

Flow Cytometric Analysis of Peripheral T lymphocyte Subpopulations in Psoriasis

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Abstract

Background : Psoriasis is a common inflammatory skin disorder, has received attention as a target for new pathogenesis and oriented therapies .

Autoimmunity and T lymphocyte subsets are suggested to be involved in the development of psoriasis.

The aim of this work is to asses the role of T lymphocyte subsets in the pathogenesis of psoriasis .

Material And Methods :We investigated the peripheral blood lymphocyte subpopulations obtained from psoriatic patient before and after treatment and from healthy controls, using two colour flow cytometry.

Results :We found highly significant suppression of total CD₃⁺ T Cells and CD₃⁺ CD₅₆⁺ NKT lymphocytes in psoriatic patient as compared to control. Also, we observed significant reduction of T helper cells in patients as compared to control.

Conclusion : The highly significant reduction of CD₃⁺ T cell and CD₃⁺ CD₅₆⁺ NKT lymphocytes proved their actively involvement in the development of psoriasis.

Introduction and aim of the work:

Psoriasis is a common genetically determined inflammatory and proliferative disease of the skin (comp RDR,1998).It occurs in 1-3% of the world's population (Ortonne,1999). The etiology and pathogenesis of psoriasis undergo intense investigation during the past decade (Kenneth *et al.*, 2003)

There are characteristic features of the disease which suggest an immunological mediated process. (Mends *et al.*, 2000)

Several direct and indirect evidence suggest that T lymphocytes play a crucial role in the pathogenesis of psoriasis (Paul *et al.*, 2001) .

The presence of T helper cells, that secrete type 1 cytokines [IFN- γ , TNF α] was demonstrated in psoriatic skin lesions. (Nickoloff., 1999)

The generation of psoriasis skin lesion has been described in animal models by a process based on T cell regulation. (Schon *et al.*.,2003) In addition, the role of T lymphocytes as key effector cells is strongly supported by work from various groups in xanthotransplantation models, injection of activated T lymphocytes from the same patient onto severe combined immunodeficiency mice resulted in the generation of psoriatic lesions (Boyman *et al.*, 2004)

Antipsoriatic therapy acts on dual populations of cytokines secreting T helper cells (Th₁ cells secrete proinflammatory cytokines [IFN- γ ,TNF α IL₂] and Th₂ which secrete inhibitory cytokines [IL₄ , IL₁₀] . (Austin *et al.*, 1999) .

Induction of immunodeviation of

psoriasis in the form of Th₂ cytokines IL₁₀ or IL₄, down regulate the activity of Th₁ and reduce psoriatic lesions by 60% - 80% . (Asadullah *et al.*, 1999) and (Ghoreschik *et al.*, 2003) .

Natural killers T cells might have a role in the imbalance between Th₁ and Th₂ cells (Seder *et al.*, 1998) NK cell clones secrete type 1 , type 2 or both types of cytokines which could influence the differentiation of Th₀ towards Th₁ or Th₂ cells (Doherty *et al.*, 1999) CD₃ , CD₅₆ cells represent one of the NK T cell populations. CD3 CD56 NK T cell were significantly decreased in peripheral blood of patients with Th₁ mediated autoimmune disease (Yanagihara *et al.*, 1999).

The aim of our study is to asses the role of different T lymphocyte subsets in the pathogenesis of psoriasis.

Patients and methods

Patients profile :

Peripheral blood samples of 19 patients with psoriasis (two erythrodermic, 17 chronic plaque psoriatic) was obtained before and after treatment from all patients. Therapeutic modalities included mono therapy and combined therapeutic regimens.

[PUVA(Psoralen +narrow band U.V.B (311), methotrexate).

After treatment, blood samples were taken at the time when the applied treatment regimen had been completed.

The control group consisted of 10 healthy persons, with informed consent for use in the study. In the control group nobody took any medicine and nobody suffered from any known acute or chronic disease.

