report of this work was presented at the meeting "Rickettsiology: The Present and the Future", Palermo (Italy), 21st–28th June 1987.

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in some of these areas among people lacking a previous history of the classic clinical triad of fever, rash and eschar at the site of tick bite (García-Curiel and Nájera-Morrondo, 1984; González et al., 1985; Gross et al., 1983; Mansueto et al., 1983; Raoult et al., 1985; Torregrossa et al., 1983).

Immunity to members of the genus *Rickettsia* is thought to be mediated primarily by cellular mechanisms (Anacker et al., 1983; Kokorin et al., 1982; Montenegro et al., 1984; Murphy et al., 1980; Ricketts and Gómez, 1908; Topping, 1940; Walker and Henderson, 1978). Previously, it has been shown that acute infection of susceptible mice with *R. tsutsugamushi* causes a decrease in the delayed type sensitivity response, and the appearance of suppressor cells responsible for this decline was postulated (Jerrells and Osterman, 1983). Furthermore, guinea pigs infected with pathogenic species of SFG rickettsiae, including *R. conorii*, show a transient and nonspecific cellular immune suppression when in vitro lymphocyte transformation and in vivo delayed cutaneous hypersensitivity responses to unrelated, non rickettsial antigens are analyzed. In this model the mechanism of suppression has been demonstrated to be not simply due to a loss of circulating lymphocytes (Oster et al., 1978).

Several reports have emphasized the immunoregulatory role of T lymphocyte subsets, and alterations in this subpopulations have been associated with human infectious diseases (Alexander et al., 1986; Bertoto et al., 1982; Bowen et al., 1985; Brahmi et al., 1982; Carney et al., 1981; Der Waele et al., 1981, 1985). In MSF, a single human case has been previously reported showing abnormal OKT4/OKT8 T lymphocyte ratio together with some signs of immunodeficiency (Roca et al., 1984). In the present investigation we have measured the numbers of peripheral blood mononuclear cells in a series of patients with MSF by means of T lymphocyte-specific monoclonal antibodies in order to evaluate, if present, the frequency, characteristics and evolution of the quantitative changes in these cell populations during the course of MSF.

Patients and Methods

Patients and control group. During the summer outbreak of MSF in 1986, 15 nonselected, consecutive adult patients (10 females and 5 males) seen at the Department of Medicine of the University Hospital in Salamanca (Spain) were included in the study. Their ages ranged between 25 and 71 years (mean ± standard deviation = 54.2±12.5). Diagnosis was based upon epidemiological (season of the year, residence in an endemic area, exposure to ticks) and clinical data (fever, characteristic rash, "tache noire"), and serological confirmation was achieved by means of immunofluorescent antibody test against *R. conorii* (single titer $\geqslant 1/640$ or fourfold increase in antibody titer). None had a significant past or present history of other pathologic events. Patients were treated with oral tetracycline since the moment the diagnosis was established, 4–10 days after the onset of symptoms.

The control group was composed of healthy individuals of comparable age and sex distribution with no history of recognizable rickettsial disease.

Methods. At the time of clinical diagnosis, the first blood sample was obtained, and a second sample was planned in the convalescent phase, four weeks later, in those cases with significant abnormalities. Peripheral blood samples were obtained by venipuncture at the same time of the day to avoid artefacts due to circadian variations of the lymphocyte subsets (Ritchie et al., 1983). Samples were transferred to tubes containing preservative free heparin (10 U/ml), platelets removed (160 g, 10 min), and resuspended blood (saline serum) was added to Lymphoprep (Nyegaard and Company, Oslo) in a 3:4 ratio (Boyun, 1969). Centrifugation (400 g, 35 min) was followed by 3 washings in medium 199 (Flow Laboratory, Irvine, Scotland) and the final suspension was made in medium 199 supplemented with 5% fetal calf serum (inactivated at 56° C, 45 min). The concentration of the white cells was adjusted to 4×10^6/ml.

