ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN—POLONIA
VOL. LVI, 37 SECTIO D 2001

Chair and Department of Dermatology, Medical University of Lublin
Chair and Department of Pathomorphology, Medical University of Lublin

DOROTA WOJNOWSKA, BARBARA LECEWICZ-TORUŃ,
GRAŻYNA CHODOROWSKA, MARIA JUSZKIEWICZ-BOROWIEC,
DANUTA SKOMRA

Expression of selected T-cell antigens in the skin infiltration in primary cutaneous T-cell lymphomas

Mycosis fungoides (MF) belongs to primary cutaneous T-cell lymphomas (CTCL). MF, the most frequent type of CTCL, usually follows an indolent chronic course with slow progression over years or decades from patches to more infiltrated plaques and eventually tumors (1, 3). In a proportion of patients lymph nodes and internal organs may become involved in the later stages of disease (1, 3, 4). Because of no unique clinical feature, the differentiation of an early stage of MF from benign inflammatory skin diseases can be difficult.

Also histological changes are often nonspecific, or only suggestive. The more advanced patch and plaque lesions of MF consist of epidermotropic, band-like infiltrates involving the papillary dermis, containing mainly small and medium-sized mononuclear cells with hyperchromatic, indented (cerebriform) nuclei and variable numbers of inflammatory cells (7, 12). Colonization of the lower layers of the epidermis by single or small groups of neoplastic cells is a characteristic finding (12). The clinical and histological picture of the early stage of MF (especially premycotic stage) is often nonspecific (7, 12). It explains the need for new diagnostic methods of the patch and plaque stages of MF.

The aim of the study was to assess T-cell phenotype of the skin infiltrate in MF and benign chronic dermatoses.

MATERIAL AND METHODS

16 patients (male: female = 10 : 6) diagnosed according to clinical and histological criteria were included in the study. 11 patients had early MF (I –IIA), 3 patients had tumor stage (IIB) (Fig.1), and 2 had erythrodermic lesions (III). The patients' age at onset of disease ranged from 47 to 76, with the mean age of 58.9.

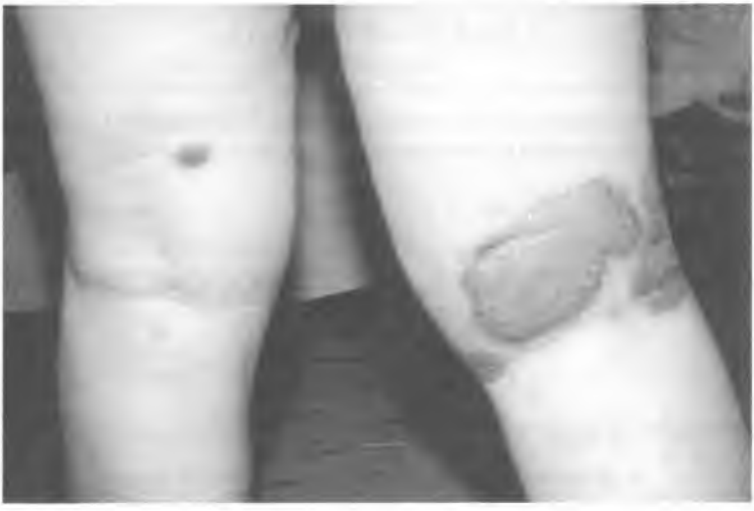

Fig.1. Tumor lesions of lymphoma (MF) on the posterior part of legs

Ten cases of benign inflammatory skin diseases (contact eczema, dermatitis atopica, nonspecific inflammation and chronic hypersensitivity reactions to UV radiation) were included in control group.

16 specimens of MF and 10 skin biopsies of benign lymphocytic (nonlymphomatous) skin conditions were examined. All lesions were diagnosed using standard clinical and histological criteria (14).

Each sample of skin lesion was frozen, cut, and immunostained using a panel of monoclonal antibodies (from DAKO) directed against T-cells (anti-CD3, -CD4, -CD5, -CD7 and -CD8), B-cells (anti- CD20) and dendritic cells (anti- CD1a). The immunoperoxidase method AB-Complex was performed (14).

Sections were examined by light microscopy and the percentage of positive cells of the infiltrate was evaluated. Quantitative results were expressed as follows : negative (-) : <5% positive cells of infiltrate; weakly positive (+) : 5-25%; moderately positive (++) : 25-50%; strongly positive (+++) : 50-100% (2).

