

Cellular Reactivity of Polymorphonuclear Leukocytes in Psoriasis and Atopic Dermatitis—Measurement of Lucigenin-dependent Chemiluminescence

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Polymorphonuclear leukocytes (PMN) of patients suffering from psoriasis and atopic dermatitis are supposed to differ in their function from the PMN of normals. However, conflicting results have been reported. Using a lucigenin-dependent chemiluminescence we investigated the response of isolated PMN from patients with atopic dermatitis (AD) and psoriasis to different stimuli. PMN of 17 patients with psoriasis and 13 patients with AD with mild to moderate disease activity were stimulated with a chemotactic peptide (f-met-phe), zymosan activated serum (ZAS), zymosan particles and phorbol myristate acetate. In the AD group we found a significantly decreased response after stimulation with ZAS in comparison to the controls. With the other stimuli tested no significant difference was detected. In comparison to normal controls psoriatic PMN showed no significant difference with any of the stimuli. Comparing patients with plaque-type and guttate-type psoriasis no significant difference was detected. We suggest that the reported hyperactivation of psoriatic PMN could be triggered by serum factors which were excluded in the present investigation. The decreased response of PMN to stimulation with ZAS from patients with AD associated with a normal reactivity to the other stimuli could be due to specific desensitization of the PMN by C5a *in vivo*. (Received December 23, 1985.)

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Altered *in vitro* function of circulating polymorphonuclear leukocytes (PMN) has been described in psoriasis and atopic dermatitis. Both diseases represent chronic inflammatory skin disorders. Accumulation of PMN in Munro's microabscesses in the stratum corneum resulting from epidermal leukocyte migration is a characteristic feature of psoriasis (1). Atopic dermatitis (AD) is characterized by many signs of immunodeficiency (2). However, there exist conflicting results on the cellular reactivity of PMN in both diseases.

The chemotactic activity of psoriatic neutrophils has been reported increased (3, 4, 5) or normal (6, 7, 8). Their adherence to nylon fibers was found to be more increased (9) or normal (8). Phagocytosis was reported by increased (5), normal (7) or decreased (10). Measurement of the oxidative burst by the nitroblue tetrazolium dye (NBT) reduction appeared to be increased (11, 12) or within normal limits (7). Schopf & Straussfeld (13) reported a stimulus-dependent increased generation of oxygen intermediates in monocytes and PMN of psoriatic patients.

In AD the chemotactic activity of PMN was reported to be decreased (14). This defect normalized when the inflammation cleared. Normal chemotactic activity was found in AD by others (15, 16). Ring & Lutz (17) described decreased release of lysosomal enzymes from peripheral leukocytes of AD patients under stimulation.

In the present study we measured the release of oxygen intermediates by PMN of patients with psoriasis and AD by a lucigenin-dependent chemiluminescence (CL, 18, 19). The results indicate that alterations of PMN function may be secondary to the disease and possibly depend on serum factors being present in the active state of the disease.

MATERIALS AND METHODS

Ficoll-Hypaque solution was obtained from Pharmacia, Freiburg, FRG. Lucigenin, phorbolmyristate acetate (PMA), f-met-phe and zymosan particles were obtained from Sigma, Munich, FRG. Culture media were from Biochrom Seromed, Berlin, FRG.

Patients

Seventeen patients suffering from psoriasis were examined. The group consisted of 12 male and 5 female patients with a mean age of 45 ± 4 years. Eleven patients exhibited symptoms of the plaque-type of psoriasis with mild to intermediate disease activity, whereas 6 patients showed the guttate type of psoriasis. The AD group (as classified by Hanifin & Rajka (21)) consisted of 13 patients (7 males and 6 females) with a mean age of 31 ± 5 years. Only patients with mild to intermediate forms of the disease were tested. None of the patients had received systemic or local steroid therapy, or therapy with UV light within 6 weeks prior to blood collection.

Controls

The control group consisted of 30 healthy non-atopic blood donors. Atopy was excluded through history and laboratory findings (21).

Isolation of PMN

Human PMN were isolated from 20 ml citrate-anticoagulated venous blood of patients and controls as described previously (22). In brief, separation was performed by Ficoll gradient centrifugation and 3 thirty second cycles of hypotonic lysis with distilled water followed by the addition of an equal volume of 1.8% NaCl and were finally suspended to a density of 5×10^6 per ml in phosphate buffered saline of pH 7.2 containing 1 mM calcium, 0.5 mM magnesium and 2 mM lucigenin. Cells were 98% PMN as judged by Pappenheim stain and more than 95% viable as tested by trypan blue exclusion. Aliquots (200 μ l) containing 1×10^6 cells each, were placed into unsealed polystyrene luminescence tubes (Lumacuvette/Abimed, Düsseldorf, FRG) and stored at 4°C for a maximum of 6 h prior to use.

Chemiluminescence measurements

Cells were warmed up to 37°C for 15 min prior to the measurements. Chemiluminescence measurements were performed in a six channel Biolumat LB 9505 (Berthold, Wildbad, FRG) interfaced via an Apple IIE Computer to an Epson RX80 graphic printer (23, 24). Measurements were made at 37°C. The cells of patients and controls were stimulated alternatively with f-met-phe 2×10^{-4} M, zymosan-activated serum (24, 1:2 final dilution), zymosan particles (2 mg/ml) or PMA (100 ng/ml). Using f-met-phe and ZAS, the peak maximum within 10 min was measured, with zymosan and PMA 30 min integral counts were measured. Usually patients and controls were tested in parallel.

Zymosan-activated serum (ZAS)

ZAS as a source of C5a was prepared by incubating 20 mg/ml washed zymosan particles with pooled normal human serum for 30 min at 37°C in a shaking waterbath. Zymosan was removed by centrifugation. ZAS was portioned and frozen at -70°C . Only one lot of ZAS was used for measurements in this study.

