The role of intradermal proliferation of T-cells in the pathogenesis of psoriasis*

Vladislav R. Khairutdinov[1]
Irena E. Belousova[1]
Alexey V. Samtsov[1]
Anastasiya F. Mikhailichenko[1]
Ekatherina Sh. Kuligina[2]
Evgeny N. Imyanitov[2,3]

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Abstract: Background: Psoriasis is a common immune-mediated chronic inflammatory disease of the skin and joints, affecting 1-3% of the population. It is generally accepted that the pathogenesis of psoriasis involves accumulation of effector T-cells within lymph nodes and their subsequent migration into the skin through the blood system. Here we provide evidence that psoriatic plaque itself may serve as a source of inflammatory T-cells.
Objective: We examined the intradermal proliferation of T-cells and the number of effector/memory (CD45RO+) T-cells in the skin of psoriatic patients at different periods of the disease.
Methods: Skin samples were obtained from 41 patients with progressive psoriatic lesions; 18 of these patients also donated skin specimens during the remission of the disease. The control group consisted of 16 healthy subjects. Ki-67 immunohistochemical staining was applied to detect proliferating cells, CD3ε served as a T-cell marker, and CD45RA and CD45RO antibodies were utilized to discriminate between naive and effector/memory T-cells, respectively.
Results: Progressive psoriatic lesions demonstrated Ki67 staining both in keratinocytes and in the CD3ε+ cells of dermal infiltrate. Median count of CD45RO+ cells per microscopic field was 15 in healthy controls, 59 in patients in remission and 208 in progressive psoriatic plaques. The observed differences demonstrated high level of statistical significance.
Study limitations: Limited number of analyzed patients..
Conclusion: Progressive phase of psoriasis is characterized by intradermal proliferation of T-cells. Spots of regressed psoriatic lesions contain high number of CD45RO+ cells, which are likely to render an immunological memory.
Keywords: Cell proliferation; Psoriasis; T-Lymphocytes

INTRODUCTION

Psoriasis is a common immune-mediated chronic inflammatory disease of the skin and joints, affecting 1-3% of the population.[1] The pathogenesis of psoriasis involves activation of Langerhans cells in the epidermis followed by their migration into the dermis; these cells subsequently relocate to draining lymph nodes and present antigens to naive T-cells.[2] This induces the differentiation of Th_0 cells into Th_1 and Th_{17} lymphocytes; the mobilization of the latter to the skin results in a formation of psoriatic plaque.[3,4] Antigen-specific differentiation is accompanied by the change of T-cell phenotype from $CD4^+CD45RA^+$ (naive) to $CD4^+CD45RO^+$ (effector/memory).[5]

This model is perfectly compatible with the primary immune response observed at the onset of the disease, e.g. in the guttate psoriasis. Indeed, the development of psoriasis frequently involves inflammation of the pharyngeal lymphoid ring caused by β-hemolytic streptococcus. The latent period of 2-3 weeks is required for the maturation of effector T-cells and hematogenous dissemination of these cells, as evidenced by multifocal lesions.[6,7]

However, the above model fails to explain some aspects of the disease relapse. For example, the recurrence of plaques is usually observed on the same ("favorite") sites, suggesting the existence of topical immunological memory within the skin. Surprisingly, regional lymph nodes always remain intact, irrespectively of the severity of psoriatic relapse. Altogether, these observations put into the question the exclusive role of lymph nodes in producing effector T-cells. We hypothesized that the psoriatic skin itself may serve as a source of antigen-specific T-cells. This report provides evidence to support this assumption.

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[1] SM Kirov Military Medical Academy – St.-Petersburg, Russia.
[2] N.N Petrov Institute of Oncology – St.-Petersburg, Russia.
[3] Saint Petersburg State Pediatric Medical University – St.-Petersburg, Russia.