The expression of CD1a and CD20 antigens were analyzed separately, using another range. Quantitative results of B cell expression in the infiltrate was evaluated as percentage of total mononuclear cell in the sample: negative (-) : the absence of B cells; weakly positive (+) : < 10% positive mononuclear cells of infiltrate; positive (++) : > 10% positive mononuclear cells of infiltrate. The amount of CD1a positive cells (Langerhans' cells) enclosed both in the dermis and the epidermis of the skin sample was evaluated as follows: negative (-) : absent or occasionally present; weakly positive (+) : small numbers; positive (+) : moderate numbers; strongly positive (+++) : numerous.

Statistical tests. Results were expressed as means ±SD. Experimental differences were evaluated by Student's test and Cochran' s and Cox's tests.

RESULTS AND CONCLUSIONS

In the present study 16 biopsies of MF and 10 biopsies of benign chronic dermatoses have been assessed and compared immunohistochemically. The phenotype of the infiltrating lymphocytes and expression of other antigens in both studied groups are given in Tables 1 and 2.

Tab.1. Expression of infiltrating cells studied antigens in MF

Case no.	Initials	Stage	CD4	CD8	CD3	CD5	CD7	CD20	CD1a
1	R.M.	IA	+++	-	+++	-	-	-	++
2	Z.H.	IA	+++	+	+++	-	-	-	++
3	T.J.	IA	+++	-	+++	+++	++	-	++
4	S.J.	IA	+++	+	+++	-	-	-	+++
5	K.T.	IA	+++	+	++	-	-	-	+++
6	P.M.	IA	+++	-	+++	+++	-	-	+
7	Z.W.	IA	+	-	+	+	-	-	+++
8	K.H.	IB	+++	+	+++	-	-	-	++
9	N.R.	IIA	+++	-	+++	-	+	-	+
10	P.S.	IIA	+++	-	+++	-	-	-	+++
11	P.K.	IIA	+++	-	+++	+++	-	-	++
12	O.C.	IIB	+++	+++	+++	-	+++	-	-
13	G.J.	IIB	+	+	+++	+++	+++	+	++
14	J.K.	IIB	+++	-	+++	+	+++	-	++
15	K.K.	III	+++	-	+++	++	++	-	+++
16	B.J.	III	+++	+	+++	++	++	-	+++

Tab. 2. Expression of infiltrating cells studied antigens in control group

Case no.	Initials	CD4	CD8	CD3	CD5	CD7	CD20	CD1a
1	B.F.	+++	+	+++	+++	+++	-	+++
2	G.J.	+++	+	+++	+++	+++	-	+++
3	M.A.	+++	++	+++	++	+++	-	++
4	M.J.	+++	+	+++	+++	+++	-	+++
5	K.H.	++	++	+++	++	++	-	++
6	B.Z.	+++	+	+++	++	++	-	+
7	P.T.	+++	+	+++	+++	+++	-	+++
8	P.Z.	+++	+	+++	+++	+++	-	+++
9	K.M.	+++	+	+++	+++	++	-	+++
10	B.J.	++	++	+++	++	++	-	++

The atypical mononuclear cell infiltrate in each of 16 patients was composed almost exclusively of T-lymphocytes, with an abnormally elevated CD4: CD8 ratio in most of the cases. In 9 specimens obtained from MF patients the absent or very weak expression of CD8 antigen was observed. 14/16 cases of MF demonstrated a predominance of helper/inducer phenotype, but in two cases a very weak expression of CD4 antigen and simultaneously a weak or absent expression of CD8 antigen were observed (see Table 1). None of the patients with MF revealed a prevalence of T-cell of suppressor/cytotoxic type. Coexpression of CD4 and CD8 antigens was observed in one case of MF. In the majority of cases of BCD, the predominance of the CD4+ lymphocytes was also detected. The study confirmed earlier reports that T-cells expressed a CD4+CD8- phenotype in at least 95 percent of cases (8, 9, 11, 12).

The examination of pan-T and major-T antigens showed some aberrations, as previously reported (2, 8, 9, 10, 11, 14). The present study confirmed the main phenotypic disturbance observed in MF was a significant decreased expression of CD7 and CD5 antigens. The loss of CD3 marker was seen less commonl; in the present study there was one case of the early MF (stage IA), where the lymphocytes of skin infiltration revealed additional aberrations (lack of CD4, CD8, CD3, CD5 and CD7).

