Acta Derm Venereol (Stockh) 1996; 76: 10–12

Soluble *Pityrosporum*-derived Chemoattractant for Polymorphonuclear Leukocytes of Psoriatic Patients

THOMAS BUNSE and GUSTAV MAHRLE

[1]*Department of Dermatology, University of Cologne, Germany*

The chemoattraction of polymorphonuclear leukocytes (PMNs) from psoriatic patients, atopic patients and healthy control persons by *Pityrosporum orbiculare/ovale* was investigated using the Boyden chamber method. The chemotactical attraction of PMNs from psoriatic patients by *Pityrosporum* (stimulation index SI = 58 ± 50) was significantly increased ($p < 0.05$) compared to PMNs from atopic patients (SI = 20 ± 17) and control persons (SI = 26 ± 24). This effect seems to be specific for *Pityrosporum*, since the chemotactical response to *Staphylococcus epidermidis* was not increased in psoriasis. The chemotactical factor produced by *Pityrosporum* is hydrophilic and is destroyed by acid hydrolysis, indicating its protein nature. The yeast *Pityrosporum* may thus play a role in the koebnerization of psoriasis. *Key words: chemotaxis; Boyden chamber; atopic eczema.*

(Accepted August 5, 1995.)

Acta Derm Venereol (Stockh) 1996; 76: 10–12.

T. Bunse, Römerstraße 432, D-47441 Moers, Germany.

The yeast *Pityrosporum orbiculare/ovale* is known to cause pityriasis versicolor (1), *Pityrosporum* folliculitis (2) and *Malassezia* intertrigo (3) and is generally accepted to play a role in seborrheic eczema (4). Anthralin is a potent inhibitor of *Pityrosporum* in vitro (5). There are two findings which indicate that *Pityrosporum* may also be involved in the pathogenesis of psoriasis. Cell fragments of *Pityrosporum* topically applied to the skin of psoriatic patients induced new psoriatic plaques in 100% of the cases (6). Furthermore, it has been observed that *Pityrosporum* folliculitis can convert into psoriatic lesions (7).

On the other hand, skin colonization with *Pityrosporum* is not increased in psoriasis (8). Therefore, an overgrowth with *Pityrosporum* does not seem to be a necessary prerequisite for its role in the pathogenesis of psoriasis and other skin disorders. For atopic and seborrheic eczema, for instance, it has been demonstrated that the crucial point is not the severity of skin colonization with *Pityrosporum*, but rather an altered reactivity of the host towards this yeast (4, 9, 10).

In the present study we investigated whether a special reactivity of the host towards *Pityrosporum* also exists in psoriasis. Since the infiltration of the dermis with polymorphonulear leukocytes (PMNs) is one of the earliest signs of psoriasis, we decided to focus on the chemoattraction of PMNs by *Pityrosporum*.

PATIENTS AND METHODS

Patients

For the experiments with *Pityrosporum* as chemoattractant, 20 patients (11 females, 9 males, age 44.2 ± 18.5 years) suffering from chronic plaque psoriasis and 9 patients (4 females, 5 males, age 33.9 ± 15.6 years) with atopic dermatitis were included in the study. Except for the skin disorder the patients were healthy; in particular, they suffered from no current infections. The control group consisted of 17 healthy persons (11 females, 6 males, age 37.7 ± 13.4 years) from the clinical staff.

For the experiments with *Straphylococcus epidermidis* the patient group consisted of 21 psoriatic patients (5 females, 16 males, age 45.9 ± 13.4 years) and the control group of 23 healthy individuals (11 females, 12 males, age 39.9 ± 10.9 years).

Preparation of chemoattractants

The *Pityrosporum orbiculare/ovale* strain CBS 6001 (Centraalbureau for Schimmelcultures, Delft, Netherlands) was grown at 34°C under constant shaking in Sabouraud 2% glucose-broth supplemented with 0.25 g glycerol monostearate, 0.1 g yeast extract and 7.5 ml Tween 80 per 100 ml. After 24 h of incubation the yeast cells were harvested by centrifugation, washed three times and resuspended in RPMI 1640-medium (Gibco BRL, Effenstein-Leopoldhafen, Germany) to give a final inoculum of 15,000 cells per µl. For comparison of normal, psoriatic and atopic PMNs this suspension was used immediately. For comparison of the chemotactical activities of LTB_4 and *Pityrosporum*, the yeast cells were incubated at 34°C for 3 h. This suspension, its supernatant and the centrifuged yeast cells, resuspended in RPMI 1640-medium, were applied as chemoattractants. LTB_4 was dissolved in RPMI 1640-medium (1 ng/ml). Furthermore, the supernatant was extracted with diethylether, the ether evaporated and the extract resolved in RPMI 1640-medium. For acid hydrolysis 180 µl of 1 N HCl was added to 1 ml of the supernatant. After 5 min at 100°C the supernatant was neutralized by the addition of 180 µl 1 N NaOH.

