SHORT REPORTS

Phenotypes of Peripheral Blood Leukocytes in Melkersson-Rosenthal Syndrome

LARS RÖNNBLOM,[1,4] OLOF SJÖBERG,[5] PER-ERIC EVRIN[2] and GUNNAR NETHANDER[3]

[1]*Department of Internal Medicine,* [2]*Department of Clinical Chemistry and* [3]*Department of Oral Surgery, Central Hospital, Boden,* [4]*Department of Immunology and* [5]*Department of Clinical Immunology, Uppsala University, Uppsala, Sweden*

Rönnblom L, Sjöberg O, Evrin P-E, Nethander G. Phenotypes of peripheral blood leukocytes in Melkersson-Rosenthal syndrome. Acta Derm Venereol (Stockh) 1988; 68: 154–158.

Peripheral blood leukocytes from 5 patients with Melkersson-Rosenthal syndrome (MRS) were characterized with an immunoenzymatic staining technique utilizing ten different mouse monoclonal antibodies to cell surface antigens. No gross abnormalities could be found in the various T and B cell subpopulations. However, the proportion of cells expressing the IL-2 receptor was slightly increased, indicating the occurrence of activated lymphocytes in the circulation. Furthermore, in all 5 patients a large proportion of a non-phagocytic, Leu-M1-expressing, granulocyte-like cells of low density was found, probably reflecting the presence of activated granulocytes in the blood in MRS patients. The findings suggest that activation of both the lymphocyte and the granulocyte systems may be of importance in the pathogenesis of MRS. (Received May 7, 1987.)

L. Rönnblom, Department of Internal Medicine, Central Hospital, S-961 85 Boden, Sweden.

The Melkersson-Rosenthal syndrome (MRS) is characterized by recurrent orofacial swellings which subsequently lead to permanent, recurrent paralysis of the facial nerve and *linqua plicata* (1). Cheilitis granulomatosa of Miescher, characterized by oro-facial lesions only, is regarded as an oligosymptomatic form of MRS, since both show the same histopathological changes (2, 3).

Neither the etiology nor the pathogenesis of MRS is known. Laboratory studies of inflammatory parameters have shown that most MRS patients do not differ from healthy individuals (1, 3, Rönnblom et al., unpublished results). However, in some MRS patients, various abnormalities have been found, such as elevated immunoglobulin levels, moderate leukocytosis, and slightly increased erythrocyte sedimentation rate. It has also been noted that some MRS patients suffer from various allergic symptoms (3). Thus there are scanty findings suggesting an involvement of the immune system in MRS.

In view of these observations we wanted to investigate the different cellular components of the immune system in blood from MRS patients. In the present study we have characterized the proportions of different peripheral blood leukocytes (PBL) from 5 patients with MRS, using a panel of monoclonal antibodies. Antibodies to various subsets of T lymphocytes as well as to non-T cells have been used. An increased proportion of leukocytes expressing the interleukin-2 (IL-2) receptor was demonstrated. However, the most unexpected finding was that of an atypical granulocyte.

MATERIALS AND METHODS

Patients and controls

Five patients, considered to have MRS, were included in the study. The diagnostic criteria were recurrent orofacial swellings of the labial and/or the oral tissues and the presence of non-caseating epithelioid

cell granulomas on histologic examination of tissue sections stained with hematoxylin-eosin and/or periodic acid-Schiff. No sign of disseminated granulomatous disease could be found. The disease duration varied from 5 to 40 years. In 4 of the patients the blood samples were collected during bouts of increased disease activity. Normal blood donors served as controls.

Antisera

The monoclonal antibodies used for detecting cellular antigens and the present knowledge about their specificities are summarized in Table I. The antibodies were used at a dilution of 1 : 20 except for the pan-B antibody (1 : 10).

Surface marker studies

PBL were isolated from heparinized blood by centrifugation on Ficoll-Isopaque followed by treatment of the cells with carbonylated iron powder to remove phagocytic cells (4). Immunoenzymatic staining was then performed as described elsewhere (5). In short, the PBL were first fixed on glass slides using 1% paraformaldehyde. The slides were then incubated with the proper dilution of the monoclonal antibody diluted in phosphate-buffered saline with 1% bovine serum albumin for 30 min. After washing, the slides were subsequently incubated with biotinylated horse anti-mouse IgG diluted 1 : 400 followed by a complex of avidin and biotinylated horseradish peroxidase H and a carbazol-containing buffer according to the instructions of the manufacturer (Vectastain; Kemila, Stockholm, Sweden). No staining was observed when the primary antibody was omitted.

Statistics

Student's *t*-test was used for the statistical analysis.

RESULTS

T lymphocyte subsets in MRS

Table II shows the proportion of PBL reacting with different T lymphocyte specific monoclonal antibodies in patients with MRS. No gross deviations in the relative frequency of T cells or T cell subpopulations were recorded. However, the proportion of cells reactive with the anti-IL-2 receptor antibody was slightly increased vis-à-vis PBL from normal blood donors.

