Assessment of Perforin Expression in Peripheral Blood Lymphocytes in Psoriatic Patients During Exacerbation of Disease

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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+). Keywords: psoriasis; perforin; cytotoxic T lymphocytes; NK cells.

INTRODUCTION

Psoriasis is an inflammatory papulosquamous skin disease which is immunologically characterized by increased keratinocyte 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 infiltrated 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 armamentarium 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 investigated, 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/Hyphaque 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 1×10⁶ peripheral blood lymphocytes per sample in fluorescent-activated cell sorting (FACS) buffer. Cell viability was checked using trypan blue.

Monoclonal antibodies

A murine anti-human 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 (10⁶ 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 was added and the cells were incubated for 15 min at room temperature.
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 (fluorescein-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 10⁶ 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 cytolytic 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 CD4+ 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
lymphocytes 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 cytolysis 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 CTRL, 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 com-
pared 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 lympho-
cytes 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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REFERENCES

immunodermatology, 3rd edn. St. Louis, MO: Mosby, 1996:
301–315.

2. Krueger JG, Krane JF, Carter DM, Gottlieb AB. Role of growth
factors, cytokines, and their receptors in the pathogenesis of

disease of abnormal keratinocyte proliferation induced by T

4. Bos JD, Hagaens C, Das PK, Kreig SR, Voorn WJ, Kapse-
ngen ML. Predominance of “memory” T cells (CD4+, CD82+)
over “naïve” T cells (CD4+, CD45R) in both normal and


of perforin in lymphocyte-mediated cytolysis. Adv Immunol

7. Podack ER. Functional significance of two cytolytic pathways

8. Rukavina D, Laskarzin G, Rubesa G, Strbo N, Bedenicki I,
Manestar D, et al. The age-related decline of perforin
expression in human cytotoxic T lymphocytes and natural killer cells. Blood
1998; 92: 1–12.

Podack ER. Perforin expression in peripheral blood lymphocytes
in rejecting and tolerating kidney transplant recipients. Transplantation

10. Rubesa G, Podack ER, Sepic J, Rukavina D. Increased perforin
expression in peripheral blood lymphocytes in multiple sclerosis
patients in exacerbation of disease. J Neuroimmunol 1997; 74:
198–204.


Purification of three cytotoxic lymphocyte granule serine
proteases that induce apoptosis through distinct substrate and

13. Shiver JW, Su L, Henkart PA. Cytotoxicity with target DNA
breakdown by rat basophilic leukemia cells expressing both

14. Hameed A, Lowrey DM, Lichtenheld M, Podack ER. Charac-
terization of three serine esterases isolated from human
interleukin-2 activated killer cells. J Immunol 1988; 141:
3142–3147.

from skin of patients with psoriasis. J Am Acad Dermatol 1993;

16. Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L.
Psoriasis: a T-cell-mediated autoimmune disease induced by

17. De Boer OJ, van der Loos CM, Hamerlink F, Bos JD, Das PK.
Reappraisal of in situ immunophenotypic analysis of psoriasis
skin: interaction of activated HLA-DR+ immunocompetent cells
and endothelial cells is a major feature of psoriatic lesions. Arch

18. Shimizu M, Higaki Y, Higaki M, Kawashima M. The role of
granzyme B-expressing CD8-positive T cells in apoptosis of
keratinocytes in lichen planus. Arch Dermatol Res 1997; 289:
527–532.