Materials

Anti CD₄ FITC (Clone MT 310), anti CD₈ PE and anti CD₅₆ FITC, Anti CD₃ FITC and anti CD₃ PE [Clone UCHTI]. Immunostaining and flowcytometry:

Peripheral blood, anticoagulated with EDTA, was collected each blood sample

(50 Microlitres) was stained with two monoclonal antibodies one conjugated with FITC and the other with PE (20 Microlitres from each) at room temperature, in the dark for 20 min . Erythrocytes were lysed with lysing solution. After two washes with PBS [phosphate buffer saline], the cells were resuspended in PBS for immediate analysis or were fixed 2% paraformaldehyde over night storage before analysis then analysed by flowcytometer in a Beckman coulter. EPICS XL flowcytometer equipped with argon ion laser 488 nm, soft system II ver 3 .

Statistical analysis:

Data was expressed as mean \pm SD comparison between 2 means was performed using student's test, P value < 0.05 was considered significant, whereas P value < 0.01 was considered highly significant.

Results:

The lymphocyte subsets of psoriatic patient before and after treatments and in healthy control were analysed.

It has been shown that the percentage of total CD₃⁺ T lymphocytes was highly significantly decreased in patient before treatment as compared to healthy control (57.89 \pm 6 in patient before treatment versus 72.09 \pm 2.16 in healthy control, P = 0.0000) (Table 1) .

Similarly, the percentage of CD₃⁺ , CD₅₆⁺ T lymphocytes were highly significantly reduced in psoriatic patient before treatment in comparing to healthy control (1.29 \pm 0.4 before treatment versus 4.58 \pm 0.7 in control, P = 0.0000) (Table 1)

A suppression in the percentage of T helper lymphocytes was observed in psoriatic patient before treatment as compared to control (31.42 \pm 3.99 before treatment versus 39.13 \pm 2.15 in control, P < 0.0001) (Fig1)

The cytotoxic CD₈ lymphocytes showed non-significant reduction in patient before treatment versus healthy control (25.51 \pm 4.25 before treatment versus 29.17

± 1.83 in healthy control, $P = 0.024$) (Table 1) .

Comparing the lymphocyte subsets of patients before and after treatment showed that both total CD_3^+ T lymphocytes and CD_3^+ , CD_{56}^+ cells were significantly increased in patient after treatment as compared to patient before treatment. (The percentage of total CD_3^+ before treatment

57.89 ± 6 versus 63.46 ± 5.5 after treatment, $P = 0.0000$) (Table 2 , Fig 1)

(CD_3^+ , CD_{56}^+ lymphocytes before treatment 1.29 ± 0.4 versus 1.89 ± 0.5 after treatment $P < 0.0001$) (Fig 2)

No significant change was observed in the percentage of CD_4 and CD_8 T lymphocytes, before and after treatment. (Table2)

Table (1) :- Shows the percentage of lymphocyte subsets in psoriatic patients before treatment and in healthy controls.

