Impact of systemic psoriasis treatment on the lesional T–lymphocyte subtypes

K. Adamicová, Ž. Fetisovová, J. Péč, P. Cingel, K. Parobeková and I. Chromej

ABSTRACT

Objective In 19 patients suffering from severe psoriasis and who were treated by Cyclosporin A (CyA), CD4 and CD8 positive T-cells were studied.
Materials and methods. Skin from affected areas was examined by immunohistopathology for the presence of CD4 and CD8 positive T-cells before and 4 weeks after the start of treatment.
Results. Significant decrease in CD4 positive T-cells and a non-significant decrease in CD8 positive T-cells were observed after the initiation of treatment. CD4/CD8 ratio dropped from the values of 2.10 to 1.68. Dramatic decrease in PASI score was observed, from the average value of 23.34 to that of 11.97.
Discussion. The authors compare and discuss the results of a few similar cytophotometric studies found in the available literature. The decreases in T-lymphocyte subsets correlate with clinically well-known treatment effect of CyA and suggest underlying immunopathologic process.

KEY WORDS

psoriasis, PASI score, cyclosporin A treatment, lesional CD4, CD8 lymphocytes

Introduction

Pathogenesis of psoriasis still remains unclear. Attention has been traditionally focused on abnormalities of keratinocyte proliferation and differentiation. Inflammatory and immunological reactions in the affected skin have been proposed as a suspected trigger only recently (1). The latest knowledge in the field of immunology of psoriasis and interaction processes between the cells at the site of inflammation suggest a possible autoimmune background of this disease (2).

The cells of the immune system bear on their surfaces hundreds of molecules specific for particular development stage and functional state. Until now, more than 260 various types of molecules have been identified on the surface of human leucocytes, but only a few decades of these are associated with a known structure and function. Sets of monoclonal antibodies were produced to target antigenic glycoprotein molecules on the surface of cells involved in immune response. In 1982, the Paris nomenclature of these glycoprotein molecules, denoted as CD (cluster of differentiation) markers, was approved (3,4).

Healthy dermis contains a certain number of T-lym-

Tab 1 CD4 and CD 8 positive T-cells measured before (1st excision) and after (2nd excision) the start of treatment with CyA in 19 patients suffering from severe form of psoriasis. The ratio CD4/CD8 was calculated and the clinical status was expressed as PASI score.

	1st excision				2nd excision			
No.	**CD4 -1**	**CD8 -1**	**CD4:CD8**	**PASI - 1**	**CD4 -2**	**CD8 -2**	**CD4:CD8**	**PASI -2**
1	24	8	3	38,8	40	10	4,00	18,8
2	104	41	2,53	26	26	23	1,13	14,9
3	68	83	0,81	28,8	48	33	1,45	15,5
4	100	20	5	25	74	49	1,51	3,6
5	25	11	2,27	15,3	20	14	1,42	9,3
6	27	35	0,77	42	21	18	1,16	18,2
7	30	30	1	6,3	25	27	0,92	4,6
8	100	70	1,42	13	69	45	1,53	9,6
9	40	40	1	25,8	29	18	1,61	22,2
10	22	10	2,2	25,8	20	12	1,66	10,4
11	21	12	1,75	14	18	21	0,85	6,0
12	84	62	1,35	13,9	57	29	1,96	6,0
13	96	42	2,28	17,4	82	31	2,64	5,8
14	31	26	1,19	15,6	27	19	1,42	13,3
15	97	36	2,7	27,5	51	22	2,31	15,2
16	120	45	2,66	22,4	36	31	1,16	5,1
17	93	49	1,9	29,9	94	58	1,62	21,1
18	178	54	3,29	13,2	125	78	1,60	12,6
19	162	58	2,79	42,8	142	69	2,05	15,6
Mean:	**74,84**	**38,52**	**2,1**	**23,3**	**52,8**	**31,9**	**1,68**	**11,97**

