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Chair and Department of Dermatology, Medical University of Lublin
Department of Clinical Immunology, Medical University of Lublin

BARBARA LECEWICZ-TORUŃ,
ALDONA PIETRZAK, JACEK ROLIŃSKI

CD45RO and CD45RA cells in peripheral blood in psoriatic patients preceded by an infection

The mononuclear infiltrate found in the skin in a variety of inflammatory dermatoses was characterised by a predominance of T-helper-inducer lymphocytes (Thi), CD4+/CD45RA-/CD45RO+, a population of cells responsible for maintaining and promoting immune reactions. Therefore, the predominance of Thi lymphocytes seen in the dermatoses studied (atopic dermatitis, contact dermatitis, lichen planus, psoriasis, discoid lupus erythematosus), implies a mechanism by which differential subset distribution is seen at the sites of dermal inflammation (9). Immunophenotypic studies of skin biopsies taken from evolving lesions of psoriasis demonstrate that T lymphocytes accumulate early in both the dermal and epidermal compartments of the skin. Their influx into the skin precedes that of neutrophils, a leukocyte that was previously thought to play a major role in the pathophysiology of the disease. Other studies have demonstrated that the T-cells present in psoriasis are T helper cells with memory phenotype UCHLI+, CD45RA- which have previously been exposed to an antigen and are activated as determined by expression of HLA-DR and IL-2 receptors (3, 9). Onuma (11) stated that in psoriasis among the infiltrating cells in the epidermis, CD4 -positive cells were dominant in the early phase; CD8-positive cells were dominant in the chronic phase, resulting in a markedly decreased CD4/CD8 ratio in the latter.

Baker et al. (3) demonstrated that in the group of psoriatic patients with extensive lesions a significant reduction in the number of total and T helper/inducer cells (Th) but not in T suppressor/ cytotoxic cells (Ts) was observed in the peripheral blood. The Th/Ts ratio in early guttate lesions was the same as in the blood and significantly lower than in the plaque lesions. The authors suggested that there was an active selective recruitment of Th cells into the established psoriatic lesions (3). Recruitment into psoriatic lesions may sometimes contribute to the reduction of Th-cell numbers in the blood. Boehncke et al. (5) have stated the presence of VLA-1+ epidermal lymphocytes suggests a long persistence of activated T-cells in psoriatic epidermis which might contribute to the chronic inflammatory nature of this disease.

De Rie et al. (12) found that not only lesional but also clinically uninvolved perilesional skin showed an increased number of T-cells. In lesional psoriatic skin, however, T-cells showed predominantly the CD4 phenotype, while in perilesional skin CD8 positive T cells were dominant. They noticed that the absolute number of T-cells, their CD27, CD28 and CD45RA expression, and the influx of CD8 positive T-cells, indicate that perilesional psoriatic skin is different from normal

and lesional psoriatic skin; and secondly, the data on CD27 and CD28 suggest that not only lesional but also perilesional psoriatic skin is subject to continuous antigenic stimulation, thus leading to decreased CD27 and CD28 expression on skin T-cells. Veale et al. (14) observed significantly more CD45RO T-cells and blood vessels in patients with psoriatic arthritis compared with both psoriasis alone, and with normal controls ($p < 0.02$).This study suggests that increased numbers of CD45RO T--cells, greater vascularity, the presence of B-cells, and increased numbers of DR+ epidermal cells are markers for arthritis in patients with psoriasis. Wakita et al. (13) have demonstrated that the majority of infiltrating CD4+ T-lymphocytes in psoriatic lesions are CD45RO+, LFA-1+, VLA-4+; an observation consistent with a skin homing T-cell subset (8). Also Cai et al. (6) showed in their works that specialised dermal microvascular endothelial cells (DMEC) in psoriatic lesions promote the selective adherence of the CD4 CD45RO helper T-cell subset.

OBJECTIVE

The aim of this study was to check if the proportion of CD45RO and CD45RA cells in peripheral blood in patients with psoriasis preceded by an infection is down-regulated by the process and if it differs from healthy controls.

MATERIAL AND METHODS

Subjects. A group of patients with psoriasis preceded by an infection was examined. The means (±SD) of patients' age was 34.5±13.9 years. The mean age of the control group was 31.7±11.1 years. The type of psoriasis was pint point, guttate and/or nummular.

