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BLOOD LYMPHOCYTE SUBPOPULATIONS IN MYCOSIS FUNGOIDES AND THEIR FUNCTIONS *IN VITRO*

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Abstract. T-lymphocyte subpopulations, PWM stimulated *in vitro* Ig production by mononuclear cells, and spontaneous cell-mediated cytotoxicity (SCMC) were determined in patients with Mycosis fungoides (MF) as well as in sex- and age-matched normal controls. We were able to confirm our earlier findings of a significantly higher frequency of T-lymphocytes with Fc-receptors for IgG (Tg cells) and a proportion of T lymphocytes with Fe receptors for IgM (Tm cells) not significantly differing from normal controls. As defined by monoclonal antibodies, the proportion of T-helper cells (Leu3A) was significantly lower, whereas the T-suppressor cells (Leu2A) were in the range of normal controls. The PWM stimulated and spontaneous Ig synthesis *in vitro* was lower in MF patients whereas there was no difference in the SCMC activity, in comparison with normal controls. The lower frequency of T-helper cells in the peripheral blood may be explained by their migration to the skin.

Key words: T-lymphocyte; Suppressor cells; Helper cells; Natural killer cells

Lymphocytes with T-cell phenotype have been found in skin infiltrates of patients with Mycosis fungoides (MF) and this disease is therefore generally considered to be a T-cell derived lymphoma (2). We have demonstrated in our previous study that the proportion of T-cells with Fc-receptors for IgG is higher in MF patients than in normal controls (14). Recently Reinherz et al. examined T-cell subsets with Fc-receptors for IgG (Tg-cells) and IgM (Tm-cells) by means of monoclonal antibodies specific for helper and suppressor subpopulations (13). The results demonstrated a poor correlation between T-cell subsets as defined by monoclonal antibodies and Fc-receptors, respectively. In view of this report, we considered it of interest to re-examine the distribution of lymphocyte subsets in Mycosis fungoides by using monoclonal antibodies against antigens characteristic of different lymphocyte subpopulations. We have also studied spontaneous cell-mediated cytotoxicity (SCMC) and pokeweed mitogen (PWM) stimulated Ig synthesis *in vitro*. These experiments supplied additional information on lymphocyte subsets involved in such specific functions.

MATERIAL AND METHODS

Patients

Nine patients with MF classified according to the criteria published by the Scandinavian Mycosis Fungoides Study group (3) were examined concomitantly with sex- and age-matched healthy donors (Table I).

Immunological methods

The methods used for cell separation, identification of Tg- and Tm-cells, determination of PWM stimulated Ig production *in vitro* and evaluation of SCMC have been published elsewhere (4, 5, 6).

Monoclonal antibodies

The monoclonal antibodies used in this study were directed against helper/inducer T-cells (Leu3A) and suppressor/cytotoxic T-cells (Leu2A). They were purchased from Becton Dickinson Facs systems (490-B Lakeside Drive, Sunnyvale, CA 94086, USA).

Immunofluorescence

T-cell subset typing was performed by indirect immunofluorescence, using the above-mentioned monoclonal antibodies and FITC-conjugated sheep anti-mouse IgG (National Bacteriological Laboratory, Stockholm, Sweden) absorbed with human serum by means of a column containing mixed polymerized human serum and Sepharose 6B.

T-cells (10^6) were mixed with Leu3A or Leu2A antibodies in an optimal dilution determined in preliminary experiments (Leu3A: 6 μg/ml, Leu2A 1 μg/ml) in phosphate-buffered saline (PBS) containing 5% fetal calf serum (FCS) in a total volume of 50 μl, and incubated for 40 min at +4°C. Then the cells were washed twice with cold medium as above and mixed with FITC-conjugated sheep anti-mouse IgG antibodies diluted 1 : 10 in PBS with 5% FCS in a total volume of 50 μl and incubated for 40 min at +4°C. Then the cells were washed twice with cold medium and stored on ice before reading with incident

