Inflammation, Vol. 22, No. 5, 1998

NEUTROPHIL FUNCTIONS AND IL-8 IN PSORIATIC ARTHRITIS AND IN CUTANEOUS PSORIASIS

DOMENICO BIASI,[1] ANTONIO CARLETTO,[1] PAOLA CARAMASCHI, PAOLO BELLAVITE,[2] TELLI MALEKNIA,[1] CINZIA SCAMBI,[1] NOVELLA FAVALLI,[1] and LISA MARIA BAMBARA[1]

[1]*Institute of Medical Pathology,*
[2]*Clinical Chemistry and Microscopy, University of Verona*
Ospedale Policlinico, 37134 Verona, Italy

Abstract—The aim of this study is to determine some functions of neutrophil in patients affected by psoriatic arthritis and to compare them to those of patients affected by cutaneous psoriasis and to normal controls. We used a model of experimental cutaneous inflammation allowing to separate a cluster of purified and viable PMN cells. Then we analyzed, within the three groups, the IL-8 concentration in serum and in the supernatant obtained from the inflammatory site to gather data on the possible pathogenic role played by this cytokine in psoriatic arthritis. We studied neutrophil functions in patients with cutaneous psoriasis and psoriatic arthritis, in acute phase, in comparison with healthy control subjects. We investigated in vivo neutrophil migration by Senn's skin window technique and measured adhesion assay and superoxide production in circulating and migrating neutrophils after different stimuli. We also measured IL-8 concentration in serum and in the supernatant obtained from the inflammatory site, artificially created through the skin window scrape. Neutrophil migration in vivo was significantly higher in both groups of patients than in controls. In the presence of fMLP, blood cells showed a burst of superoxide release, which was significantly more pronounced in patients when compared to healthy controls. Neutrophils from skin window scrape showed a much higher response to fMLP as compared to blood cells of all subject groups, but no differences were observed between patients and controls. No correlation was found between the three groups in adhesion ability under basal condition or in response to different stimuli by circulating and migrating neutrophils. Our results also show a great increase of IL-8 in the exudate from patients compared to controls. Our study shows that there is no difference in neutrophil functions between patients with psoriatic arthritis and cutaneous psoriasis; moreover we suggest that the source of high IL-8 levels are neutrophils rather than the keratinocytes.

0360-3997/98/1000-0533$15.00/0

INTRODUCTION

Psoriasis is a complex chronic inflammatory cutaneous disease, sometimes linked to arthritis; in its pathogenesis, vascular nonspecific alterations, activated T cells, nonspecific activation of keratinocytes activation and neutrophils (PMN) are associated (1).

Since PMN infiltration inside psoriatic plaques represents the typical histologic pattern of this disease (2), a number of studies were designed to determine the functional alterations of PMNs and their possible involvement in the history of this disease. The literature reports studies about in vivo and in vitro chemotaxis, phagocytosis, oxidative metabolism on PMNs obtained from peripheral and, in few cases, from exudate created by skin window scrape (3–13).

The comparison of the results obtained from the above studies is quite difficult because of the diversity of the applied techniques; however it seems that in vitro chemotaxis (3–5), in vivo leukocyte migration (7), as well as the production of the superoxide triggered by a certain stimuli (8–13) and phagocytosis (7) are increased in patients versus normal subjects.

More recent data (14–16) are focused on identification of the main chemotactic agent responsible for PMN accumulation, showing the role of IL-8 that seems to be produced in situ by epidermic keratinocytes and possibly by PMN themselves (17).

The linkage between cutaneous psoriasis and psoriatic arthropathy is quite complicated and its pathogenesis remains to be determined; identifying on a cellular and immunological level the developing mechanisms of the cutaneous pathology, from those concerning the articular pathology, is a main issue.

Since an activation of PMN may be a common denominator of both forms of disease or, conversely, may represent a differentiating factor of their cellular patterns, the characterization of PMN function in patients affected by cutaneous psoriasis and psoriatic arthropathy is of primary interest in this field.