Percentages of the following cells were studied: CD3+ (T-lymphocytes), CD19+ (B-lymphocytes), CD4+8+ (double positive lymphocytes CD4+, CD8+), CD4+ (T helper lymphocytes), CD8+ (T suppressor lymphocytes), NK (natural killer cells), CD25+3+ (T lymphocytes with alpha chain of IL-2R), CD25+ (all lymphocytes with alpha chain of IL-2R), CD45RO+ (memory cells of all lymphocytes), CD45RO+ CD4+ (memory cells of T helper lymphocytes) and expression of the antigens mentioned above on the same cells.

Peripheral blood samples were obtained at diagnosis from 28 patients with psoriasis and from 22 healthy donors. The cell surface antigens in each case were determined on fresh cells at the time of sample submission. Mononuclear cells were isolated by density centrifugation on Lymphoprep (Nycomed, Norway) and washed twice in phosphate buffered saline (PBS) containing 1% bovine serum albumin. Double colour immunofluorescence studies were performed using combinations of phycoerythrin (PE) and fluorescein isothyocyanate (FITC) conjugated with monoclonal antibodies. Monoclonal antibodies were obtained from Ortho Diagnostic Systems (Germany), Becton Dickinson (Germany) and/or Dako (Denmark). The characteristics of antibodies has been presented in detail in our previous study (8).

All samples were measured on a Cytoron flow cytometer (Ortho Diagnostic Systems). 10,000 cells were analysed per test. In order to quantify the levels of fluorescence, the mean fluorescence intensity and fluorescence signal strength of the study antigens was calculated. The mean fluorescence intensity and fluorescence signal strength of histogram was measured from the upper limit of the negative control.

Statistical analysis. Data are expressed as mean ±SD. Mann-Whitney U-Test was used for the comparison of the groups. Differences were considered significant at $p<0.05$.

RESULTS

The results of the study are presented in Tables 1 and 2. The mean percentage CD45RO+ cells in the psoriatic group (3.46±3.74) was significantly higher ($p<0.05$) than in the controls (1.51±1.96). The mean expression CD45RO (expressed as mean fluorescence intensity; MIF) in the controls did not differ from the mean expression in the psoriatics. The percentage of CD45RA in both groups was not statistically important ($p>0.08$) even though in the psoriatic group its value was almost doubled. There were no differences in CD45RA expression between the psoriatic and control groups stated. In the psoriatic group no statistically significant differences between the values of any parameters investigated before and after the treatment were stated.

Table 1. Patients with psoriasis preceded by infection compared to the control group

Parameter	Controls	Psoriasis	Probability
Percentage of CD45RO	1.51+-1.96	3.46+-3.74	p<0.05
Expression of CD45RO	75,82+18,78	79.56+23.75	=1.0
Percentage of CD45RA	1.59+2.1	3.0+5.6	p>0.08
Expression of CD45RA	72.74+14.05	77.57+22.32	p>0.9

Table 2. Patients with psoriasis preceded by an infection prior to treatment and post-treatment

Parameter N=28	Psoriasis before treatment	Psoriasis after treatment	Probability
Percentage of CD45RO	3.61+3.93	3.67+3.93	>0.6
Expression of CD45RO	76.48+23.47	71.71+19.63	>0.3
Percentage of CD45RA	3.12+5.98	2.54+2.48	>0.5
Expression of CD45RA	74.32+21.66	72.05+20.34	>0.3

DISCUSSION

The results of our study indicate both increased percent of CD45RO memory cells and the increased expression of this antigen on the lymphocytes of the peripheral blood patients with psoriasis when compared with the control group.

CD45 is an abundant transmembrane molecule, expressed by all leukocytes, whose intracellular domain has tyrosine phosphatase activity (2). CD45 merely activates Lck (2). It is indisputable that phosphorylated Tyr 505 is a good substrate for CD45 and that in the absence of CD45 this is the major site of Lck hyperphosphorylation (2). Several other more indirect approaches have also challenged the notion that the role of CD45 *in vivo* is simply to up-regulate Src-family kinase activity (2). Pervanadate, an inhibitor of CD45 activity as well as other phosphatases, induces a variety of T-cell activation events such as increased tyrosine phosphorylation of cellular substrates, intracellular Ca 2+ elevation and up-regulation of interleukin 2 receptor (2). The importance of the cells with expression of antigen CD45+ is observed indirectly.

M o r e l et al., R h i z o v a et al. and A b r a m s (1) have observed the effect of treatment of psoriatic subjects with anti-CD4 monoclonal antibody. They observed the administration of antibodies using immunohistochemical methods. Lesional skin samples demonstrated, among others, a gradual improvement in parakeratosis, papillomatosis and acathosis; partial decrease in epidermal T-cell infiltrate and no major changes in the dermal infiltrate composed of CD3+, TCR alpha beta+, CD45RO+, HLA-DR+T-cells. The authors emphasised that immunohistochemical changes differ from those induced by cyclosporin A or an 8-methoxypsolaren plus long wave UV light (PUVA) therapy (10).