METHODS

Patients and biopsies

The study included 41 patients with active psoriasis vulgaris (mean age 43.9±12.5 years) who did not receive any systemic or topical treatment at least within 4 weeks prior the examination. Punch biopsies (6 mm) were obtained from the periphery of psoriatic plaques. 18 of these patients underwent second biopsy in the same skin spot at the time of disease remission. Control samples from 16 healthy individuals included the material leftover after plastic surgeries (mean age 36.1±12.6 years). All recruited subjects were required to sign an informed consent form.

Antibodies and immunohistochemistry

The antibodies used for immunohistochemistry are listed in table 1. The numbers of $CD45RA^+$, $CD45RO^+$, $Ki67^+$ and $CD3\varepsilon^+Ki67^+$ cells in tissue sections were counted at $200^\times$ magnification on three non-contiguous random grids under high-power field (size 720 × 530 μm = 0.38 mm^2). The image analysis was assisted by the "UTHSCSA ImageTool 3.0" software. Based on the obtained data, median count of positive cells per field was calculated.

Statistical analysis was performed using the IBM SPSS Statistics, version 20. Wilcoxon-Mann-Whitney test was used to evaluate the differences between the samples of the skin from progressive psoriatic lesions, remission of psoriasis and healthy individuals. A *p*-value of less than 0.01 was considered significant.

RESULTS

Median count of $Ki67^+$ cells per field in the derma of the groups of patients with progressive disease, patients in remission and healthy individuals was 40, 7, and 3, respectively (Table 2). In addition, abundant Ki67 staining was detected in keratinocytes of the basal and lower spinous layers of the epidermis of progressive psoriatic lesions (Figure 1). As expected, the number of proliferating Ki67-positive cells was significantly higher in the derma samples from the patients with progressive psoriatic lesions compared with samples from the patients in remission; meanwhile, the derma of healthy individuals contained very few scattered Ki67 positive cells (Table 2, Figure 1).

Double staining with Ki67 and T-cell marker CD3ε ($CD3\varepsilon^+Ki67^+$) confirmed that approximately 30% of all proliferating cells in the dermis of progressive psoriatic lesions appeared to be T-cells (Table 2 and Figure 2). No proliferating T-cells were detected in the skin of healthy individuals (Figure 1). The skin of patients with psoriasis in remission contained just single proliferating T-cells.

Naive and effector/memory T-cells can be distinguished by the CD45RA/CD45RO staining. We found that the skin of psoriatic patients in acute phase contained significantly higher number of $CD45RO^+$ effector/memory T-cells (median count: 208) as well as $CD45RA^+$ naive T-cells (median count: 25) compared with patients in remission (median counts 59 and 8, respectively) and healthy controls (median counts 15 and 3, respectively). Interestingly, the spots of regressed psoriatic lesions also contained remarkable number of $CD45RO^+$ cells (Table 2, Figure 1).

Table 1: Antibodies for immunohistochemical analysis

Antibody	Cell marker	Antigen (clone)	Antibody dilution	Antigen unmasking	Manufacturer
CD3ε	T-cell	F7.2.38	1:600	Tris-EDTA buffer, pH 9,0	Thermo Fisher Scientific, USA
CD45RA	Naive T-cell	4KB5	1:200	Without processing	Thermo Fisher Scientific, USA
CD45RO	Effector/memory T-cell	UCHL1	1:150	Citrate buffer, pH 6,0	Dako, Denmark
Ki67	Proliferating cell	SP6	1:600	Tris-EDTA buffer, pH 9,0	Thermo Fisher Scientific, USA

Table 2: Immunohistochemical characteristics of the derma of progressive psoriatic lesions, remission of psoriasis and healthy individuals