Few data are available about the PMN role in the psoriatic arthropathy: they only regard in vitro chemotaxis and their results are controversial (18, 19).

Purpose of this study is to determine some functions of PMN cell in patients affected by psoriatic arthritis and to compare them to those of patients affected by cutaneous psoriasis and to normal controls. We used a model of experimental cutaneous inflammation, that we have been extensively studied for the last few years, allowing to separate a cluster of purified and viable PMN cells (20–28).

By the skin window scrape (Senn) (20) we studied the adhesion and the superoxide production caused by different stimuli in PMN cells coming from the inflammatory site and in those coming from the peripheral blood stream.

Then we analyzed, within the three groups, the IL-8 concentration in serum and in the supernatant obtained from the inflammatory site, artificially created

through the skin window scrape; our aim was to gather data on the possible pathogenic role played by this cytokine in psoriatic arthritis.

MATERIALS AND METHODS

We studied 15 patients affected by psoriatic arthritis, (9 males and 6 females, mean age 39.2 ± 10.4, range 24–58 years), 15 patients with psoriasis vulgaris, (11 males and 4 females, mean age 43.35 ± 11.3, range 26–63 years) and 22 sex-and age matched control subjects.

The diagnosis of psoriasis was performed by a dermatologist.

All the patients and controls gave their consent.

The patients were enrolled into the study during a 1-year period (1996) at the Institute of Special Medical Pathology of the University of Verona.

All patients presented in an acute phase disease and they did not receive any pretreatment. All our patients presented psoriasis vulgaris or psoriasis associated with features of an inflammatory arthritis: 3 had arthritis of the distal interphalangeal joints, 4 had spondyloarthropathy and 8 asymmetric oligoarthritis; no patient had rheumatoid factor. All patients were examined for the in vivo study on the PMN migration by the skin window scrape technique (20) and its following modifications (21). The determinations of the PMN superoxide production, of PMN adhesive ability and of IL-8 were then performed in blood samples and of skin window exudate from each subject.

Reagents. The chemotactic peptide fMLP, zymosan and phorbol myristate acetate (PMA) were purchased from Sigma Chemical Company, St. Louis; cytochrome c from Boehringer, Mannheim, Germany; purified serum bovine albumin and human albumin from Behring Institut, Marburg, Germany. Percoll was from Pharmacia, Uppsala. Sterile 96-well microtiter plates with flat-bottomed wells (Linbro type) were from Flow Laboratories. Hank's balanced salt solution (HBSS) and reagents were of the highest purity available.

Zymosan, opsonized with a pool of normal human sera (STZ), as described by Metcalf et al. (11), was stored in aliquots at −20°C. In order to avoid contamination, a possible cause of artifactual activation or priming of the cells, sterile apyrogenic solutions and disposable plasticware were used in all experiments, which were carried out, whenever possible, under laminar flow hood. Reagents were prepared using pyrogen-free water or 0.9% NaCl solutions.

Cell Preparation. PMNs were obtained from blood and from skin window exudates of subjects after having obtained their informed consent. Blood PMNs were prepared from 40 ml of ethylene diamine tetraacetate-anticoagulated blood by centrifugation over Percoll gradients (29, 30). Cells were finally suspended in HBSS containing 5 mM glucose and 0.2% human serum albumin (HGA) and kept at room temperature until use. A few minutes before use, 100× concentrated solutions of $CaCl_2$ and $MgSO_4$ were added to the cell suspensions at the final concentration of 0.5 mM, and 1 mM respectively. Exudate PMNs were isolated according to the method described by Senn (20), with modifications (21). An area of volar surface, without skin lesions, of nondominant forearm was disinfected with ether and an abrasion of 1 cm^2 was obtained with a rotating sterile abrasive cylinder operated by a milling cutter (minidrill, Saint Julien en Genevois, France). The abrasion did not cause bleeding since only the epidermis was removed; the wet and transudating surface of derma was exposed. A bell-shaped, sterile and disposable plastic skin chamber with circular adhesive base (FAR Italia, Verona, Italy) was put on the skin abrasion and fixed with a fenestrated sticking plaster. The chamber has on its top a 5 mm-wide hole equipped with a plug. One ml of autologous serum was then injected into the chamber and twenty-four hours later the exudate was collected by aspiration. The exudate cells (>95% PMN, >98% viable, as assessed by trypan-blue exclusion test) were then centrifuged at 1200 rpm, washed twice with phosphate buffered saline (PBS) and finally