Detection of surface antigens was achieved by the indirect immunofluorescence antibody test using commercial monoclonal antibodies of the OKT series (Ortho Diagnostic System Inc., Raritan, NJ; Coulter Immunology, Hialeah, Florida) which react with the following markers: CD3 (T lymphocytes), CD4 (helper-inducer T lymphocyte subset), CD8 (suppressor-cytotoxic T lymphocyte subset), CD38 (activated T lymphocytes) and CD20 (B lymphocytes) (Foon et al., 1982; Nadler et al., 1981; Reinherz and Schlossmann, 1981; Stashenko et al., 1980; Talle et al., 1983; Terhorst et al., 1980). Cells were incubated with the appropiate monoclonal antibody (30 min at 4° C), washed twice in PBS (0.1% sodium azide, 2% bovine serum albumin) and immunofluorescence staining was accomplished with a second incubation with FITC goat antimouse conjugate (Meloy, Springfield, VA). Cells were washed, mounted in slides with glycerol, and cell ratios were calculated after counting under a Leitz Orthoplan ultraviolet microscope. Total leucocyte counts were quantified in a Coulter Model S Plus IV/VD.

Results

All the 15 patients included in the study suffered a non-severe form of MSF and recovered without complications. Temperature returned to normal levels between 48–72 h after antibiotic therapy was started, and the remaining symptoms (myalgia, headache, malaise) disappeared during the following days. The laboratory pattern was also characteristic showing variable levels of hypoalbuminemia and hyponatremia together with other laboratory manifestations previously reported in MSF (Ruiz et al., 1984; Ruiz-Beltrán et al., 1985).

During the acute phase, patients showed total lymphocyte counts of $2132.30 \pm 737/\mu l$ (mean values $\pm$ standard deviation) of which $8.1 \pm 2.3\%$ were $CD20^+$ (B lymphocytes) and $64.7 \pm 7\%$ were $CD3^+$ (T lymphocytes). Table 1 shows the percentages of the counts of the T lymphocyte subpopulations in the acutely ill patients and in control donors.

No significant alteration in the numbers of total lymphocytes was detected in any of the patients. Three of them (case 9, case 10 and case 12) (Table 2) showed a reversal in the $CD4^+$ / $CD8^+$ cell ratio during the acute stage of the disease (0.72, 0.58 and 0.70, respectively). The absolute counts of $CD8^+$ lymphocytes were elevated, and the absolute numbers of $CD4^+$ lymphocytes decreased when compared with the counts in the convalescent phase. $CD4^+$ / $CD8^+$ lymphocyte ratio was >1 in all the controls.

Follow-up investigation of the cases showing abnormalities in the acute phase demonstrated four weeks later (Table 2) a persistently inverted $CD4^+$ / $CD8^+$ ratio in patient 10, whose convalescent $CD4^+$ / $CD8^+$ ratio (0.75) was closer to normal than the acute phase ratio (0.58). The other two patients with acute changes showed recovery in their second sample with normalized abso-

Table 1. Characterization of T lymphocyte subsets in acute MSF

	Total lymphocytes (/μl)	$CD3^+$ (%) (T)	$CD4^+$ (%) (T helper-inducer)	$CD8^+$ (%) (T suppressor-cytotoxic)	$CD38^+$ (%) (T activated)
Patients	2132.30±737*	64.7±7	36.6±10.3	26.9±8.1	2.1±4.6
Control donors	2600±750	70±8	50±9	27±5	<5

*Mean values ± standard deviation

Table 2. Peripheral lymphocyte populations in MSF patients (acute and convalescent phase) showing altered ratio of $CD4^+$ / $CD8^+$ T lymphocytes

	$CD3^+$ (%) (T)	$CD4^+$ (%) (T helper-inducer)	$CD8^+$ (%) (T suppressor-cytotoxic)	$CD38^+$ (%) (T activated)	$CD20^+$ (%) (B)	Lymphocytes (/μl)
Case 9						
acute	70	35	48	18	6	1390
convalescent	72	49	20	0	11	3100
Case 10						
acute	66	21	36	0	6	2040
convalescent	51	24	32	0	10	2000
Case 12						
acute	60	23	33	3	10	2830
convalescent	68	41	22	2	7	2830

lute numbers of the lymphocyte subsets and $CD4^+$ / $CD8^+$ ratios of 2.45 (case 9) and 1.86 (case 12).