Staphylococcus epidermidis was grown at 34°C under constant shaking in Müller–Hinton broth. After 24 h of incubation the bacteria were harvested by centrifugation, washed three times and resuspended in RPMI 1640-medium to give a final inoculum of 15,000 cells per µl.

Preparation of PMNs

Twenty ml of heparinized blood from patients or control persons were allowed to stand for 60 min. Six ml of the plasma were layered on the top of 8 ml Ficoll Paque (Pharmacia LKB, Uppsala, Sweden). After centrifugation at 1,500 rpm for 20 min the buffy coat was harvested and suspended in RPMI 1640-medium. The contaminating monocytes were removed by hypotonous lysis and the remaining PMNs washed twice and resuspended in RPMI 1640-medium to give a final inoculum of 5,000 cells per µl. The trypan blue exclusion test showed more than 98% of the PMNs to be viable.

Measurement of chemotaxis

Chemotaxis was measured according to the Boyden chamber method. The lower compartment of the chamber was filled with 200 µl of the chemoattractant, the upper compartment with 200 µl of PMN suspension. The two compartments were seperated from each other by a Millipore membrane (Millipore, Eschborn, Germany) with a pore size of 3 µm. After 3 h of incubation at 37°C in an atmosphere with 100% relative humidity and 5% CO_2, the PMNs which had passed the membrane and dropped into the lower part of the Boyden chamber were counted in a Neubauer chamber. The stimulation index SI was defined as the number of PMNs which had passed the membrane in the presence of a chemoattractant divided by the number of PMNs which had passed the membrane in the absense of chemoattractants.

 ISSN 0001-5555

Statistics

Statistical analysis was perfomed according to Student's *t*-test.

RESULTS

When *Pityrosporum orbiculare/ovale* was used as chemoattractant, the PMNs from psoriatics showed a significantly higher ($p<0.05$) stimulation index ($SI=58\pm50$) than did the PMNs from control persons ($SI=26\pm24$) (Table I). On the contrary, the stimulation index of PMNs from atopics did not differ significantly from the control value. With *Staphylococcus epidermidis* as chemoattractant the stimulation index of PMNs isolated from psoriatic patients was not increased compared to the stimulation index of control PMNs.

The chemoattraction of PMNs from psoriatic patients and controls by a suspension of *Pityrosporum* cells was comparable with the chemoattraction by LTB_4 at a concentration of 1 ng/ml, as shown in Table II. Strong chemotactical effects were achieved with both *Pityrosporum* supernatant and washed *Pityrosporum* cells. However, the effect of washed *Pityrosporum* cells on PMNs from psoriatics (59.4% of the LTB_4-effect) was markedly higher than on control PMNs (20.5% of the LTB_4-effect). After ether extraction the aqueous phase retained most of the chemotactical stimulus. Acid hydrolysis almost completely destroyed the chemotactical activity of the *Pityrosporum* supernatant.

Table I. *Chemoattraction of the PMNs of psoriatic and atopic patients by Pityrosporum orbiculare/ovale and Staphylococcus epidermidis*

n.s.: not significant.

Subjects	Chemoattractant	Stimulation index SI ± SD	Significance compared to controls
Controls ($n=17$)	*Pityrosporum*	26 ± 24	–
Psoriatics ($n=20$)	*Pityrosporum*	58 ± 50	$p<0.05$
Atopics ($n=9$)	*Pityrosporum*	20 ± 17	n.s.
Controls ($n=23$)	*Staph.epidermidis*	41 ± 35	–
Psoriatics ($n=21$)	*Staph.epidermidis*	38 ± 43	n.s.

Table II. *Chemoattraction of the PMNs of controls and psoriatic patients by different preparations of Pityrosporum (P) compared to leukotriene B_4 (LTB_4) (1 ng/ml)*

Chemoattractant	Controls	Psoriatics
LTB_4	100.0%	100.0%
P-suspension*	73.8%	87.7%
P-supernatant**	45.0%	43.5%
Washed P-cells***	20.5%	59.4%
P-supernatant after ether extraction	10.5%	39.8%
Ether extract of P-supernatant	2.7%	2.3%
P-supernatant after acid hydrolysis	2.4%	0.4%

**Pityrosporum orbiculare/ovale* grown for 3 h in RPMI 1640-medium;
**Supernatant after centrifugation of P-suspension;
***Pellet of P-suspension washed three times and resuspended in RPMI 1640-medium.

