THE DENSITIES OF T CELL SUBSETS, LANGERHANS CELLS, AND NATURAL KILLER CELLS IN PSORIATIC PATIENTS AND IN PATIENTS TREATED WITH PUVA

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SUMMARY :

Purpose : *Psoriasis is a chronic inflammatory skin disease of unknown etiopathogenesis. Several treatment modalities have been used, among which psoralen/ultraviolet A radiation (PUVA) has yielded successful results.* ***Method:*** *We investigated epidermal and dermal total T lymphocyte (CD3+), helper inducer T lymphocyte (CD4+), supressor / cytotoxic T lymphocyte (CD8+), Langerhans cells (CD1a+) and natural killer (CD56+) cell densities, immunohistochemically, in patients with chronic psoriatic plaques and in patients with recovered plaques after PUVA in order to assess the possible immunologic effects of this treatment.* ***Results :*** *In this study, both epidermal and dermal CD3+ and CD4+ cells were found to be higher in the study group compared with the control group ($p<0.017$). CD8+ cells were increased in number in the dermis of the patient group ($p<0.017$), while a significant increase was not noted in the epidermis. CD1a+ cells were also more abundant in the patient group ($p<0.017$). CD56+ cells were higher in number in psoriatic epidermis and dermis ($p<0.017$).* ***Conclusion:*** *We thought that T cell subsets may play an important role the complex immunopathogenesis of psoriasis.*

Key Words: *Psoriasis, PUVA, T Cell Subsets, Langerhans Cells,Natural Killer Cells.*

INTRODUCTION

Psoriasis is a well known skin disease with a chronic and relapsing course (1). Although the exact immunopathogenesis of psoriasis is unknown, immunologic and genetic factors have been implicated in its etiology (2, 3). Psoriatic plaques are characterized by epidermal hyperplasia and the presence of acute and chronic inflammatory cells (3). The epidermis and dermis of an active psoriatic plaque contain increased numbers of several different cells of the immune system, including lymphokine-secreting activated T cells, activated antigen-presenting cells (APCs) (Langerhans cells, other dendritic cells and macrophages), polymorphonuclear leukocytes, and hyperproliferating keratinocytes (4, 5). The activation of APCs, keratinocytes or dermal cells may result in induction of antigen presentation, cytokine release, and enhanced T-cell activation. The enhanced level of antigen presentation by APCs within the epidermis or dermis results in CD4+ cell (Th) activation and lymphokine release (4, 5). Lymphokines, in turn, may have direct effects on keratinocytes, resulting in hyperproliferation and accelerated differentiation (4). PUVA radiation is known to cause a decrease in T lymphocytes and T lymphocyte subsets, both locally and systematically (2, 6). This study

investigated epidermal and dermal CD3+, CD4+, CD8+, CD1a+, and CD56+ cell densities immunohistochemically, in patients with chronic psoriatic plaques and in patients with recovered plaques after PUVA.

MATERIAL AND METHODS

Two groups of psoriatic patients were studied. The first group included 23 chronic plaque type psoriatic patients. None of the patients had received either local or systemic treatment for at least one month nor had previously undergone photochemoterapy. The second group of 18 psoriatic patients were treated by PUVA and had recovered psoriatic plaques. The control group consisted of 15 healthy subjects. Punch biopsy specimens with a diameter of about 0.5 cm were taken from the three groups of patients. Each specimen was washed in saline, embedded in tissue-tek, and stored frozen at - 55° C until use. Cryostat sections, 4 mm thick, were placed on poly-L-Lysine treated glass slides and fixed in cold acetone for 10 min. After washing in tris buffered saline (TBS) the slides were incubated with blocking normal geat serum for 20 min. Primary antibodies were applied for 1 h at room temperature. The monoclonal mouse antibodies were as follows: CD3+(Dako-4B5), CD4+(Dako-MT310), CD8+(Dako-DK5), CD1a+(Dako-NA 1/34), CD56+(Dako-T199). Further steps were carried out using alkaline phosphatase antialkaline phosphatase (APAAP) (Dako-K670) kit. After several washings with TBS, the sections were subsequently incubated with biotinylated link antibody for 30 min and then with alkalin phosphatase labeled streptavidin for additional 30 min. Naphtol phosphate, as substrate, and new fuchsin, as chromogen, were applied for demonstration of the immune reactions. After being washed with TBS, the slides were counterstained with hematoxylin. Fresh frozen tonsil was used as positive control tissue (7).