Table I. *Patients with Mycosis fungoides (MF)*

Cases 1–4 included in earlier study (14) as numbers 1, 3, 4 and 5

Case no.	Sex	Age (y.)	MF stage
1 (AL)	M	75	III
2 (ALF)	F	60	II
3 (IR)	F	57	II
4 (KM)	M	74	II
5 (SD)	M	75	IV
6 (KE)	M	75	II
7 (GA)	F	70	II
8 (SM)	M	69	II
9 (MG)	F	58	IV

light specific for FITC fluorescence using a Leitz Ortoplan microscope. Alternate illumination with conventional light was applied and at least 200 cells were counted.

Statistical analysis

The statistical method used was Student's *t*-test.

RESULTS

The total number of peripheral blood lymphocytes in patients with MF did not differ from that of healthy persons of the same age and sex (data not presented). The percentages of total T-lymphocytes (rosetting with neuraminidase-treated sheep red cells, SRBC) and of various T-cell subsets are shown in Table II. The proportion of Tg cells was significantly higher and the proportion of T-helper cells (Leu3A) was significantly lower than that of normal controls. The proportions of total T-cells, Tm- and T-suppressor cells (Leu2A), on the other hand, did not differ from those of normal donors.

The spontaneous and PWM-stimulated production of IgG and IgM *in vitro* by mononuclear cells from patients and controls, respectively, is set out in Table III. It can be seen that significantly smaller IgG and IgM amounts were present in supernatants from mononuclear cells derived from MF patients than from those of normal donors. The Ig production in non-stimulated cultures was somewhat lower in the patient group.

The SCMC activity of non-fractionated lymphocytes in both patients and controls is presented in Table IV. No significant difference in SCMC activity, as expressed in percentage of specific ^{51}Cr release, was found between MF patients and controls. However, there was a slightly lower SCMC activity in the patient group than in the controls. Similar results were obtained for both Chang cell line and K562 cell line and for all the lymphocyte: target cell ratios.

DISCUSSION

Our previous findings of a higher frequency of Tg-cells and a normal proportion of Tm-cells in Mycosis fungoides could be confirmed in the present study (14). T-suppressor cells as defined by monoclonal antibodies did not differ in proportion from controls, whereas the frequency of T-helper cells (Leu3A) was lower. The reason for this difference is not known. It might be so, that the cells with these different surface markers do not belong to the same populations, or at least do not overlap com-

Table II. *Percentage of total T cells (T), Tg, Tm, Ts and Th cells in MF patients and controls*

Tg=T-cells with Fc-receptors for IgG, Tm=T-cells with Fc-receptors for IgM, Ts=T-suppressor cell defined by monoclonal antibodies Leu2A, Th=T-helper cell defined by monoclonal antibodies Leu3A

Case no.	MF patients					Controls				
	T	Tg	Tm	Ts	Th	T	Tg	Tm	Ts	Th
1	76	43	35	20	34	81	28	39	12	54
2	82	48	51	21	48	81	28	72	22	50
3	69	47	50	23	41	83	39	59	17	52
4	88	29	50	52	22	87	23	40	33	47
5	80	52	37	18	18	78	35	58	17	37
6	88	30	56	12	51	84	28	50	20	50
7	73	43	68	13	48	89	34	63	20	56
8	72	48	40	23	31	87	38	44	17	58
9	80	37	31	N.D.	N.D.	82	22	46	N.D.	N.D.
Mean ± S.D.	79±7	42±8**	46±12	23±13	37±12*	84±4	31±6	52±11	20±6	51±7

*$p<0.02$. **$p<0.01$.