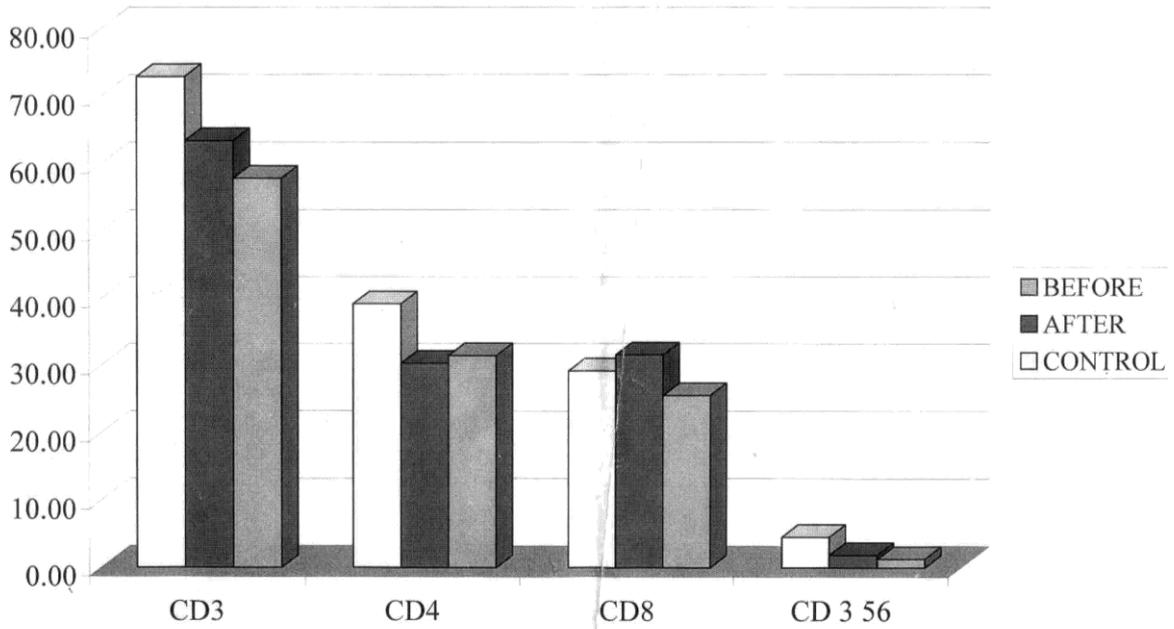
	Control	Patient before treatment	P value
CD_3^+	72.09 ± 2.16	57.89 ± 6	0.0000
$CD_3^+ CD_{56}^+$	4.58 ± 0.7	1.29 ± 0.4	0.0000
CD_4	39.13 ± 2.15	31.42 ± 3.99	< 0.0001
CD_8	29.17 ± 1.85	25.51 ± 4.25	0.0243

Table (2):- Shows comparative study between lymphocytes subsets before and after treatments of psoriatic patients.

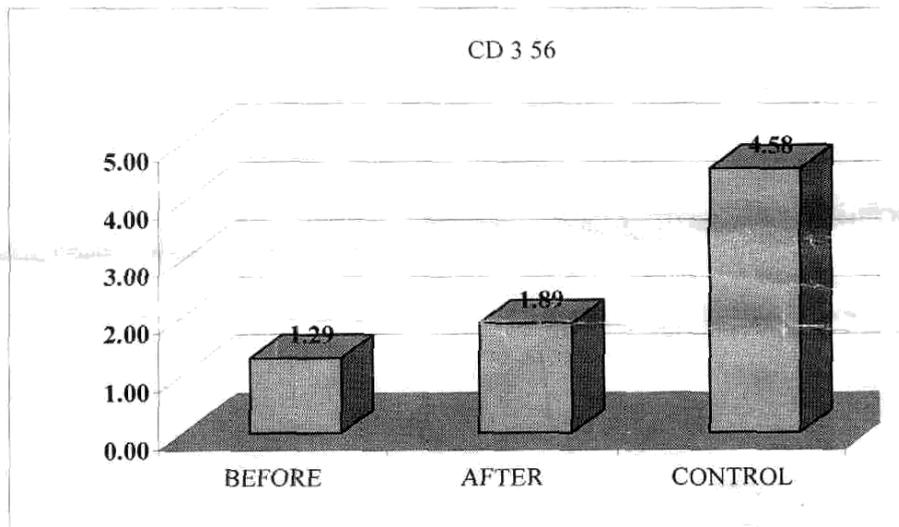
	Before	Patient after treatment	P value
CD_3^+	57.89 ± 6	63.46 ± 5.5	0.0000
$CD_3^+ CD_{56}^+$	1.29 ± 0.4	1.89 ± 0.5	< 0.0001
CD_4	31.42 ± 3.99	32.28 ± 3.10	0.0074
CD_8	25.51 ± 4.25	27.56 ± 4.57	0.022

Flow cytometric analysis of peripheral.....