Group	Patients with progressive psoriatic lesions (Pr)	Psoriasis patients in remission (R)	Healthy individuals (H)	p-value**		
				Pr vs R	Pr vs H	R vs H
Number of positive cells, X (x0.25-x0.75)*: Ki67+	40 (22-54)	7 (4-13)	3 (2-6)	9.7×10^{-13}	1.33×10^{-14}	8.9×10^{-11}
CD3ε+Ki67+ T-cells	12 (5-17)	2 (1-3)	0	3.3×10^{-15}	1.3×10^{-14}	2.2×10^{-5}
CD45RA+ (naïve T-cells)	25 (15-30)	8 (3-11)	3 (1-4)	1.7×10^{-13}	4.4×10^{-13}	2.5×10^{-6}
CD45RO+ (effector/memory T-cells)	208 (152-268)	59 (41-73)	15 (11-21)	3.5×10^{-11}	6.7×10^{-14}	2.8×10^{-8}
Number of examined samples	41	18	16			

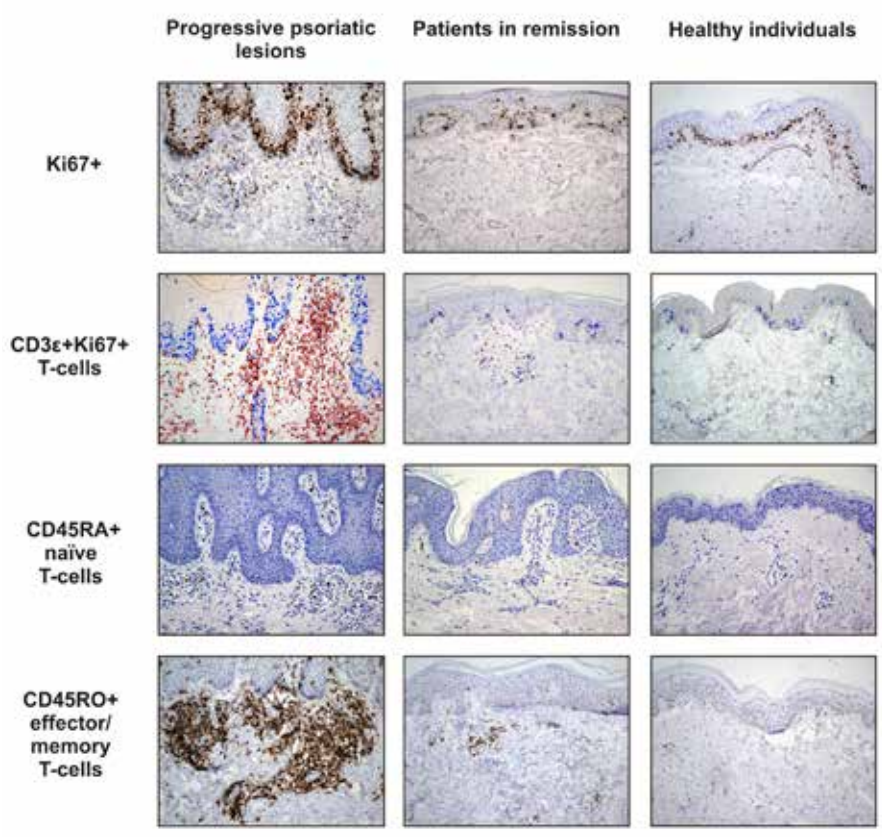

Figure 1: Immunohistochemical staining for Ki67+, CD3ε+Ki67+, CD45RA+, and CD45RO+ cells in the progressive psoriatic lesions, seemingly intact skin in remission and skin of healthy individuals (Hematoxylin & eosin x200)

