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Leukocyte Migration in vivo and in vitro in Patients with Psoriasis

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Leukocyte migration in vivo was studied with a skin chamber technique in 21 patients with active psoriasis vulgaris and 18 with cleared psoriasis vulgaris. Measuring over 24 h, no difference was found between healthy volunteers and most patients with active psoriasis, although a subgroup of patients with long-lasting relapses showed subnormal migration values. In patients with cleared psoriasis on the other hand the in vivo leukocyte migration values were increased. In addition, leukocyte migration in vitro under agarose was studied, but no difference was found between healthy controls and patients with psoriasis, active or cleared. *Key words: Chemotaxis; Skin chamber.* (Received December 29, 1986.)

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The pathogenesis of psoriasis is unknown but several studies have indicated a role of polymorphonuclear leukocytes (PMNs). There are several reports of an enhanced chemotactic activity of PMNs in psoriasis (1, 2, 3, 4) while other investigators have found normal (5, 6) or decreased (7, 8) chemotactic activity. Studies of the in vivo migration of polymorphonuclear leukocytes in psoriasis have also given conflicting results (9, 10, 11, 12).

The present study was undertaken in an effort to elucidate further the in vivo and in vitro migration of polymorphonuclear leukocytes in psoriasis. Results are presented indicating an abnormal leukocyte migration in psoriatic patients.

MATERIAL AND METHODS

Subjects studied

A total of 21 patients with active psoriasis vulgaris (14 ♀ and 7 ♂) with a mean age of 47, and 18 patients with cleared psoriasis vulgaris (10 ♀ and 8 ♂) with a mean age of 42 were investigated for in vivo leukocyte migration with a skin chamber technique. The patients with active disease had nummular psoriasis with discs and plaques involving 5–25% of the cutaneous surface. During the months before the investigation the disease was spreading in all patients and the only treatments used during this period were emollient creams and ointments, and also topical corticosteroids. Forty-one healthy volunteers (25 ♀ and 16 ♂, aged 21–56, mean 34) served as a control group.

In addition the in vitro migration of PMNs isolated from the psoriatic patients, was studied, in nine patients with active psoriasis and in 13 with cleared psoriasis, using a migration under agarose technique. The migration of PMNs from a healthy volunteer was investigated in parallel in every agarose experiment.

The patients were not receiving any systemic treatment during the study.

Skin chamber technique

The skin chamber technique was applied as described earlier (13). Three small lesions, each with an area of 0.07 cm^2 were produced in the epidermis on the volar side of the forearm with the suction blister technique in patients and in healthy volunteers. In patients with active psoriasis, the lesions were made in uninvolved skin. After removal of the blister tops, a collection chamber containing three

separate compartments was placed over the lesions and filled with autologous serum, 0.5 ml in each compartment. The leukocytes were allowed to migrate into the collection chamber and the cells were harvested after 24 h. In some experiments two chambers were used and were harvested after 12 and 24 h respectively. The total number of cells in each compartment was determined in an automatic cell counter (Linson). The value for leukocyte migration was the mean of the values for the three compartments. Due to leakage of the chambers only two compartments were available on the occasion.

Chemotaxis in vitro under agarose

Chemotaxis was studied with a migration under agarose method described earlier (13). Leukocytes were isolated from heparinized blood by sedimentation on dextran. After being washed twice, they were suspended in tissue culture medium 199 (National Bacteriological Laboratory, Stockholm, Sweden) to a concentration of 5×10^{10}/l. In the agarose plates six pairs of wells were punched into the agar. The inner wells were filled with 10 µl of the leukocyte suspension and the outer wells were filled with either 10 µl of a chemo attractant or 10 µl of tissue culture medium 199. Two different attractants were used, a chemotactically active *Escherichia coli* culture filtrate (BF) and a zymosan activated serum (ZAS). To prepare ZAS, 1 ml of normal human serum was incubated with 0.025 g zymosan (Schwartz/Mann Division of Becton Dickinson & Co., Orangeburg, New York, USA) for 30 min at 37°C. After centrifugation the serum was decanted. Incubation of the agarose plates was carried out for a period of 3 h. After fixation with methanol and formalin the chemotaxis was quantified by measurement of a greatly enlarged projection of the migration pattern.

Statistics

The statistical significance of differences was calculated with the Student's *t*-test when the groups included more than ten patients and the Mann Whitney U-test when the patients were fewer than ten.