On microscopic examination, only membranous staining were considered to be positive. The positively stained infiltrating cells were observed both in the epidermis and dermal papilla. Positive cells were counted per 5 microscopic fields at a magnification of x 200, and the mean number of cells were determined.The mean values of the infiltrating cell populations were revaluated comparatively in nontreated 23 cases, treated 18 cases, and 15 control cases, and the differences between the infiltrating cell populations were determined.

Mann-Whitney U test was used for statistical analysis when comparing two groups. The conformity of the data of each group to normal distribution was tested with the Kolmogorov-Smirnov test. The three groups were compared to one another with Kruskal Wallis H test, and when a difference was detected, three paired comparisons were done and a p value of 0.017 was used, according to Bonferoni's correction (8).

RESULTS

CD4+,CD8+,CD1a+,CD56+,CD3+ cells were evaluated in the epidermis and dermis of 23 patients with chronic plaque type psoriasis (16 males, 7 females; mean age 39.3±16.6 y; mean disease duration 9.8±4.1 y), 18 patients with psoriasis vulgaris whose lesions disappeared with PUVA treatment (10 males, 8 females; mean age 40.05±16.3 year; mean cumulative dose 955.9±298.2 J/cm^2), and 15 healthy subjects (11 males, 4 females; mean age 30.3±12.08 y) (Fig. 1, 2). The ranges and median values of the cell numbers infiltrating epidermis and dermis of all three groups are shown on table 1. Results of statistical analysis were as follows:

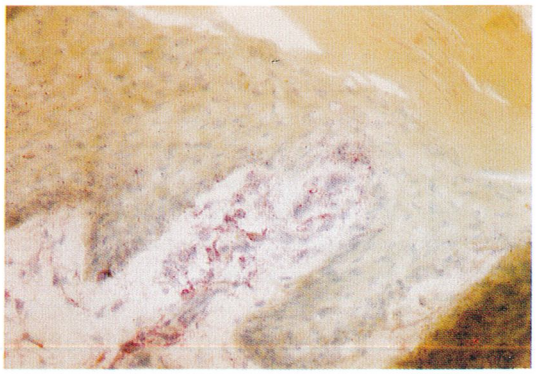

Fig - 1 : (Naphtol-New Fuchsin x 200) CD4+ cells in papillary dermis are seen a patient treated with PUVA (CD4 - APAAP).

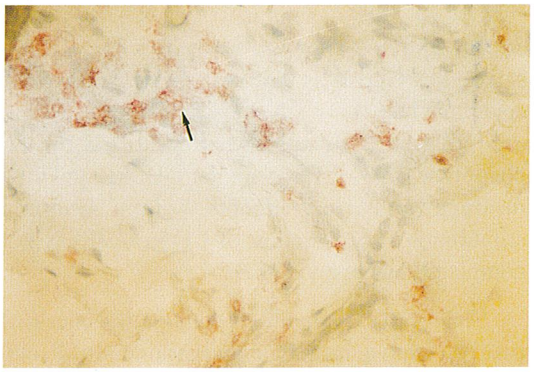

Fig - 2 : (Naphtol-New Fuchsin x 400) Membranous staining with CD4+ cells in the same patient at a higher magnification (CD4 - APAAP).

CD3+ cells: Both epidermal and dermal levels were statistically higher in the psoriasis group than in both PUVA treated and control groups ($p<0.017$). No difference was seen between the epidermal and dermal levels of PUVA treated and control groups ($p>0.017$).

CD4+ cells: Both epidermal and dermal levels of the psoriasis group were higher than the control group, ($p<0.017$); epidermal levels were higher than PUVA treated group, but dermal levels were not different ($p>0.017$)*. Epidermal levels of the PUVA treated group were not different from those of the control group ($p>0.017$), but dermal levels were higher ($p<0.017$).

CD8+ cells: Both epidermal and dermal levels of the psoriasis group were not different than the PUVA treated group ($p>0.017$). Dermal levels were higher in the psoriasis group compared with the control group ($p<0.017$), but epidermal levels were not different ($p>0.017$). Epidermal levels were not different between the PUVA treated and control groups ($p>0.017$), but dermal levels was lower in the PUVA treated group ($p<0.017$)*.