Antigenic markers on non-T-cells

All MRS patients had a normal B lymphocyte count as defined by the pan-B antiserum (Table III). The proportions of cells expressing the HLA-DR antigen and cells reactive with

Table I. *Monoclonal antibodies used*

Antibody	Company	Specificity
Anti-Leu-1	BD[a]	T cells
Anti-Leu-2a	BD	T cytotoxic/suppressor cells
Anti-Leu-3a	BD	T helper/inducer cells
Anti-Leu-7	BD	Large granular lymphocytes
Anti-Leu-M1	BD	Monocytes, granulocytes
Anti-Leu-M3	BD	Monocytes
Anti-pan-B	Dakopatts[b]	B cells
OKIa1	Ortho[c]	HLA-DR
OKT9	Ortho	Transferrin receptor
Anti-IL-2 rec.	BD	IL-2 receptor

[a] Becton Dickinson Laboratory Systems, Stockholm, Sweden.
[b] Dakopatts, Hägersten, Sweden.
[c] Ortho Diagnostic Systems, Sollentuna, Sweden.

Leu-7 antibodies (including the natural killer cells) were also normal. The frequencies of cells positive with OKT-9 and Leu-M3 antibodies were very low (1.4±1.3% and 0.8±1.3%, respectively).

In contrast, a large proportion of the PBL were stained with a monoclonal antibody against the M1 antigen (Table III). This antigen is restricted to granulocytes and monocytes. Normally very few such cells are present in PBL prepared from normal blood donors. The M1-positive cells from the patients looked like neutrophil granulocytes on routine May-Grünewald-Giemsa-stained specimens. They were also negative with the monocyte specific antiserum anti-Leu-M3 (results not shown). Thus, the M1-positive cells noted are probably granulocytes, but differ from normal granulocytes in having a lower buoyant density and poor capacity to phagocytose iron powder. Furthermore, we noted weak HLA-DR expression on the Leu-M1-positive cells (results not shown).

Table II. *Proportions of different T lymphocyte subsets in peripheral blood leukocytes from MRS patients*

Patient no.	Proportion of cells positive for			
	Leu-1 (%)	Leu-2a (%)	Leu-3a (%)	IL-2 rec. (%)
1	68	21	43	ND[a]
2	77	26	46	7
3	68	23	45	4.5
4	68	24	47	0.3
5	77	31	46	2.2
Patients	72±5 (5)[b]	25±4 (5)	45±2 (5)	3.5±2.9 (4)
Controls	76±8 (30)	29±9 (30)	49±6 (30)	0.5±0.5 (15)
p-value	NS[c]	NS	NS	<0.001

[a] ND = Not done.
[b] Mean ± SD (number of patients).
[c] NS = Not significant ($p>0.05$).

Table III. *Expression of non-T cell antigenic markers on peripheral blood leukocytes from MRS patients*

Patient no.	Proportion of cells positive for			
	pan-B (%)	Leu-7 (%)	Leu-M1 (%)	OKIa1 (%)
1	9	18	30	11
2	13	5	20	16
3	19	20	15	10
4	6	30	39	9
5	4	24	11	13
Patients	10±6 (5)[a]	19± 9 (5)	23±11 (4)	12±3 (5)
Controls	11±5 (10)	15±10 (15)	1± 1 (10)	13±5 (10)
p-value	NS[b]	NS	<0.001	NS

[a] Mean ± SD (number of patients).
[b] NS = Not significant ($p>0.05$).

DISCUSSION

In the present study we have demonstrated that patients with MRS have an increased frequency of PBLs expressing the IL-2 receptor, although the proportions of T cells and T cell subpopulations are normal. Furthermore, an atypical granulocyte is present in large numbers in the blood from the patients.

The IL-2 receptor is a marker of activated T cells (6). Such cells also carry HLA-DR antigens (7). We have previously demonstrated that the majority of the infiltrating cells in the oral lesions in MRS are helper/inducer T cells, although some T suppressor/cytotoxic lymphocytes are also present. Virtually all the mononuclear infiltrating cells carry HLA-DR antigens, indicating local accumulation of activated T cells (8). Such a pattern is similar to that found in other diseases where immune reactions are thought to play an important role (9, 10). The finding of the IL-2 receptor expressing lymphocytes in the systemic circulation gives further support to our earlier conclusion that an activated immune system contributes to the pathogenesis of MRS. The circulating activated lymphocytes indicate that the deviations in the immune system are more general and are not limited to the oral lesions.

Although activated (IL-2 receptor bearing) T cells were present in the blood, the proportion of HLA-DR-expressing lymphocytes was not increased. The reason for this is probably that the number of activated HLA-DR-positive T cells was too low to significantly alter the overall proportion of HLA-DR-bearing cells.

The large proportion of Leu-M1-positive polymorphonuclear leukocytes among the PBL from patients with MRS was unexpected. Several features of these cells indicate that they are activated granulocytes, i.e. have poor phagocytic capacity, low buoyant density and HLA-DR expression (11). Activated T cells produce interferon-γ which can induce HLA-DR expression on various cells (12). Therefore it is likely that the HLA-DR expression on the Leu-M1-positive cells is caused by activated T cells.