DISCUSSION

The results demonstrate that PMNs from psoriatic patients show a stronger chemotactical response to *Pityrosporum orbiculare/ovale* than PMNs from healthy controls. Obviously, this effect is specific for *Pityrosporum*, since *Staphylococcus epidermidis*, also a common saprophyte on human skin, did not reveal a chemotactical effect on the PMNs of psoriatic patients exceeding that of control PMNs. The mere inflammation of the skin cannot be the reason for the increased chemoattraction of psoriatic PMNs by *Pityrosporum*. Otherwise, chemotaxis would also have been increased in atopic dermatitis, another inflammatory skin disorder. This, however, was not the case, as the results of this study have shown.

In earlier investigations PMNs from psoriatic patients were rarely found to show an increased chemotaxis (11, 12.) In most cases the chemotaxis was unchanged (13, 14) or even reduced (15–18) in psoriasis. This underlines the specificity of the *Pityrosporum* effect on psoriatic PMNs reported in this study. The present results are consistent with the observation that cell fragments of *Pityrosporum* applied epicutaneously to the skin of psoriatic patients provoke new psoriatic plaques in 100% of the cases (6). Nevertheless, it has still to be proven whether other bacteria or yeasts, such as streptococci or *candida* species, which are known to trigger psoriasis, also enhance chemotaxis.

In further experiments we showed that the chemotactical factor produced by *Pityrosporum* is hydrophilic and can be degraded by acid hydrolysis, indicating its protein nature.

In conclusion, this study provides further support for the hypothesis that skin colonization with *Pityrosporum* may also play a role in the koebnerization of psoriasis.

REFERENCES

1. Faergemann J, Fredrikson T. Experimental infections in rabbits and humans with *Pityrosporum orbiculare* and *P. ovale*. J Invest Dermatol 1981; 77: 314–318.
2. Bäck O, Faergemann J, Hörnqvist R. *Pityrosporum* folliculitis: a common disease in the young and middle aged. J Am Acad Dermatol 1985; 12: 56–61.
3. Katoh T, Kagawa S, Ishimoto M. *Malassezia* intertrigo, a new clinical entity. Mycoses 1988; 31: 558–562.
4. Bergbrant I-M, Faergemann J. Seborrheic dermatitis and *Pityrosporum ovale*: a cultural and immunological study. Acta Derm Venereol (Stockh) 1989; 69: 332–335.
5. Bunse T, Mahrle G. Anthralin is a potent inhibitor of *Pityrosporum orbiculare/ovale* in vitro. Acta Derm Venereol (Stockh) 1992; 72: 72–73.
6. Lober CW, Belew PW, Rosenberg EQ, Bale G. Patch tests with killed sonicated microflora in patients with psoriasis. Arch Dermatol 1982; 118: 322–325.
7. Elewski B. Does *Pityrosporum ovale* have a role in psoriasis? Arch Dermatol 1990; 126: 1111–1112.
8. Bunse T, Mahrle G. Der Effekt von Anthralin auf die Hautbesiedelung von Psoriatikern mit *Pityrosporum*. Z Hautkr 1992; 67: 324–329.
9. Bergbrant I-M, Faergemann J. The role of *Pityrosporum ovale* in seborrheic dermatitis. Semin Dermatol 1990; 9: 262–268.
10. Broberg A, Faergemann J, Johansson S, Johansson SGO,

Strannegård I-L, Svejgaard E. *Pityrosporum ovale* and atopic dermatitis in children and young adults. Acta Derm Venereol (Stockh) 1992; 72: 187–192.

11. Langner A, Chorzelski TP, Fraczkowska G, Jablonska S, Szymanczyk J. Is chemotactic activity in polymorphonuclear leukocytes increased in psoriasis? Arch Dermatol Res 1983; 275: 226–228.
12. Wahba A, Cohen HA, Bar-Eli M, Gallily R. Enhanced chemotactic and phagocytic activities of leukocytes in psoriasis vulgaris. J Invest Dermatol 1978; 71: 186–188.
13. Green CM, Ferguson J, MacLeod TM, Millar BW, Raffle EJ. Polymorphonuclear leucocyte chemotaxis in response to leukotriene B4 in treated and untreated psoriatics. Dermatologica 1989; 178: 20–22.
14. Scheja A, Bruze M, Forsgren A. Leukocyte migration in vivo and in vitro in patients with psoriasis. Acta Derm Venereol (Stockh) 1987; 67: 427–432.
15. Guillor B, Coupe M, Alirezai M, Bureau JP, Giulhou JJ. Studies of polymorphonuclear migration into psoriatic skin using a new in vivo method. Arch Dermatol Res 1989; 280: 477–480.
16. van de Kerkhof PCM, Chang A. Migration of polymorphonuclear leukocytes in psoriasis. Skin Pharmacol 1989; 2: 138–154.
17. Isoda M. Leukocyte chemotactic activity and circulating immune complexes in psoriasis. J Dermatol 1987; 14: 576–579.
18. Lammers AM, van de Kerkhof PCM. Response of polymorphonuclear leukocytes to topical leukotriene B4 in healthy and psoriatic skin. Br J Dermatol 1987; 116: 521–524.