CD56+ cells: Both epidermal and dermal levels of the psoriasis group were higher than both PUVA treated and control groups ($p<0.017$), but there was no difference between the epidermal and dermal levels of the PUVA treated and the control groups ($p>0.017$).

		CD3+		CD4+		CD8+		CD56+		CD1a+
		E	D	E	D	E	D	E	D	E
PSORIASIS GROUP (N=23)	RANGE	0 - 9	0 - 38	0 -7.4	0 - 26	0 -3.6	0 - 26	0 - 3	0 - 8.6	0.2-15.2
	MEAN	2.15	7.37	1.32	4.38	0.75	2.85	0.5	1.35	2.41
PUVA GROUP (N=18)	RANGE	0 - 2	0-11.2	0 -1.4	0 - 9	0 -1.0	0 -6.4	0 -0.6	0 -3.8	0 - 5
	MEAN	0.37	1.61	0.15	1.62	0.12	1.11	0.06	0.5	0.53
CONTROL GROUP (N=15)	RANGE	0 -0.5	0 - 1	0 - 1	0 -0.6	0 - 1	0-0.44	0-0.20	0-0.33	0 - 1.0
	MEAN	0.14	0.43	0.14	0.14	0.11	0.10	0.05	0.09	0.33

E: Epidermis D: Dermis

Table 1: The distributions and mean values of CD cells in the groups of psoriasis, PUVA and control groups.

CD1a+ cells: Epidermal levels were higher in psoriasis group compared with the control group and PUVA treated group ($p<0.017$), and were higher in the PUVA treated group than the control group ($p<0.017$)*.

*Since the groups were not homogenous (did not conform to normal distribution), statistical results do not correlate with the mean values.

DISCUSSION

Although the exact cause of psoriasis is unknown, several immunological changes play a crucial role in the complex pathogenesis of psoriasis (1-6, 9, 10). Many hypotheses have been postulated concerning its pathogenesis. Initiation of the psoriatic lesion may be due to either microbial or physical damage to the skin, or to systemic or local antigenic, autoantigenic, or superantigenic substances. The activation of APCs, keratinocytes, or dermal cells can result in induction of antigen presentation, cytokine release, and enhanced T cell activation. The enhanced level of antigen presentation by APCs (in particular macrophages, but possible dendritic cells and LC) within the epidermis or dermis results in CD4+ cell activation and lymphokine release (4, 5). T cell derived lymphokines (especially INF-γ, IL-2, IL-1,IL-4,IL-6,IL-8,IL-9,IL-12) appear to play an important role in the immunopathogenesis of psoriasis (4, 5, 6, 11). These lymphokines may have direct effects on keratinocytes resulting in proliferation, accelerated differantiation, and/or chemotaxis, as well as NK activation, and TNF-α release (1, 4, 11, 12). Cytokines could also induce the expression of cell adhesion molecules, such as intercellular adhesion molecule-1(ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1) in psoriatic lesions (1). This expression is not a static process; it can be modified by many extracellular and intracellular factors. Factors known to upregulate the expression and/or adhesiveness of adhesion molecules include exposure to proinflammatory cytokines such as IL-1, TNF , or INF-γ, adhesion to extracellular matrix protein, T cell activation, and conversion of T lymphocytes from naive to memory cells (1, 4).

Recent studies have investigated expression of different surface antigens on epidermal and dermal infiltrating cells in psoriasis. CD3+ is considered to be the most specific marker of mature T lymphocytes (13). Subsets of mature T lymphocytes can be distinguished on the basis of expression of the CD4 and CD8 differantiation antigens. T cells expressing the phenotype CD4+ CD8- have been referred to as helper / inducer T cells (CD4+ cells); similarly CD4 - CD8+ T cells are called cytotoxic / supressor T cells (CD8+ cells) (11). CD1a+ is the most spesific marker of epidermal LCs (1, 14). Natural killer (NK) cells are a population of lymphocytes identified by the phenotype CD3 - CD16+ and/or CD56+, and they produce numerous effects upon immune stimulation. The function of CD56+ cells is not completely understood (12).