The presented disturbances were observed not only in the advanted stages of MF, as confirmed earlier reports, but in the majority of patients with an early stage of CTCL. The assessment of aberrant immunophenotypes at a very early stage of MF supports the idea of phenotypic heterogeneity from the onset (8, 10, 11, 14). However no correlation was found between the degree of phenotypic aberration and duration or stage of disease. The present study and earlier findings confirmed the aberrant phenotypes with loss of one or more T-cell markers was a common feature (2, 8-11, 14). These results reported above demonstrate that lymphoid infiltrates found in BCD and MF can reveal immunophenotypic differences. These findings are similar to those previously reported and we agree with others that the loss of expression of one (especially CD7) or more T-cell antigens may be a little helpful in differentiating cutaneous reactive T-cell hyperplasias from early primary cutaneous T-cell lymphomas (6, 8, 10, 11). Of practical importance is the observation that CD7 and CD5 antigens can be often absent on T-cells in the lymphomatous infiltration (see Figs 2 and 3), but rarely in benign inflammatory infiltrations. Previous investigations and our results confirm that in most cases CTCL cells display unusual CD4+CD8-CD7- phenotype. The presence of analogous disturbances of T-cells of peripheral blood has been performed in proceeding analyses (5, 13).

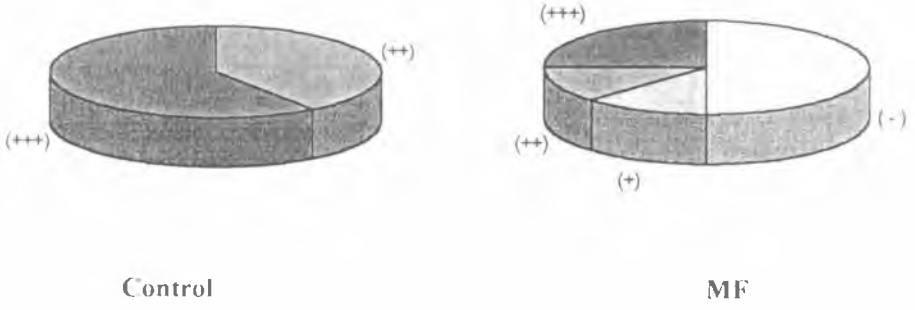

Fig. 2. Expression of CD5 antigen in dermal infiltrate

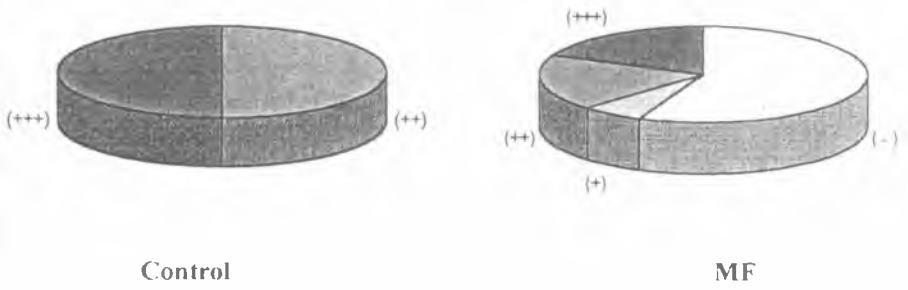

Fig. 3. Expression of CD7 antigen in dermal infiltrate

The expression of the CD1a (Langerhans' cells) was similar in both studied groups, but in a few cases of MF the lack or weak expression of this antigen was observed.

In the dermal infiltrate B cells were present in very small numbers. Infiltration by B-cells was not substantial in the studied cases, only one sample obtained from patient with advanced MF (IIB) revealed weakly positive expression of CD20 antigen. CD20+ cells showed a preference for the peripheral, deeper parts of the infiltrate.

The present study showed that specific alterations of expression of CD7, CD5 and CD3 antigens may appear in patients with MF, but these abnormalities are generally not seen in benign inflammatory skin diseases, except pityriasis lichenoides chronica (8). Observation of clinical features, histopathologic picture and immunophenotypic characterization of skin infiltrate revealed that no correlation between the loss of antigenicity and the prognosis was detected (11). No aberrant phenotypes were found on lymphocytes in specimens from a variety of nonlymphomatous skin diseases. The results of the present study are partly confirmed by previous reports (6, 11).

The detection of aberrant phenotypes can give additional support for a diagnosis that is already based on combination of clinical, histological and cytologic features.