Discussion

The humoral arm of the immune response has an important role in resistance to infection with many bacterial species. However, intracellular bacteria such as rickettsiae demand a more complex mechanism of antimicrobial activity. Thus, in diseases caused by SFG rickettsiae, antibodies have been shown to be incompetent to control infection, and they have only shown protective activity in experiments in which the microorganisms were reacted with them prior to inoculation (Anacker et al., 1983) or when immune serum was given before infection (Ricketts and Gómez, 1908; Topping, 1940). Also, guinea pigs inoculated intradermally with *R. typhi*, a typhus group rickettsia, were not protected from development of the cutaneous lesion when they received immune serum. They were protected when splenic cells from immune donors were given prior to infection (Murphy et al., 1980). On the other hand, the importance of cellular immunity has been demonstrated against *R. conorii* and other SFG rickettsiae in different experimental animal models: adoptive transfer of lymphocytes (Kokorin et al., 1982) and the use of mice genetically deficient in T cells (Montenegro et al., 1984) or treated with antilymphocyte serum (Walker and Henderson, 1978).

T helper-inducer lymphocytes might exercise their antirickettsial activity through gamma interferon by activation of macrophages to inhibit rickettsial growth or to kill rickettsiae (Jerrells et al., 1985) or by an undefined effect on endothelial cells inhibiting the organisms (Wisseman and Waddell, 1983). Nevertheless, the role of other lymphocyte subpopulations seems less clear. A T cell mediated cytotoxic mechanism has been reported to act against murine fibroblast cells infected with *R. typhi* or *R. tsutsugamushi* (Rollwagen et al., 1986), but the importance of the T cytotoxic lymphocyte subpopulation has not been described for in vitro infections by SFG rickettsiae nor for any rickettsial infection in vivo.

The presence of suppressor cells could explain the relatively poor response in the lymphocyte proliferation test of lymph node lymphocytes collected from *R. akari*-infected mice against this antigen (Jerrells et al., 1986) or the loss of protective capacity of splenic lymphocytes in mice inoculated with *R. conorii* (Kokorin et al., 1982). These and other experiments (Jerrells and Osterman, 1983; Oster et al., 1978) have suggested the presence of a T cell mediated suppressor activity in rickettsial infections.

A patient with MSF (confirmed serologically by a complement fixation test) has been reported as having during the course of his disease a transient increase in the level of antibodies against several viruses and a cutaneous delayed hypersensitivity test with dinitrochlorobenzene that was repeatedly negative (Roca et al., 1984). When T lymphocyte subsets were enumerated, a transient reduction of the helper-inducer / suppressor-cytotoxic (OKT4 / OKT8) ratio was observed until the 10th–16th week of the disease.

In our series, three of fifteen patients (20%) with confirmed *R. conorii* infection showed a reversal in the T-lymphocyte helper-inducer / suppressor-cytotoxic ratio that recovered, in two of them, before the fifth week of the disease. This reversal was the consequence of both an increase in the $CD8^+$ cell population and a decrease in the $CD4^+$ subset. When present, the disturbance in the T cell subpopulation counts was not severe. The altered ratios were significantly less perturbed than those observed in other disease states associated with alterations in this balance which also generally show a longer persistence of the inverted ratio (Carney et al., 1981).

The genesis and the meaning of these quantitative changes are unclear. The cells identified by the monoclonal antibodies form functionally heterogenous groups (e.g. $CD8^+$ cells with suppressor or cytotoxic activity). On the other hand, the lymphocyte subsets observed in peripheral blood of patients with MSF correlate with the lymphocyte subsets in local lesions caused by *R. conorii.* A predominance of T helper-inducer lymphocytes was detected during a recent study (Herrero-Herrero et al., 1987) at the level of the perivascular inflammatory infiltrates in six *taches noires* in response to the invasion of the endothelium by *R. conorii.*

In the course of MSF no particular increased incidence of superinfection by other pathogens has been reported. Furthermore, our cases no. 9, 10 and 12 did not show a more prolonged disease course nor other clinical data suggesting a severe form of rickettsial infection (Devrient et al., 1985; Ruiz-Beltrán et al., 1985). Thus, although further studies regarding the immunological behavior of these patients have to be completed, the appearance of altered T lymphocyte helper-inducer / suppressor-cytotoxic ratio seems to be incidental and without clinical significance during human MSF.

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