suspended in HGA and kept at room temperature until use. Before use, the cell suspensions were supplemented with $CaCl_2$ and $MgSO_4$ as described above for blood cells.

Oxidative Metabolism Assay. Superoxide anion was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome c in a microplate assay system. The microplate assay for O_2^- production was performed according to previously published methods (29), with the following modifications. The wells were coated with fetal bovine serum as described (30), then were supplemented with 25 μl of 0.6 mM cytochrome c in HGA containing 0.5 mM $CaCl_2$ and 1 mM $MgSO_4$ (HGACM) and with 25 μl of the stimulants diluted in HGACM at a concentration exactly 4 times higher than that required in the assay. This was done because each agent is diluted 4 times in the final incubation mixture (as noted). The plate was then brought to 37°C, and 50 μl of the neutrophil suspensions (2×10^5 cells), prewarmed at 37°C, were added to each well using a multichannel pipette and the plates were incubated at the 37°C for the desired time. At the time indicated, the plates were rapidly transferred into a microplate reader (Reader 400 SLT Labs Instruments) and the reduction of cytochrome c was measured at 550 nm using 540 nm as reference wavelength, and using 0.037 optical density units as standard for 1 nmole of reduced cytochrome c (29).

Adhesion Assay. For adhesion measurements, after the assay of cytochrome c reduction, the plates were transferred to an automatic washer (Easy Washer 2 SLT Labs Instruments) and subjected to two washing cycles with PBS at room temperature. Adherent cells were quantified by measuring the membrane enzyme acid phosphatase and the percentage of adhesion was calculated on the basis of a standard curve obtained with known numbers of PMNs (29).

IL-8 Assay. The IL-8 concentration in the serum and in the supernatant obtained from the skin window scrape of the subjects was determined using a double-ligand ELISA method as suggested by Bazzoni et al. (31).

Samples and recombinant IL-8 standards (0.02–10 ng/ml) were incubated for 2h at 37°C in microtiter plates coated with a mouse anti-IL-8 monoclonal antibody. After washing, a goat anti-IL-8 monoclonal antibody conjugated to alkaline phosphatase was added, and finally, the activity was determined with *p*-nitrophenylphosphate.

Statistics. Statistical analysis was carried out using the nonparametric Wilcoxon test.

RESULTS

Figure 1 shows the data concerning the PMN migration in controls and in patients. The migration was $61.8 \pm 12.4 \times 10^6$ PMN/cm^2/24 h in healthy subjects, $84.8 \pm 9.1 \times 10^6$ PMN/cm^2/ 24 h in patients suffering from psoriasis vulgaris ($P < 0.001$) and $84.7 \pm 20.1 \times 10^6$ PMN/cm^2/24 h in patients with psoriatic arthritis ($P < 0.001$).

Table 1 reports data on superoxide production by circulating and migrating PMNs, under basal conditions and after specific stimuli (fMLP, STZ, or PMA). Unstimulated production was very low in all groups and in both blood and skin window neutrophils. In the presence of fMLP, blood cells showed a burst of superoxide release, which was significantly more pronounced in patients as compared to healthy controls. Skin window showed a much higher response to fMLP as compared to blood cells of all subject groups, but no differences were observed between patients and controls.