Studies to explain the immunopathogenesis of psoriasis have largely concentrated on T cell subsets. Although the results have been controversial, activation of CD4+ cells appears to play a key role. Onuma has reported that CD4+ cells increase in early phases and CD8+ increase in late phases within dermis, compared with controls (1). According to Valdimarsson's hypothesis, triggering factors such as foreign antigens or trauma provoke activation and proliferation of CD4+ cells by way of APCs (especially LC). The resultant increase in cytokine release, in turn, brings about hyperproliferation of keratinocytes (6). In our study, both epidermal and dermal CD3+ and CD4+ cells were found to be higher in the study group compared with the control group. These findings suggests a role for CD4+ cells in the pathogenesis of psoriasis. CD8+ cells, on the other hand, were increased in number in the dermis of the patient group, while a significant increase was not noted in the epidermis. In accordance with the suggestions of Onuma, the increase in the number of CD8+ cells in the chronic phase of the psoriatic dermis may be due to the release of lymphocyte and/or macrophage/monocyte-induced lymphokines/cytokines. Epidermal LCs were also more abundant in the patient group. LC found in the epidermis are best characterized by dendritic cell population, and they play an important role in the immune response of the skin as antigen presenting cells. On the other hand, the role of LC within the lesional skin is unclear (4, 5, 14). LC produce IL-1,IL-8 and TNF- (15). IL-1 promotes the influx and activation CD4+ cells (6). Many investigators have suggested that the density of LC is increased in psoriatic epidermis (4-6, 15, 16). The higher density of LCs in psoriatic lesions is associated with the increase in CD4+ cells and assumes a role

in the pathogenesis.

Many lymphokines such as IL-1,IL-2,IL-4,IL-12 activate NK cells.On the other hand, NK cells produce cytokines such as IL-1, and may play a role in the complex pathogenesis of psoriasis (12). We found an increase in the number of CD56 + cells in psoriatic epidermis and dermis. The increased NK density may be related to cytokine production of lymphocytic (especially CD4+ cells) or nonlymphocytic origin, and additional cytokine release from NK may contribute to the pathogenesis.

The major effect of photochemotherapy on the psoriatic epidermis is concentrated on to DNA synthesis. The data in the literature about the effects of photochemotherapy on the immune system vary considerably and at varied points the of immune traffic. Photochemotherapy affects the immune functions of keratinocytes, fibroblasts, T lymphocytes, and APCs by chancing their cytokine production. (2, 17-19). The observation that PUVA-induced clinical improvement of the psoriatic lesions was accompanied by a marked reorganization of the dermal infiltrate consisting mostly of T cells has highlighted the fact that these cells are the possible target of photochemoterapy (2, 20, 21). Photochemoterapy treated psoriatic patients have been reported to develope a highly significant reduction in the percentage of circulating CD3+ and CD4+ cells (2, 22). It has been show that PUVA-induced resolution is associated with depletion of epidermal CD4+ and CD8+ cells (6). In addition, irradiated subjects demonstrated increased nonspesific supressor cell activity ,decreased NK cell activity, and a decrease in the CD4+ to CD8+ cell ratio compared to controls (17). Our results confirmed the fact that PUVA has an inhibitory effect in epidermal and dermal CD3+ cells. This inhibitory effect is more pronounced on CD4+ cells in the epidermis and on CD8+ cells in the dermis. These results indicate that photochemoterapy has a global inhibitory effect on T cells, induces reorganization of epidermal and dermal T-cell subsets, changes the pattern of T-cell originated cytokine (probably IL-1) release, and thus hampers the psoriatic process through the new immune state. UV irradiation limits the survival of LC, decreases their density, and changes their morphology or functions such as ICAM-1 expression or cytokine release (17-19, 23-25). The clinical remission provided by PUVA in psoriasis was shown to correlate a decrease in LC (6). Although the effect of photochemotherapy on NK cells has not been well defined, irradiated subjects demonstrated decreased NK cell activity (17). In this study, we found that NK cell density is decreased in epidermis and dermis, and LC density is decreased in epidermis by PUVA therapy.

Our results suggest that PUVA inhibits NK cells activated by T lymphocyte- originated cytokines, and LC activated by the antigenic stimulus. The inhibitory mechanism might be related to a direct cytotoxic effect of PUVA or an indirect effect resulting from an altered immune balance.

In conclusion, an altered immune balance regarding both epidermal and dermal components appears to mediate the pathogenesis of psoriasis. The activation of CD3+ cells, especially CD4+ cells, and LC probably plays a key role in this mechanism, but NK cells also seem to have a contributory effect. PUVA may bring about the suppression of this immune response, in addition to its inhibitory effect on epidermal DNA synthesis.

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