REFERENCES

1. Arndt et al.: Cutaneous Medicine and Surgery. An Integrated Program in Dermatology. W.B. Saunders Company, vol. 2, Philadelphia 1996.
2. Bachelez H. et al.: Detection of clonal T-cell receptor g gene rearrangements with the use of the polymerase chain reaction in cutaneous lesions of mycosis fungoides and Sézary syndrome. Arch. Dermatol., 131, 1027, 1995.
3. Barcos M.: Mycosis fungoides. Diagnosis and Pathogenesis. Hematopathology, 99, 452, 1993.
4. Braun-Falco O. et al.: Dermatology. Springer-Verlag, Berlin 1984.
5. Collenberg C. et al.: Monitoring of the disease progress in Sézary syndrome by CD7- cell using flow cytometry. Br. J. Dermatol., 134, 980, Letter 1996.
6. Mitchie S. A. et al.: Expression of T-cell receptor antigens in mycosis fungoides and inflammatory skin lesions. J. Invest. Dermatol., 93, 116, 1989.
7. Nickoloff B. J.: Light-microscopic assessment of 100 patients with patch/plaque-stage of mycosis fungoides. Am. J. Dermatopathol. 10, 469, 1988.

8. Preesman A. H. et al.: Immunophenotyping on simultaneously occurring plaques and tumors in mycosis fungoides and Sézary syndrome. Br. J. Dermatol., 129, 660, 1993.
9. Ralfkiaer E. et al.: Phenotypic characterization of lymphocyte subsets in mycosis fungoides: comparison with large plaque parapsoriasis and benign chronic dermatoses. Am. J. Clin. Pathol., 84, 610, 1985.
10. Ralfkiaer E. et al.: Immunophenotypic studies in cutaneous T-cell lymphomas: clinical implications. Br. J. Dermatol., 129, 655, 1993.
11. Van der Putte S. C. J. et al.: Aberrant immunophenotypes in mycosis fungoides. Arch. Dermatol. 124, 373, 1988.
12. Warnke R. et al.: Atlas of Tumor Pathology. Tumors of the Lymph Nodes and Spleen. Armed Forces Institute of Pathology, Washington 1995.
13. Wojnowska D. et al.: Ekspresja antygenów limfocytów T krwi obwodowej w chłoniakach skórnych T-komórkowych – doniesienie wstępne. Przegl. Dermatol., 5, 415, 1996.
14. Wood G. S. et al.: Leu-8 and Leu-9 antigens phenotypes: Immunologic criteria for the distinction of mycosis fungoides from cutaneous inflammation. J. Am. Acad. Dermatol., 14, 1006, 1986.

2001.03.14

SUMMARY

Skin biopsy specimens obtained from 16 patients with mycosis fungoides (MF) were examined immunohistochemically to assess phenotype of T-cells. Cases of benign chronic skin disorders (BCD) were included in control group. Examination of skin biopsies obtained from patients with MF revealed aberrant immunophenotypes of T-cells (loss of one or more of T-cell markers CD7, CD5, CD8, and rarely CD3). The expression of CD1a antigen both in the dermis and epidermis of the studied groups was similar and normal.

No correlation between this loss of antigenicity and the prognosis was observed. These disturbances were seen in most of the cases of the early MF, but not seen in inflammatory skin diseases. In conclusion, the presence of aberrant immunophenotype of T-cells in skin infiltration may be useful in the diagnosis and differentiation of MF.

Ekspresja wybranych antygenów T-komórkowych w nacieku skórnym w pierwotnie skórnych chłoniakach T-komórkowych

W celu oceny fenotypu komórek T w nacieku wycinki skórne pobrane ze zmian od 16 chorych na ziarniniaka grzybiastego (*mycosis fungoides*, MF) poddano badaniom immunohistochemicznym. Grupę kontrolną stanowiły biopsje otrzymane od pacjentów z łagodnymi, przewlekłymi dermatozami. Ocena nacieku w wykwitach chłoniakowych wykazała obecność aberracji immunofenotypu limfocytów T (utrata jednego lub więcej antygenów T-komórkowych, tj. CD7, CD5, CD8, rzadziej CD3). Ekspresja antygenu CD1a zarówno w naskórku, jak i w skórze właściwej w obu grupach była podobna i w większości przypadków prawidłowa.

Nie obserwowano korelacji pomiędzy utratą ekspresji badanych antygenów a rokowaniem. Przedstawione nieprawidłowości występowały w większości przypadków wczesnego stadium MF, ale przeważnie nie stwierdzano ich w grupie zapalnych chorób skóry. Wykazanie aberracji fenotypu komórek T w nacieku skórnym może być pomocne w rozpoznawaniu i różnicowaniu MF.