Priming of superoxide production in skin window cells versus blood cells and priming in blood of patients versus controls were stimulus (fMLP) spe-

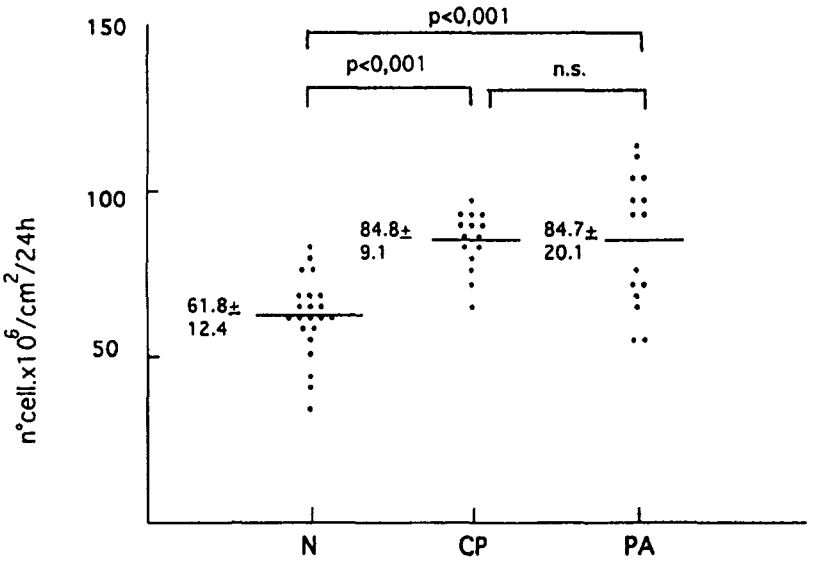

Fig. 1. PMN migration in 22 normal subjects (N), in 15 patients affected by Cutaneous Psoriasis (CP) and in 15 patients with Psoriatic Arthritis (PA).

Table 1. Superoxide Production, Under Basal Condition and in Response to Different Stimuli, by PMN from Blood (B) and from Skin Window Exudate (SW) in Healthy Control Subjects (N = 22) and in Patients Suffering from Cutaneous Psoriasis (N = 15) and Psoriatic Arthritis (N = 15).

Stimulant	Controls	Cutaneous Psoriasis	Psoriatic Arthritis
None			
B	0.3 ± 0.3	0.4 ± 0.3	0.5 ± 0.4
SW	0.7 ± 0.6	0.7 ± 0.6	0.9 ± 0.8
fMLP 10^{-7} M			
B	6.7 ± 3.5	10.2 ± 3.1[a]	10.3 ± 4.5[a]
SW	18.1 ± 4.7	18.9 ± 1.7	17.7 ± 5.7
STZ 0.1 mg/ml			
B	7.5 ± 2.5	8.6 ± 3.7	7.9 ± 2.9
SW	7.7 ± 2.3	8.6 ± 3.9	8 ± 3
PMA 10 ng/ml			
B	22.2 ± 5.4	23.1 ± 3.4	23.9 ± 4.3
SW	23.2 ± 3.5	23.4 ± 3.5	24.5 ± 5.3

Values are nmoles $O_2^-/10^6$ PMN ± standard deviation. Incubations of resting cells, fMLP stimulated and PMA stimulated cells were carried out for 10 min; incubations of STZ stimulated cells were carried out for 40 min. [a] $P < 0.001$.

Table 2. Adhesion of PMN from Blood (B) and from Skin Window Exudate (SW) Under Basal Condition and After Different Stimuli in Control Subjects ($N = 22$) and in Patients Suffering from Cutaneous Psoriasis ($N = 15$) and Psoriatic Arthritis ($N = 15$). Values are Percentages of Adherent PMN ± SD. Incubations in All Conditions Were Carried Out for 40 Minutes.