RESULTS

Twenty patients with active psoriasis and 18 with cleared psoriasis were investigated with skin chambers filled with autologous serum and harvested after 24 h. The corresponding control group consisted of 41 healthy volunteers. As shown in Fig. 1, the group of cleared psoriatic patients had higher leukocyte migration values ($p<0.005$, *t*-test) compared to the healthy volunteers, while the group of patients with active psoriasis, showed no difference compared to the control group. A significant difference, however, was noted when the patients with active psoriasis were divided into subgroups according to the duration of the relapse, and compared to the healthy volunteers. Two patients were excluded from this subdivision, because the duration of the relapse was unknown. Seven patients had a relapse duration of more than five months (Table I) and these patients had decreased values $p<0.001$ compared with the healthy volunteers. On the other hand, no statistically significant difference was noted when the patients with active psoriasis were divided into subgroups according to the affected area, and compared with the healthy volunteers.

In eight patients with active psoriasis, seven of whom are included in Table I, an attempt

Table I. *Eighteen patients with active psoriasis divided into subgroups according to the length of the relapse*

	Duration of relapse (months)	Number	Mean leukocyte migration (10^6 cells/collection compartment)
Patients	<2	4	4.4
	2–5	7	6.7
	>5	7	2.8
Controls	0	41	5.5

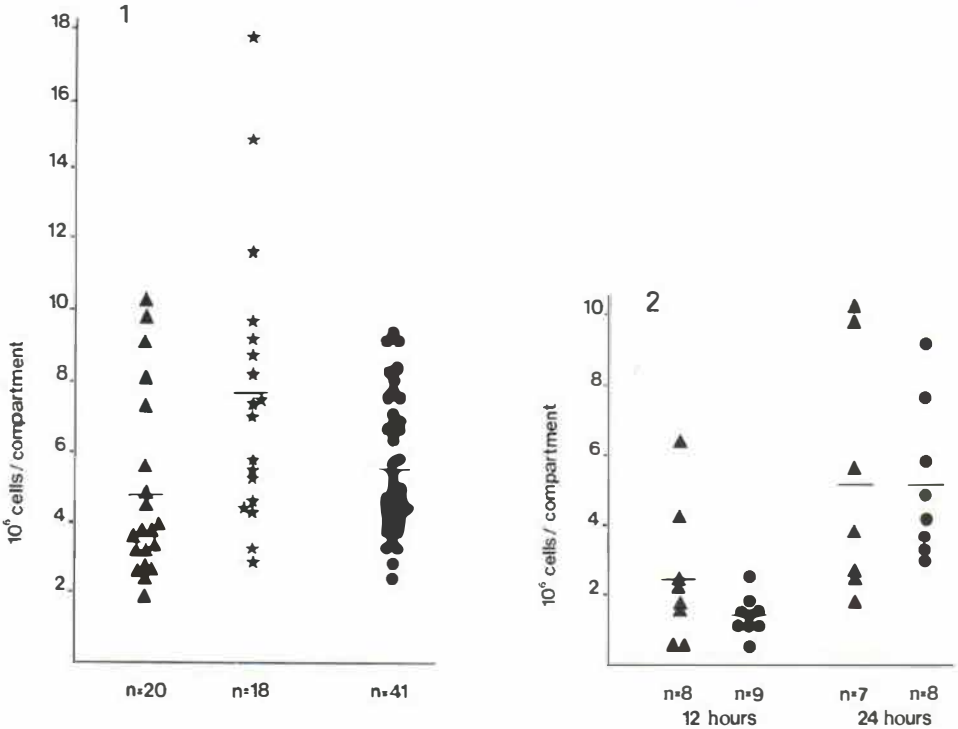

Fig. 1. Leukocyte migration (10^6 leukocytes/collection compartment) into skin chambers containing autologous serum, harvested after 24 h, in 20 patients with active psoriasis (▲), 18 patients with cleared psoriasis (★) and 41 healthy volunters (●).

Fig. 2. Leukocyte migration (10^6 leukocytes/collection compartment) into skin chambers containing autologous serum, harvested after 12 and 24 h, in eight patients with active psoriasis (▲) and 9 healthy controls (●).

was made to evaluate the importance of the chosen harvesting time of 24 h. The patients were investigated with two skin chambers containing autologous serum and harvested at 12 and 24 h respectively. Nine healthy volunteers were investigated in the same way and served as a control group. One patient and one volunteer had to be excluded at 24 h because of leakage from the chambers. The result is shown in Fig. 2. No difference was found between patients with active psoriasis and healthy controls. However, at 12 h there was a tendency to higher values for active psoriasis (NS). The three lowest values belonged to three patients with long lasting relapses.

In the agarose gel experiments no difference was seen in migration between PMNs from patients with active psoriasis (Table II) or cleared ones (Table III) compared with healthy persons.