(Fig 1) CD3,CD4, CD8 & CD3 56 AMONG STUDIED GROUPS



(Fig 2) CD3 56 AMONG STUDIED GROUPS



Discussion:

Psoriasis is an instructive model for studying interactions of immigration immunocytes with resident epithelial and mesenchymal cell. This disease throw high lights on the pathogenic importance of T cells (Spadro *et al.*, 2004)

In our current study, we assessed the levels of Total CD₃⁺ T lymphocytes , CD₃⁺ CD₅₆⁺ NKT cells , CD₄⁺ T helper cells and CD₈⁺ cytotoxic T Cells in psoriatic patients before and after treatment in comparing to the control group. We observed highly significant reduction in psoriatic CD₃⁺ T lymphocytes before and after treatment as compared to their levels in healthy control group. Since we didn't study CD₃⁺ T lymphocytes in the skin, we couldn't exclude that the reduction of psoriatic CD₃⁺ T lymphocytes might come from their differential homing to skin . Michael *et al.* (2005). demonstrated the complex pathological tissue alterations in psoriatic skin and showed a mixed inflammatory infiltrate that contains activated CD₃⁺ T lymphocytes within psoriatic dermis and epidermis.

Another very important finding in our study was highly significant reduction in the number of CD₃⁺ CD₅₆⁺ T lymphocytes in peripheral blood of psoriatic patient before treatment in comparing to healthy control and significantly increased after different therapies but remain significantly lower than those found in healthy control, this in agreement with KORECK *et al.* (2002) who suggested that the decrease of CD₃⁺ CD₅₆⁺ lymphocytes may represent an intrinsic characteristic feature of psoriatic patient. This hypothesis is supported by the fact the CD₃⁺ CD₅₆⁺ lymphocytes of psoriatic patient after treatment didn't reach the values found in healthy control.

Another possible explanation may be represented by early activation of CD₃⁺ CD₅₆⁺ lymphocytes by antigen involved in the disease, followed by apoptosis , this is evidenced by Thomas and Kupper. (2005) who showed migratory patterns of CD₃⁺ CD₅₆⁺ lymphocytes to home in the skin and their interaction with psoriatic autoantigen .

The study has also shown a suppression in the proportion of CD₄ T helper cells in psoriatic patient before treatment in comparing to control. This was explained by Michael *et al.* (2005) who observed the trafficking pattern of helper T cells from peripheral blood to psoriatic dermis and epidermis.

Conclusion:

Our results suggest that reduced total CD₃⁺ T cells and marked suppression of CD₃⁺ CD₅₆⁺ T cells in psoriatic patient can be of importance in the development of psoriasis . As such, it may help in the development of novel immune therapies for psoriasis.

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تحليل خلايا ت الليمفاوية فى الدم بوسطة جهاز الفلوسيتومتيرى فى مرضى الصدفية

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يعتبر مرض الصدفية من الأمراض الشائعة، وللمناعة دورا كبير فى حدوث هذا المرض وخاصة الخلل فى الخلايا ت الليمفاوية. وقد أجريت هذه الدراسة على مجموعة من مرضى الصدفية قبل وبعد العلاج وذلك لتحليل هذه الخلايا بواسطة جهاز الفلوسيتومتيرى بعد أخذ عينة من الدم وقد أخذت عينات من بعض الأشخاص الأصحاء كمجموعة ضابطة .

ولقد وجد انخفاض ملحوظ فى CD_3^+ cells الكلية وأيضا انخفاض فى $CD_3^+ CD_{56}NKT$ وذلك فى مرضى الصدفية بالمقارنة بالمجموعة الضابطة . وقد وجد أيضا انخفاض ملحوظ فى خلايا ت المساعدة فى مرضى الصدفية مقارنة بالمجموعة الضابطة ومن ذلك يتضح مدى أهمية هذه الخلايا فى حدوث مرض الصدفية .