Stimulant	Controls	Cutaneous Psoriasis	Psoriatic Arthritis
None			
B	7.6 ± 2.6	7.9 ± 2.4	7.7 ± 1.9
SW	15 ± 5.9	14.9 ± 2.2	15.4 ± 6.5
fMLP 10^{-7} M			
B	23.4 ± 4.8	24.3 ± 3.3	24.7 ± 5.3
SW	29.6 ± 7.7	29.2 ± 4.7	31.7 ± 7.8
STZ 0.1 mg/ml			
B	22.6 ± 9.6	23.7 ± 3.6	24.2 ± 8.6
SW	27.4 ± 5.9	28.3 ± 3.9	29 ± 6.3
PMA 10 ng/ml			
B	54.9 ± 9.8	52.3 ± 3.8	51.5 ± 5.2
SW	52.1 ± 6.9	52.3 ± 3.9	50.9 ± 5.3

cific, because these differences were not apparent when two different stimulants, namely STZ and PMA, were employed.

Table 2 shows the results for adhesion of circulating and migrating PMNs under basal condition and after stimuli. At variance with the results of oxidative metabolism, adhesion of PMN was observed even in the absence of stimulants and this phenomenon was increased in skin window cells as compared to blood cells. In the presence of stimulants, adhesion increased in all conditions. A slight difference of adhesion in favor of skin window cells was detected, but it was largely accounted for by the difference present in unstimulated cells. There was no difference between normal subjects and patients.

Our results also show a great increase of IL-8 in the exudate from patients (both groups, with psoriatic arthritis and with cutaneous psoriasis) compared to controls: the average was 1.85 ± 0.52 ng/ml in patients with psoriatic arthritis, 1.94 ± 0.65 ng/ml in patients with cutaneous psoriasis and 0.65 ± 0.32 ng/ml in controls (Figure 2).

The relationship between the IL-8 in the supernatant obtained from the skin window scrape and the amount of migrated cells was 23 ± 0.06 pg/10^6 cells in patients with psoriatic arthritis, 24 ± 0.08 pg/10^6 cells in patients with cutaneous psoriasis and 11 ± 0.07 pg/10^6 cells in controls. Therefore, migrating PMN in patients produce twofold as much IL-8 as compared to healthy controls ($P < 0.0005$).

The IL-8 concentrations in the peripheral blood were undetectable in all subjects.

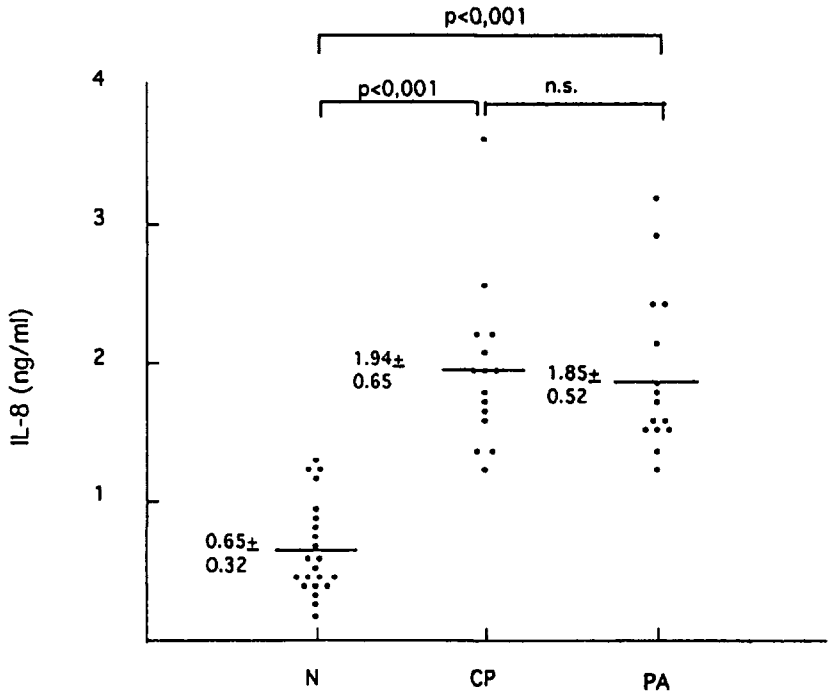

Fig. 2. IL-8 concentration in supernatant from skin window exudate in 22 normal subjects (N), in 15 patients affected by Cutaneous Psoriasis (CP) and in 15 patients with Psoriatic Arthritis (PA).