phocytes which can multiply significantly under pathologic (inflammatory) conditions (5). Major pathological abnormalities in psoriasis occur in epidermis with disturbed balance in proliferation and differentiation of keratinocytes. Hyper-and parakeratosis in the psoriatic sites is accompanied by inflammation in the dermis. Related to development of psoriasis and the effects of some systemic drugs used in the treatment, the dynamics of T-lymphocytes and their subpopulations of helper (CD4) T-cells and suppressor/cytotoxic (CD8) T-cells might be closely connected with disease progression and a long-term outcome. In our pilot study, the absolute and relative presence of T-lymphocyte subtypes, CD4 and CD8 T-cells, has been monitored in situ, before and during the treatment of severe forms of psoriasis with cyclosporin A (CyA). CyA (Sandimmun Neoral, Novartis) is a lipophilic undecapeptide isolated from Tolypocladium inflatum. It is an effective immunosuppressive drug with selective and reversible inhibitory effect on the T-lymphocyte functions mainly due to the inhibition of interleukin-2 production, and has been proved as effective in the prophylaxis and treatment of autoimmune dermatoses including psoriasis vulgaris (6). It has been used in the treatment of psoriatic arthropathy, moderate and severe forms of psoriasis, including psoriatic erythrodermia and pustular psoriasis.

TAB.2 Correlations coefficients of CD4, CD8 count, CD4/CD8 ratio and PASI score in the course of treatment.

CD4	0,8389
CD8	0,5456
CD4:CD8	0,299
PASI	**0,6561**

Material and Methods

19 patients (11 males, 8 females) with severe psoriasis were included into the study. The age of patients varied from 32 to 58 years with an average of 42. The extent and severity of disease were measured by means of PASI score (psoriasis area and severity index). The study protocol was approved and monitored by the Ethical committee at Martin Faculty Hospital. The procedure was explained to the patients and their consent was obtained.

The skin specimens were excised from the lesion

margins using 5 mm needle, the tissue was processed by the method of frozen cuts and monoclonal antibodies specific against CD4 marker of helper/inducer T-cells, and CD8 marker of suppressor/cytotoxic T-cells (Dako Co.) were applied. Under magnification of 480x, fixed number of 10 visual fields of the dermis was evaluated by a semi quantitative method. After morphological evaluation of T-lymphocytes with positive reaction to the added antibody, the cells were counted. The skin specimens were processed and evaluated under the same conditions before the start of systemic treatment, and at four weeks after starting CyA therapy in the initial dosage of 3mg/kg/day. The final evaluation consisted of comparing clinical outcome of treatment with CyA measured by PASI and relative and absolute presence of CD4 and CD8 positive T-cells in the psoriatic lesions. Two selection paired t-test was used as statistics for the level of significance of observed data.

Results

Table 1 is a summary of data obtained in the group of 19 patients divided according to the time of excision. Before the start of CyA treatment, the number of CD4 positive T-cells was within the range of 22-178, with the average of 74.8 per 10 fields of vision. CD8 positive T-cells were less, their values ranged from 8 to 83, with the average of 38.5 per 10 fields of vision. The CD4/CD8 ratio at the time of the pretreatment excision was about 2.1. PASI score was 23.3 on average, ranging from 6.3 up to 42.8.

After 4 weeks of CyA treatment, the number of T-cells and their subtypes were decreased. The CD4 positive T-cells decreased to the value of 52.8 on average, the CD8 positive T-cells decreased to the value of 31.9 on average. The decrease of CD4 positive T-cells was statistically significant at the level of $p < 0.05$. The decrease of CD8 positive T-cells was not statistically significant. Decrease in CD4/CD8 ratio from the value of 2.10 to 1.68 was slightly exceeding the limit of significance, staying within the limits of trend with p=0,055. The remarkable decrease in PASI score from the average value of 23.34 to the average value of 11.97 was statistically significant at the level of $p = 0.00000292$.

Discussion

The assumption of the autoimmune origin of psoriasis is mainly due to the conspicuous presence of an increased number of T-lymphocytes in lesions and to the observation of disease transfer by bone marrow transplantation or its cure by the same procedure (7).