Statistical analysis

Statistical analysis was performed by the Student's *t*-test.

RESULTS

As shown in Table I there was no significant difference in the background activity of psoriatic PMN compared with normals. Under stimulation with the chemotactic peptide f-met-phe and ZAS as a source of C5a no significant differences were found either. Also the phagocytosis of zymosan particles and triggering the PMN with PMA did not lead to any detectable difference between the PMN from psoriatic patients and those of controls. When comparing patients showing the guttate type and the plaque-type of psoriasis no significant differences were found (Table II).

With PMN from atopic dermatitis patients no significant differences were detected for the background activity and the cellular reactivity under stimulation with f-met-phe,

zymosan and PMA (Table I). Only stimulation with ZAS led to a significantly decreased response ($p < 0.01$).

DISCUSSION

In the present study the oxidative response of PMN from patients with psoriasis and atopic dermatitis (AD) was measured. Only patients with mild to intermediate disease activity were tested. The induction of the oxidative burst was assessed by a lucigenin-dependent chemiluminescence (18, 19). CL is thought to be the result of the generation of reactive oxygen species which are produced as a result of activation of a membrane NAD(P)H-oxidase (18, 20). Release of toxic oxygen radicals may be considered as a measure of the microbicidal and tissue destructive potential of the PMN. The described system is more sensitive than other convenient methods for measuring the oxidative burst. To evaluate different ways of activation PMN were stimulated with four distinct stimuli, the chemotactic peptide f-met-phe, ZAS as a source of the complement split product C5a, zymosan particles and PMA.

PMN of patients with psoriasis did not show any significant difference in their response to the tested stimuli. Our results are in agreement with the data presented by Geerdinck et

Table I. Cellular reactivity of PMN in atopic dermatitis and psoriasis

Stimulus	CL-response		
	Psoriasis	Atopic dermatitis	Controls
Spontaneous ^a	9,684 ± 1,550 ^c (17) ^d	13,014 ± 2,108 (13)	11,471 ± 2,306 (30)
f-met-phe ^b	14,554 ± 2,461 (17)	11,171 ± 2,760 (13)	13,776 ± 1,667 (30)
ZAS ^b	59,483 ± 9,824 (17)	33,467 ± 6,111 (13) ^c	64,176 ± 7,327 (30)
Zymosan ^a	615,857 ± 84,313 (16)	471,604 ± 68,035 (13)	619,846 ± 55,819 (29)
PMA ^a	444,393 ± 64,130 (16)	470,666 ± 49,308 (13)	557,068 ± 48,821 (29)

^a Values are expressed as integral-counts × 10⁻³ over a period of 30 min.

^b Values are expressed as peak-CL (cpm × 10⁻³).

^c Values represent mean ± SEM.

^d Number of probands in parenthesis.

^e $p < 0.01$.

Table II. Cellular reactivity of PMN in guttate type and plaque type psoriasis

Stimulus	CL-response	
	Guttate-type	Plaque-type
Spontaneous ^a	12,777 ± 3,495 ^c (6) ^d	7,997 ± 1,343 (11)
f-met-phe ^b	15,869 ± 4,241 (6)	13,836 ± 3,150 (11)
ZAS ^b	65,062 ± 19,434 (6)	56,439 ± 11,533 (11)
Zymosan ^a	595,349 ± 126,104 (6)	628,161 ± 116,665 (10)
PMA ^a	404,310 ± 87,581 (6)	468,443 ± 90,769 (10)
uflnj1		

^a Values are expressed as integral-counts × 10⁻³ over a period of 30 min.

^b Values are expressed as peak-CL (cpm × 10⁻³).

^c Values represent mean ± SEM.

^d Number of probands in parenthesis.

al. (25) who reported that patients with mild psoriasis did not show an alteration of "the metabolic burst". In contrast to our results Csato et al. (11, 12) reported increased NBT reduction by zymosan-stimulated PMN of patients with psoriasis supporting the concept of "activated granulocytes". However, Lipopolysaccharide-induced NBT-reduction was reported to be within normal limits (7). Schopf & Straussfeld (13) described an increased luminol-dependent CL under stimulation with PMA which they did not observe with opsonized zymosan. These findings differ from our results, possibly due to differences in the assay system. So, luminol-enhanced CL was used, which in contrast to lucigenin-dependent CL is dependent on the presence of myeloperoxidase (26).

The data presented in our paper did not support the concept of an intrinsic abnormality of the PMN in psoriasis. The alterations of PMN described by several authors could possibly be due to the presence of serum factors (27, 28) or to increased in situ generation of chemotactically active fragments of complement (8, 29). Also, defects in opsonization capacity should be considered (10, 30).

In the AD group the response to ZAS was significantly decreased. However, with the other tested stimuli, no significant differences were detected. Hanifin et al. (31) reported that the described effect of PMN chemotaxis (14) was due to a circulating "inhibitor" of chemotaxis present in plasma during acute flares of atopic dermatitis. This phenomenon could be explained by specific deactivation (32, 33, 34) of the PMN in the skin of the patients by the complement split product C5a. There is evidence in AD for activation of the complement cascade (35) possibly triggered by immune complexes (36).

Since the reported observations only reflect the situation in the skin of the patients, the use of skin chambers (37) and others appropriate systems could be of major advantage to evaluate the function of PMN "in loco" and to further investigate the role of cytokines (22) and other factors which may locally activate or deactivate the PMN in the patients. Based on our results we suggest that there is no intrinsic defect of the oxidative response of circulating PMN in psoriasis and AD. However, the presence of serum factors should be considered.

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