R h i z o v a et al. (1) suggested that the observed changes are secondary to down-regulation of inflammatory cytokine production by T-cells *in situ*.

J e n s e n et al. (7) in flow cytometry analysis showed that calcipotriol does not alter the number of CD45+ cells or Langerhans' cells in psoriatic skin. These results indicate that calcipotriol alters neither the number nor the function of epidermal antigen – presenting cells in psoriatic epidermis.

Similarly to other authors, we have observed an increased percentage of CD45RO+ memory cells in the peripheral blood. Although we did not study this sub-population in the dermis, all the data published so far suggest the increased percentage of these cells in psoriatic infiltrate. It may be an intensified reaction of the lymphocytes to streptococcal antigenes, which may be responsible for triggering bacterial psoriasis and may cause the selection of T lymphocytes clone responding to these antigenes. Part of the lymphocytes of this clone become CD45RO+ immune memory cells and appears not only in the dermis but also in the peripheral blood. Circulating memory lymphocytes penetrate various tissues seeking the contact with the previously recognised bacterial antigen.

A s h w e l l et al. (2) have described that Scr-family kinases can serve as a substrate for CD45 (both negative and positive sites of tyrosine phosphorylation). The balance between these two opposing phosphorylation events regulates enzymatic activity. CD45 effects on total Src-family kinase activity might differ depending upon the cell type and the state of differentiation or activation because these variables might affect other molecules that contribute to tyrosine phosphorylation (e.g. kinases such as Csk), dephosphorylation or otherwise alter kinase activity (2). In psoriasis an increased activity of numerous kinases was observed (e.g. protein C-kinase, EGF-induced tyrosine kinase (10). B e n - B a s s a t et al. (4) observed that the inhibitors of tyrosine kinase from tyrphostyne family inhibit *in vitro* the growth of psoriatic keratinocytes. Numerous studies report normalisation of tyrosine kinase activity induced by topical (dithranol) or general (PUVA) treatment (10). G e n t e l m a n et al. (10) are of the opinion that hyperactivity of tyrosine kinase in human epidermis may be responsible for an increase in the proliferation of cells in psoriatic lesions and it leads to intensification of the disease.

An increased activity of tyrosine kinase observed in the epidermis adjacent to active psoriatic lesions can suggest an intensification of the development of the disease on the border between the clinical and sub-clinical lesions.

The lack of considerable change in the percentage of the number of memory cells before the treatment and afterwards observed in our study may be caused by a slow recovery of the homeostasis of the immune system. The study was carried out on patients with psoriasis within the period of 4-8 weeks after the application of topical treatment. However, this time may be too short for the immune system to get balanced and for the peripheral blood to normalise the lymphocytes pattern.

There may exist a correlation between an increased number of CD45 memory cells in the peripheral blood in psoriatic patients, an increased number of CD45 in infiltrates in psoriatic papulas and in the skin adjacent to psoriatic lesions as well as an increased activity of kinases. However, this requires further simultaneous studies.

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SUMMARY

A group of 28 psoriatic patients was examined before a topical treatment and afterwards. Samples of peripheral blood were obtained and analysed in a flow cytometry with respect to subpopulation of lymphocytes with a particular care paid to percentage and expression of CD45RO and CD45RA cells. The received results were compared with those from the group of 22 healthy controls. The study showed that the mean percentage of CD45RO+ cells in the psoriatic patients was significantly higher than in the control group ($p<0.005$). In the psoriatic group there were no statistically significant differences between the values of any of the parametres studied before and after the treatment.

Komórki CD45RO i CD44RA w krwi obwodowej pacjentów z łuszczycą poprzedzoną infekcją

Badano grupę 28 pacjentów z łuszczycą przed i po leczeniu lekami miejscowymi. Przeprowadzono badania krwi obwodowej w cytometrze przepływowym, analizując subpopulacje limfocytów ze szczególnym uwzględnieniem odsetka i ekspresji komórek CD45RO i CD45RA. Wartości od chorych porównano z wartościami 22-osobowej zdrowej grupy kontrolnej. Stwierdzono, że średni odsetek komórek CD45RO + w grupie chorych z łuszczycą był istotnie wyższy w porównaniu z grupą kontrolną ($p<0.05$). W grupie łuszczycowej nie stwierdzono znamiennie statystycznych różnic wartości żadnego z parametrów badanych przed i po zastosowaniu leczenia.