DISCUSSION

The leukocyte migration in psoriasis has been widely investigated. There are several reports of the intrinsic neutrophile chemotactic activity of PMN:s in psoriatic patients but the results have been conflicting. Increased chemotactic activity in vitro has been reported by several authors (1, 2, 3, 4) while others report normal (5, 6) or decreased chemotaxis (7, 8). There are also a few reports of in vivo leukocyte migration in patients with psoriasis.

Breathnach et al. (10) reported normal migration in vivo with a skin chamber harvested after 24 h, while Dubertret et al. (11) found a biphasic process in patients with active psoriasis with increased leukocyte migration for the first eight hours after application of the skin chamber followed by a period of depressed leukocyte migration. Muffel et al. (9) have reported decreased migration and finally, Tigalonowa et al. (12) found a correlation of the extent as well as the duration of the active disease and the in vivo leukocyte migration values. According to Tigalonowa et al. (12), a decrease in in vivo leukocyte migration values is found in patients with actively spreading or very extensive lesions and also in patients with short (<2 months) or long (> 5–6 months) term relapse.

The present study showed an increase in in vivo leukocyte migration over 24 h in patients with cleared psoriasis, a finding which has not been reported previously, possibly because interest has been focused on patients with active psoriasis. Further, we found normal leukocyte migration values after 24 h in patients with active psoriasis, which is in agreement with the results of Breathnach et al. (10) and Dubertret et al. (11). However, there was a tendency to increased values during the first twelve hours, a result similar to that reported by Dubertret et al. When the patients were subdivided into groups according to the duration of the relapse, there was a subgroup with decreased values, characterized by a relapse duration of more than five months. This is in agreement with Tigalonowa et al. (12) and would also support the results of Muffel et al. (9) who reported decreased in vivo leukocyte migration in psoriatic patients. However, no indication of the length of the relapses was given in the publication.

The in vitro migration of PMNs from patients with psoriasis was in the present study found to be normal, which is in disagreement with Michaëlsson (2), who reported increased migration using the under agarose method.

This study showed no correlation between the results of in vivo and in vitro leukocyte

Table II. *Migration under agarose in nine patients with active psoriasis compared to nine healthy controls*

In each experiment one patient and one healthy control is analysed. The migration is given in arbitrary migration units

	Patients Mean (range)	Controls Mean (range)
Migration against BF	2.2 (1.2–2.9)	2.3 (1.6–3.0)
Migration against ZAS	1.9 (0.9–2.3)	2.1 (1.3–2.9)
Random migration	1.0 (0.7–1.8)	1.1 (0.8–1.5)

Table III. *Migration under agarose in thirteen patients with cleared psoriasis compared to thirteen healthy controls*

In each experiment one patient and one healthy control is analysed. The migration is given in arbitrary migration units

	Patients Mean (range)	Controls Mean (range)
Migration against BF	2.4 (1.6–2.9)	2.3 (1.7–3.0)
Migration against ZAS	2.6 (1.7–3.5)	2.3 (1.8–3.0)
Random migration	1.4 (0.9–2.1)	1.4 (1.0–1.9)

migration which is in good accordance with other studies (14, 15). The lack of correlation is most probably due to the fact that in vivo migration is influenced by several factors other than the intrinsic chemotactic capacity of the leukocytes.

It is a well-known fact that both lipoxygenase and cyklo-oxygenase products of the arachidonic acid metabolism are powerful modulators of the inflammatory response. Reports of an increased amount of lipoxygenase products in the epidermis (16) have given rise to the suggestion that lipoxygenase inhibitors might be beneficial in psoriatic patients. A positive effect of a lipoxygenase inhibitor, benoxaprofen, in patients with psoriasis has been reported (17, 18) as well as an exacerbation of psoriasis induced by indomethacin (19, 20). It is speculated that such exacerbation is caused by a shift of the substrate in the arachidonic acid metabolism over to the lipoxygenase pathway.

In conclusion, this study shows increased in vivo leukocyte migration in patients with cleared psoriasis while the migration in patients with active psoriasis was more varied and not significantly altered in 24 h. The increased in vivo leukocyte migration found in patients with cleared psoriasis may be important in explaining the exacerbation of psoriasis reported to be induced by indomethacin (19, 20) and also by lithium (21). In patients with psoriasis, the discrepancy in leukocyte migration values reported in the literature might be explained by the presence of leukocyte migration inhibitors (1, 12, 22), the importance of which probably varies during the different phases of the disease. Another possible explanation is an in vivo activation of the leukocytes, as described in thermal injury (23) and in sepsis (24) leading to a deactivation and a decreased migration of the leukocytes especially in the later phases of the disease. Despite the varying results of leukocyte migration in patients with active psoriasis found in the present study and also reported by others, altered leukocyte migration is a probable and important factor to consider when in search of an efficacious treatment of the disease.

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