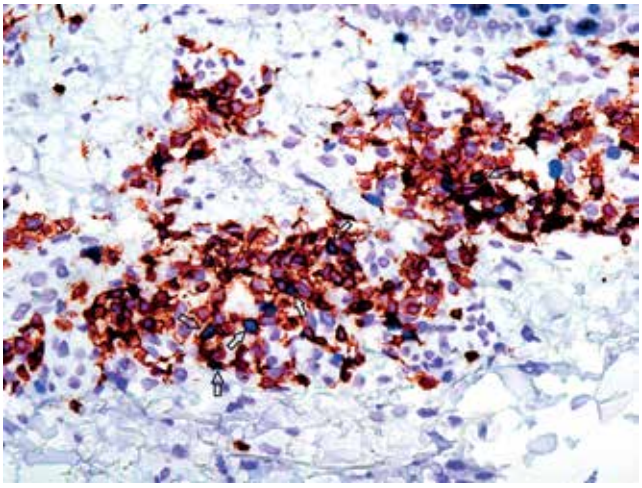

Figure 2: Double immunohistochemical staining for CD3ε+Ki67+ cells (Hematoxylin & eosin x600)

Thus, the obtained data suggest that proliferation of T-cells in psoriatic patients does not necessarily involve lymph node tissue, but can occur directly in the progressive psoriatic lesions. Apparently, topical effector/memory T-cells ($CD45RO^+$) serve as a primary source of lymphocyte expansion.

DISCUSSION

Some of the earlier studies reported that psoriatic lesions are virtually devoid of Ki-67-positive dermal T-cells, however later, in 1990, Nickoloff and Griffiths revealed Ki-67-positive T-cells in psoriatic lesions.[8,9] Ki-67 expression was shown to occur in T-cells after their migration into the epidermis and interaction with keratinocytes; however, immunohistochemical analysis demonstrated that more than 95% of the dermal T-cells in psoriatic lesions were Ki-67 negative, suggesting that they were in a resting or non-cycling (G_o) state. The authors concluded that T cells proliferate extra-cutaneously and then migrate to the skin.[10]

Our study demonstrates that progressive psoriatic lesions harbor significant number of proliferating T-cells. Therefore, the skin of psoriatic patients can operate as a lymphoid organ. These results do not conflict with the literature data. Local activation of T-cells is considered to be an important feature of psoriatic skin lesions. Pro-inflammatory cytokine IL17A, being secreted by Th17 cells, is capable to initiate the formation of ectopic lymphoid organs upon chronic inflammation.[11,12] IL17A stimulates the release of chemokines CXCL13 and CCL19 by fibroblasts and induces the development of lymphoid follicles; the neutralization of IL17A leads to reduction of the size of ectopic lymphoid foci.[11,13] It was also shown that dendritic cells derived from psoriatic plaques induce in vitro proliferation of T-lymphocytes more effectively than either psoriatic blood-derived or normal skin-derived dendritic cells.[14]

The formation of the ectopic foci of lymphoid tissue, which

resemble lymphoid follicles, is characteristic for the tissues suffering from chronic inflammation. These foci are usually referred to as tertiary lymphoid organs (TLO). Unlike the lymph nodes, TLO are not encapsulated and therefore engaged in direct interaction with the neighboring tissues. TLO are a landmark of many autoimmune diseases and related conditions, being observed in inflammatory tissues from patients with rheumatoid arthritis, Hashimoto's thyroiditis, recipients of transplanted organs, etc. [15-17]

CONCLUSION

Our results suggest that the formation of the cellular infiltrate in the skin of patients with psoriasis may occur not only through migration of the immune cells from the peripheral blood, but also by intradermal T-Cell proliferation. Moreover, we found that an excess of $CD45RO^+$ T-cells persists in the regressed psoriatic plaques (i.e., in the seemingly intact skin) in psoriatic patients at remission. These memory T-cells may remain silent for some periods of time, but become activated upon various stimuli and trigger the development of psoriatic lesion. The existence of the intradermal memory T-Cell explains why psoriatic plaques almost always relapse at the sites of the previously regressed lesions. Therapeutic targeting of the specific populations of silent intradermal T-cells appears to be a promising approach for prolonging remission of the disease in psoriatic patients.❑

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MAILING ADDRESS:
Khairutdinov Vladislav Rinatovich
Ul. Akademika Lebedeva, 6
Sankt-Peterburg, Russia, 195009.
Email: haric03@list.ru

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