There are very few data concerning the role played by 
cell-mediated cytotoxicity, particularly at the molecular 
level, in the course of psoriasis. Both cytotoxic T 
lymphocytes (CTL) and natural killer cells contain in 
their granules the cytolytic protein perforin, a mediator in 
cell-mediated cytotoxicity reactions. The aim of this 
study was to analyze perforin expression in various 
sets and subsets of perforin-positive peripheral blood 
lymphocytes in 17 patients with chronic psoriasis vulgaris in 
the exacerbation phase. The results were compared with those of an 
age- and sex-matched healthy control group (n = 21). Perforin 
(intracellular antigen) and cell surface antigens were detected using 
the simultaneous double-staining method. We found a 
significant increase in perforin (P) expression in the patient 
group for CTL (CD3+P+ cells), which are located mostly in the 
CD8+ population of T lymphocytes (CD8+P+). Key 
words: psoriasis; perforin; cytotoxic T lymphocytes; NK cells.

INTRODUCTION
Psoriasis is an inflammatory papulosquamous skin disease 
which is immunologically characterized by increased ker- 
tinocyte proliferation, infiltration of inflammatory cells, 
especially T lymphocytes, and production of inflammatory 
cytokines, mostly of Th1 phenotype (1).

Immunological changes characteristic of psoriasis occur at 
the systemic and local levels, in the region of the psoriatic 
lesion in the skin (2). It is generally considered that T 
lymphocytes play an important role in the pathogenesis of 
psoriasis. Skin lesions in psoriatic patients are strongly 
infilitrated with T lymphocytes and, depending on the stage 
of disease, CD4+ or CD8+ cells predominate. Variations in 
the levels of these cells are observed in the peripheral blood 
(1, 3, 4).

There are very few data concerning the role of lymphocyte 
cell-mediated cytotoxicity (CMC), particularly at the 
molecular level. Both cytotoxic T lymphocytes (CTL) and 
natural killer (NK) cells are mediators in CMC reactions. 
These cells are endowed with a potent cytolytic armamentari-

um located in the cytoplasmic granules, containing the 
cytolytic molecule perforin (pore-forming protein) and 
associated serine esterases (granzymes). In unstimulated 
peripheral blood lymphocytes perforin is constitutively 
expressed in NK and gamma/delta TCR+ cells (high level 
of expression) and in the subpopulation of CD8+ cells 
expressing CD11b antigen (moderate expression). However, 
perforin can be induced in the CD8+CD11b- subpopulation 
and in some CD4+ T cells (5, 6). Perforin is a mediator in 
CMC reactions and the main function of granzymes is to 
mediate target DNA degradation upon gaining entry into the 

target cell through the presence of perforin pores (7). 
According to a search of the literature, the role of perforin-
mediated cytotoxicity in psoriasis has not yet been investi-
gated, either in the peripheral blood or in lesions.

The aim of this study was to analyze perforin expression in 
various sets and subsets of perforin-positive peripheral blood 
lymphocytes, which are known to have different functions.

MATERIALS AND METHODS

Patients
This study was approved by the Ethics Committee of the Medical 
Faculty, University of Rijeka. For flow cytometric analyses 2 groups 
of patients were recruited: (i) 17 patients (8 males, 9 females; 23–60 
years) who were in the early exacerbation phase of chronic psoriasis; 
and (ii) 21 healthy persons (laboratory personnel and volunteer blood 
donors; 9 males, 12 females; 20–57 years) who served as controls.

Lymphocyte preparation
Heparinized venous peripheral blood (10 ml) was layered onto Ficoll/ 
Hypaque density gradient and centrifuged for 20 min at 800 g. Cells 
accumulating at the interface were washed twice in RPMI 1640 and 
resuspended at a final concentration of 1×10^6 peripheral blood 
lymphocytes per sample in fluorescent-activated cell sorting (FACS) 
buffer. Cell viability was checked using trypan blue.

Monoclonal antibodies
A murine antihuman perforin monoclonal antibody (MoAb) δG9 
(IgG2b) was purified from Balb/c ascites (produced by E. R. P.). The 
following MoAbs were obtained conjugated to phycoerythrin from 
Becton Dickinson (Mountain View, CA): Leu-4 (anti-CD3), Leu-3a 
(anti-CD4), Leu-2a (anti-CD8), Leu-11b (anti-CD16) and Leu-19 
(anti-CD56).

Simultaneous detection of cell surface and intracellular antigens by 
flow cytometry
Our procedure for the simultaneous detection of intracellular 
(perforin) and cell surface membrane antigens has been described 
elsewhere (8). Briefly, peripheral blood lymphocytes were cultured 
overnight at 37°C in complete RPMI. Non-adherent cells were 
aliquoted (10^6 per aliquot), washed in FACS buffer and then fixed in 100 
μl PBS containing 4% paraformaldehyde, pH 7.4, for 10 min at 
room temperature. After 2 washes in FACS buffer, the cells were 
permeabilized with saponin buffer (0.1% saponin) (Sigma, Poole, UK) 
for 20 min at room temperature. Anti-perforin MoAb in saponin
buffer (3 μg/100 ml) was added to the cell suspension at a final concentration of 8–10 μg per sample and incubated for 30 min at room temperature. A second antibody (fluorescin-conjugated goat anti-mouse IgG; Becton Dickinson) was added for a further 30 min at room temperature. Cell surface antigens were then labeled by incubating for 30 min at room temperature with phycoerythrin-conjugated MoAbs after the integrity of the membranes was restored by a 10-min incubation in FACS buffer. In some experiments, only cell surface labeling was performed. An irrelevant isotype-matched murine MoAb was used as a negative control.