Similar atypical granulocytes have also been found in other autoimmune and infectious diseases (12; and Sjöberg, unpublished results). Therefore it is possible that the occurrence of activated granulocytes in the peripheral blood is a common finding in diseases characterized by chronic granulomatous lesions or in other longstanding inflammatory conditions.

In conclusion, our data suggest that activation of both the lymphocyte and the granulocyte systems plays an essential role in the development of MRS. This is further supported by the presence of similar changes in other diseases of suspected autoimmune origin or in which deviations in the immune system are important.

ACKNOWLEDGEMENT

Excellent technical assistance was given by Gun Bjare.

REFERENCES

1. Hornstein OP. Melkersson-Rosenthal syndrome: A neuromucocutaneous disease of complex origin. Curr Probl Dermatol 1973; 5: 117–156.
2. Miescher G. Über essentielle granulomatöse Makrocheilie (Cheilitis granulomatosa). Dermatologica 1945; 91: 57–85.
3. Worsaae N, Christensen KC, Schmidt M, Reibel J. Melkersson-Rosenthal syndrome and cheilitis granulomatosa. Oral Surg 1982; 54: 404–413.
4. Sjöberg O, Inganäs M. Detection of Fc receptor-bearing lymphocytes by using IgG-coated latex particles. Scand J Immunol 1979; 9: 547–552.
5. Karlsson-Parra A, Forsum U, Klareskog L, Sjöberg O. A simple immunoenzyme batch staining method for the enumeration of peripheral human T lymphocyte subsets. J Immunol Methods 1983; 64: 85–90.
6. Uchiyama T, Broder S, Waldmann TA. A monoclonal antibody (Anti-Tac) reactive with activated

and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac(+) cells. J Immunol 1981; 126: 1393–1397.
7. Ko HS, Fu SM, Winchester RJ, Yu DTY, Kunkel HG. Ia determinants on stimulated human lymphocytes. Occurrence on mitogen and antigen-activated T cells. J Exp Med 1979; 150: 246–255.
8. Rönnblom L, Forsum U, Evrin P-E, Gillnäs T, Nethander G. Intralesional T lymphocyte phenotypes and HLA-DR expression in Melkersson-Rosenthal syndrome. Int J Oral Maxillofac Surg 1986; 15: 614–619.
9. Klareskog L, Forsum U, Wigren A, Wigzell H. Relationships between HLA-DR-expressing cells and T lymphocytes of different subsets in rheumatoid synovial tissue. Scand J Immunol 1982; 15: 501–507.
10. Traugott U, Reinherz EL, Raine CS. Multiple sclerosis. Distribution of T cells, T cell subsets and Ia-positive macrophages in lesions of different ages. J Neuroimmunol 1983; 4: 201–221.
11. Venge P, Strömberg A, Braconier JH, Roxin L-E, Olsson I. Neutrophil and eosinophil granulocytes in bacterial infection. Sequential studies of cellular and serum levels of granule proteins. Br J Haematol 1978; 38: 475–483.
12. Epstein LB. The comparative biology of immune and classical interferons. In: Cohen S, Pick E, Oppenheim JJ, eds. Biology of the lymphokines. New York: Academic Press, 1979; 443–514.
13. Feltelius N, Hällgren R, Sjöberg O. T lymphocytes in ankylosing spondylitis and the influence of sulphasalazine treatment. Clinical Rheumatology [in press].

A Case of Confluent and Reticulate Papillomatosis (Gougerot–Carteaud) with an Unusual Location

ANN BROBERG and JAN FAERGEMANN

Department of Dermatology, University of Gothenburg, Sahlgren's Hospital, Gothenburg, Sweden

Broberg A, Faergemann J. A case of confluent and reticulate papillomatosis (Gougerot-Carteaud) with an unusual location. Acta Derm Venereol (Stockh) 1988; 68: 158–160.

A 15-year-old female with a brown hyperkeratotic plaque in the pubic region confirming with the diagnosis of confluent and reticulate papillomatosis (CRP) is presented. The lesion disappeared rapidly after 3 weeks of treatment with 50% propylene glycol in water. The etiological role of *Pityrosporum orbiculare* in CRP is discussed. *Key words: Pityrosporum orbiculare; Propylene glycol.* (Received July 9, 1987.)

A. Broberg, Department of Dermatology, Sahlgrenska sjukhuset, S-413 45 Göteborg, Sweden.

Confluent and reticulate papillomatosis (CRP) was first described by Gougerot & Carteaud in 1927 (1). It consists of grayish-brown pigmented papules that later coalesce, most often localized in the intermammary and interscapular regions, neck and abdomen. It usually starts shortly after puberty, primarily in females. The lipophilic yeast *Pityrosporum orbiculare*, the course of pityriasis versicolor has been associated with CRP (2, 3). The etiology remains unclear but many observations are in favour of the role of *P. orbiculare* in CRP (4). Several investigations now indicate that *P. orbiculare* and *P. ovale* are round and oval variations of the same lipophilic yeast (2, 4).

CASE REPORT

A 15-year-old girl was admitted to the Department because of a brown hyperkeratosis in the pubic region (Fig. 1). She had had the lesion for one year. It consisted of a brown reticulated and