In a well-developed psoriatic lesion, the focal T-lymphocytes and their subsets multiply. The CD4 positive T-cells are mostly auxiliary T-lymphocytes engaged in Th1 type of immunoreaction. They interact with antigen-presenting cells and are connected with class II histocompatibility complex (MHC). They might play an important role in precise determination of epitopes triggering the immune response. T-lymphocytes positive for CD8 on their surface belong to the MHC I class and play a role in principal recognition of self and non-self antigens as a part of the first defense reaction against infections and in maintaining integrity of the epithelium layer.

Dermal infiltrate consists mostly of CD4 positive T-cells, while the T-lymphocyte infiltrate found among epidermal cells consists mostly of CD8 positive T-cells (8). Retention of the lymphocytes in the epidermis is due to their bond with keratinocytes which express adhesive ICAM-1 molecule (7). In our specimens, lymphocytes were found in the epidermis only occasionally and in insignificant quantity, therefore we did not evaluate them further. This condition may be the result of local therapy with corticosteroids used by psoriatic patients. In a recent study, cytomorphometry of peripheral blood of psoriatic patients revealed the relative presence of CD4 positive T-cells in the range of 64-85% and CD8 positive T-cells of 10-32% (8) which is in apparent contrast with their values in a healthy population. Referential normal range for CD4 positive T-cells is 33-53% and for CD8 positive T-cells is 24-50% (11). Nevertheless, the relative increase in CD4 positive and the decrease in CD8 positive T-cells in the peripheral blood of psoriatics is also reflecting the stage of disease progression, remission or relapse, as well as the effects of systemic treatment (5).

The differences in published data on the presence of activated T-lymphocytes in the peripheral blood of psoriatic patients may be also due to confounding effects of other immunologic reactions evolving in the body (9).

Only few authors studied CD4 and CD8 positive T-cells in situ, in the lesions of psoriatic patients. Baker et al. (1) have found 204 (+/- 24) CD4 positive T-cells and 119 (+/- 18) CD8 positive T-cells, with their ratio of 2.05 (+/- 0.26), in 50 fields of vision of frozen slices taken from the margins of psoriatic sites. CD4 / CD8 ratio of 1.28 was higher than that found in a healthy population. In the psoriatic lesions of patients with AIDS, CD4/C8 ratio was very low, only 0.5-0.8. Remarkable increase in the number of CD8 lymphocytes in the specimens has not been explained yet (9). The present study offers additional data on the in situ dynamics of T-lymphocyte subsets in psoriatic patients treated by CyA. The CD4/CD8 ratio measured before the treatment was similar to that of Barker et al. (2.1 vs. 2.5) (10). The decrease of absolute values in both studied T-lymphocyte subgroups can be explained by immunosuppressive effect of CyA and was reflected also in the change of their relative proportion.

It was hypothesized, that during remission of clinical manifestations of psoriasis, activated CD8 cells rela-

tively prevail and produce cytokines which help inhibit the disease process (12). Our results showed that despite of dramatic drop in PASI, no increase in CD8 positive T-cells was observed after 4 weeks of CyA treatment. Decrease in CD4/CD8 ratio after CyA treatment shows more rapid decrease in CD4 than CD8. Improved clinical status reflected in PASI decrease after 4 weeks of CyA treatment was accompanied by returning the relative numbers of T-lymphocyte subsets close to those found in healthy persons – 52.8% of CD4 positive T-cells within the reference range 33-53% (13) and 31.9% of CD8 positive T-cells within the same of 24-50% (13). The rapid and favorable effect of CyA in the treatment of psoriasis was repeatedly stressed in the literature (14, 15). The observed changes in the CD4 and CD8 positive T-cell subsets correlate with the treatment outcome and may strongly suggest underlying immunopathology in the development of the disease (16, 17, 18).