Lymphocytes were gated on the basis of forward and side scatter. A minimum of 10^6 cells were analyzed on a FACS (Becton Dickinson) and the results are shown as contour graphs. Thresholds for positive staining were set at <2% using the negative control and percentages of positive cells were obtained by subtracting the value of the control.

**Statistical analysis**

Results were analyzed using Sigma Plot for Windows, version 1.02 (Jandel Scientific). Statistical analyses were performed using a Student's *t*-test 1-way analysis for comparison of means.

**RESULTS**

**Lymphocyte subpopulations (T and NK cells) and total perforin-positive cells**

Significant increases in CD3+ and CD8+ cells in the peripheral blood lymphocytes of psoriatic patients in the acute phase of disease were found compared to healthy controls (Fig. 1). The incidence of CD3+ cells increased from 62% to 74.4% and the incidence of CD8+ cells increased from 23.2% to 36.5% (*p < 0.001*). There was no difference between the healthy control group and the patient group in terms of either CD4+ T lymphocytes or both markers of NK cells (CD16+ and CD56+). The percentage of lymphocytes containing cytolitic molecule perforin (P+ cells) was slightly higher in the patient group than in the healthy control group, but this difference was not significant (Fig. 1). The incidence of total P+ lymphocytes was 31.4% for the patient group and 27% for the healthy controls.

**DISCUSSION**

It has been shown that the perforin-mediated cytolytic pathway plays a role in the rejection of foreign tissue, autoimmunity, control of tumor growth and viral infections and infections by intracellular pathogens (9–11), and is greatly weakened in elderly subjects (8). In the cytotoxic pathway mediated by regulated secretion of granules the formation of membrane pores by perforin is a prerequisite for apoptosis of the target cell (12, 13). In addition to perforin the main component of granules is a group of serine proteases known as granzymes (14). Their main function is to mediate target DNA degradation upon gaining entry to the target cell by means of perforin pores (7).

In the present investigation we noticed significant differences in some lymphocyte subpopulations between healthy controls and psoriatic patients. The cloning of skin-infiltrating lymphocytes from lesional skin showed that some clones are cytotoxic against autologous lymphoblastic cell lines. Cytolytic clones were mostly CD4+ and some exhibited characteristics of NK cells (15). Eruption of acute psoriatic lesions coincides with the epidermal influx and activation of CD8+ T cells, whereas spontaneous disease resolution is associated with recruitment of CD8+ cells (16). Accordingly, the absence of CD8+ cytotoxic clones in the experiments...
cited above could be the consequence of the clinical phase of the disease, when samples were collected.

Cellular infiltrates in lesional skin consist largely of HLA-DR+/IL-2R+ T cells, i.e. activated T cells (17), and perforin expression in T cells is a marker for functionally activated cytotoxic cells in situ (5, 7). Evidence of the role of granzyme B-expressing CD8-positive T cells and perforin-positive cells in the apoptosis of keratinocytes in lichen planus, which is also thought to be a T cell-mediated disease, has been obtained (18). However, perforin expression in psoriatic patients was not analyzed either in situ or in cloned cells. There was no significant difference in perforin expression in NK cell subpopulations between psoriatic patients and healthy controls. The percentage of P+ cells was slightly lower in the patient group. This might mean either that these cells are shifted to the lesional skin or that NK cells and lymphocyte-mediated antibody-dependent cytotoxicity do not play an important role in the pathogenesis of psoriasis.

Although preliminary, the results of perforin expression in the T lymphocytes of psoriatic patients are interesting. They point to the role that perforin could play in the pathogenesis of the disease, particularly in the exacerbation phase. We have examined perforin expression in CTL, both CD4+ P+ and CD8+ P+ cells, in order to directly address the question of whether a specific cell population containing perforin in its granules is operative in the disease process in vivo. The results clearly showed that CD3+ P+ cells are upregulated compared to healthy controls in the phase of disease exacerbation, and that this increase is mostly located in the CD8+ P+ subpopulation (p <0.01). CD8+ P+ cells are the subpopulation of CD8+ T lymphocytes with cytotoxic potential and rapid killing of target cells bearing MHC class I molecules and appropriate antigen (5, 7). These cells could participate directly in the switching off of the inflammatory process via HLA class I-mediated recognition of the psoriasis epitope(s) (16). Investigations of perforin expression in the T lymphocytes at various disease stages and simultaneous detection in the peripheral blood and lesional skin, which are in progress in our laboratory, could shed light on the potential role of cytolytic pathways in the clinical course of the disease.

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