Table III. *Production of IgG and IgM in PWM-stimulated and non-stimulated cultures of mononuclear cells from MF patients and controls*

Means and S.D. Range within parentheses

	PWM concentration (μg/ml)					
	3.3	1.0	0	3.3	1.0	0
	IgM (ng/ml)			IgG (ng/ml)		
Patients (9 cases)	82±45 (24–168)	72±48 (24–176)	23±10 (10–42)	137±125 (35–455)	116±119 (35–425)	73±60 (35–185)
Controls (9 cases)	218±45*** (132–280)	158±59** (86–284)	30±18 (7–68)	379±281* (135–900)	334±255* (120–830)	123±152 (35–518)

*$p<0.025$. **$p<0.0025$. ***$p<0.0005$.

pletely. This would be in agreement with findings of Reinherz et al. (13). Alternatively an increase in Tg-cells could be due to a modification of the Fc-receptor phenotype without any change in their markers as defined by monoclonal antibodies, due to some as yet unknown immunological event taking place in MF patients. It is known for example that T-lymphocytes can modify the phenotype under certain *in vitro* conditions (10, 12). The same reasoning can be applied to the observed dissociation between Tm- and T-helper cells (Leu3A).

The low *in vitro* production of immunoglobulins by mononuclear cells accords closely with the finding of an increased proportion of Tg-cells, as these cells have been shown to exert suppressor activity in PWM-stimulated Ig synthesis (7, 11). On the other hand, the demonstration of a reduced frequency of T-helper cells (Leu3A cells) might also explain these results. However, the evidence that Tg-cells are implicated in the reduced Ig production is circumstantial, Tg-cells being only one of several different cell types belonging to the suppressor repertoire, which comprises monocytes and other T-lymphocytes. The normal proportion of T-suppressor cells (Leu2A cells) certainly argues against these cells being involved in the observed suppression. However, as mononuclear cells were used in this study on Ig production, it is not possible to state with certainty which cells are implicated and whether the phenomenon observed is due to increased suppressor activity or to decreased helper activity.

One possible explanation of the present results could be a high degree of heterogeneity of suppressor and helper cells respectively, where changes in the proportion or activation of one subset can, through direct or feedback action, cause increased suppressive or decreased helper activity in connection with Ig production *in vitro*.

MF is considered to be a cutaneous T-cell lymphoma (2). Recently abnormalities of T-cell subpopulations have been reported in this disease and in the Sézary syndrome which is regarded as a leukemic form of MF. However, these reports are rather conflicting (1, 2, 7, 9, 14). Broder et al. (1) reported in the Sézary syndrome that the malignant cell is a helper cell. Hopper et al. (9), on the other hand, demonstrated in the same disease that the

Table IV. *SCMC of non-fractionated lymphocytes in MF patients and normal controls*

Means ± S.D. of specific isotope release (%). Number of patients within parentheses

	Target cells					
	Chang			K562		
Effector: Target cell ratio . . .	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1
Patients (8)	17±9	10±8	6±5	47±17	39±21	21±12
Controls (8)	23±11	15±8	8±5	53±15	44±23	30±20

Differences between mean values of patients and controls were statistically non-significant.

T-cells exert a suppressive activity. Finally, according to Gupta et al. (7) there exists a heterogeneity of the distribution of T-lymphocyte subsets in this disease. Our results differ somewhat from those of Gupta et al. (7) and Broder et al. (11), as all our patients had increased frequency of Tg-cells and the mean proportion of T-helper cells (Leu3A) was low. It is possible however that in MF helper cells migrate to the skin, which could cause a decrease of these cells in the peripheral blood. If this theory were correct it would explain the apparently conflicting reports discussed above.

It is generally accepted that Tg cells mediate SCMC (8). However, there was no higher SCMC, as could be expected in MF patients. This fact may be attributed to several different reasons. Tg-cells exert both suppressor and cytotoxic activity. It is conceivable that these cells belong to two different fractions and it is possible that only the suppressor fraction is increased in MF. Another possibility would be some kind of defect in the cytotoxic activity of Tg-cells. Finally, the failing increase in the cytotoxicity might be due to a relative shortage of T-helper cells.

It is not known at present in what way the above discussed disorders of immunological reactivity affect the pathogenesis of MF. It can be suggested, however, that MF patients display some degree of immunosuppression, at least as measured by *in vitro* activity.

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