REFERENCES

1. Hatta N, Takata M, Kawara, Hirone T, Takehara K. Tape stripping induces marked epidermal proliferation and altered TGF-alpha expression in non-lesional psoriatic skin. J Dermatol Sci 1997; 14: 154 –61.

2. Camp RDR. Psoriasis in: Textbook of Dermatology. Eds RH Champion, JL Burton, DA Burns, SM Breathanach. Vol 2, 6th ed Blackwell Science, Oxford 1998; 1589 – 649.

3. Ferenčík M, Rovenský J, Nyulassy Š. Imunológia – základné termíny a definície. Slovak Akademic Press sro, Bratislava 1999; 195.

4. Lukáš Z, Drábeková E, Feit J, Vojtíšek B. Imunohistochemiské metody v biologii a bioptické diagnostice. Sborník prací Lékaøské fakulty v Brnì. Lékaøská fakulta Masarykovy univerzity, Brno, 1997; 147.

5. McKee PH. Pathology of the Skin. 2nd ed Mosby – Wolf, London. Wiesbaden 1996; 14 –7.

6. Picascia DD. Treatment of severe resistant psoriasis with systemic cyclosporin. J Amer Acad Derm 1987; 17: 408 – 14.

7. Griffits ChEM, Kirby B. Psoriasis. M Dunitz, London, 1999; 1 – 6.

8. Brown DW, Baker BS, Ovigne JM, Hardman C, Powles AV, Fry L. Skin CD4+T cells produce interferon – gamma in vitro in response to streptococcal antigenes in chronic plaque psoriasis. J Invest Dermatol 2000; 114: 576 – 80.

9. Steigleder GK, Rasokat H, Wermmer V. Psoriasis in immunodeficiency related to HLTV – III – cellular immunity and immunohistochemical findings. Z Hautkr 1986; 61: 1971 –8.

10. Baker BS, Swain AF, Griffits,CEM. Epidermal T lymfocytes and dendritic cells in chronic plaque psoriasis: The effects of PUVA treatment. Clin Exp Immunol 1985; 61: 526 – 34.

11. Barrett JT. Textbook of Immunology an Introduction to Immunochemistry and Immunobiology. CV Mosby Comp, St Louis Washington, Toronto, 1988; s 121 – 44.

12. Fry L. Psoriasis: immunopathology and long – term treatment with cyclosporin. J Autoimmunity 1992; 5 (Suppl A): 277 – 83.

13. Petrisková J. Význam prietokovej cytometrie kože v diagnostike dermatologických ochorení. Atestačná práca, Martinské centrum imunológie, Martin 2001; 30.

14. Buchvald J, Šimaljaková, M. Cyklosporin A v terapii psoriazy. Čs Derm 1994; 69: 179 – 81.

15. Novotný F. Cyclosporin A v terapii psoriazy a atopického ekzému. Čs Derm 1999; 74: 61 – 3.

16. Glad CP, van Erp, PE, Boezman L, van de Kerkhof PC. Multiparameter flow cytometry as a tool to evaluate antipsoriatic therapy. Br J Dermatol 1997; 137: 367 – 75.

17. Griffiths ChEM. Systemic and local administration of cyclosporin in the treatment of psoriasis. J Amer Acad Derm 1990; 23: 1242 – 6.

18. Mahrle G, Schulze HJ, Brautigam M and other multicentric contributors. Anti – inflammatory efficacy of low – dose cyclosporin A in psoriatic arthritis. A prospective multicentric study. Br J Derm 1996; 135: 752 -7.

AUTHORS' ADDRESSES

Katarína Adamicová, MD, PhD, assist. professor, Department of Pathology, Jessenius Faculty of Medicine, Comenius University, Martin (JFM CU), Kollárova 2, 036 59 Martin.
Želmíra Fetisovová, MD, Department of Dermatovenerology JFM CU
Juraj Péč MD, PhD , professor and chairman, same address
Peter Cingel, Computer Centre JFM CU
Katarína Parobeková, same address
Ivan Chromej, Division of Immunology and Oncology, Novartis Bratislava