DISCUSSION

Many data are available on the PMNs' functions in patients affected by psoriasis, since accumulation of PMNs in the corneum is a typical histologic pattern of psoriasis. There are controversial data as to the in vitro increase of PMN chemotaxis (3–5) that would be normalized by therapy (9); a few authors reported an alteration of the respiratory burst in psoriasis, others demonstrated those alterations only in patients affected by pustular psoriasis (9).

There are few studies on the psoriatic arthritis whose pathogenesis itself is still unknown; these data are controversial and they took only in consideration in vitro chemotaxis; Leibovici et al. (19) maintained that the chemotaxis in psoriatic arthritis would be comparable to that of the normal subjects; on the other hand Ternowitz (18) reported an alterated chemotaxis, though not a different one, from that determined in patients affected by psoriasis without arthritis.

To our knowledge, Senn's skin window scrape has never been used in patients with psoriatic arthritis; according to our previous experiences (21–28) it

is a quite useful technique, since it allows evaluation in vivo chemotaxis under highly reproducible conditions and analysis of both cellular and humoral factors.

Our study was aimed to evaluate in vivo migration, superoxide production and adhesion of PMN coming from the blood stream and from a skin window scrape of patients with active phase psoriasis, with active phase psoriatic arthritis comparing to normal subjects.

We found a significant increase of the in vivo PMNs migration in both patients with psoriasis or psoriatic arthritis, compared to controls, and a significant increase of superoxide production from the circulating PMN, in both patients with psoriasis or psoriatic arthritis.

We also confirmed, as previous studies showed, that the PMN coming from an exudate are primed, being more responsive to fMLP (7, 21, 25–27, 32) compared to the circulating PMNs, and showing increase of the adhesive ability without stimuli (23).

The first issue to be discussed is the like results in patients by psoriatic arthritis and cutaneous psoriasis; we state that psoriatic does not seem to have any difference as to PMNs function compared to cutaneous psoriasis; in this report we do not want to deal with its controversial pathogenesis. At to the increased responsiveness to fMLP from circulating PMNs there is a curious analogy with what we found in patients affected by ankylosing spondylitis (26). We recall that has been hypothesized that long lasting bacterial products in patients with spondylitis could cause a priming on the circulating PMNs with a consequent externalization of receptor to fMLP (33, 34). Therefore we could think about a similar situation in psoriatic patients, that may be in keeping with the frequent staphylococci and streptococci infections of their cutaneous lesions (35). Our data also show that the priming effect observed in superoxide production in blood of patients as compared with normal subjects is agonist-specific, being restricted to fMLP as stimulatory agent. This may be due to the complex nature of the receptor dynamics and stimulatory pathways involved in NADPH oxidase activation, as we have already discussed in previous reports (21, 22).

Recent studies are consistent with the theory stating that increased chemotactic activity and PMN hyperaccumulation would be the consequence of an IL-8 increase in the psoriatic lesion (14–17). Our data, showing a significant increase of the IL-8 levels in the supernatant from the skin window scrape in psoriatic patients are in agreement with this view. Since serum levels do not differ in patients and controls, the increased in the skin window during psoriasis appears to be due to local factors which are probably linked to the abnormal skin responses. Our experimental setting does not permit us to establish whether the source of increased IL-8 are keratinocytes, PMN, or both. However, considering that the skin window scrape was performed on a normal skin patch and that it implies a dermal exposure (removal of most keratinocytes), we think it is correct to hypothesize that the source of high IL-8 levels could be the PMN rather

than the keratinocytes. This agrees with the finding of primed PMNs in the exudate and with the increased levels of IL-8, divided by number of cells, in both psoriatic groups, suggesting that IL-8 increase is not only due to an increased chemotaxis.

The final scenario would show a primed PMN that, once reaching the inflammatory site, secretes a high amount of IL-8 attracting other PMNs.

Acknowledgments—This work was supported by a grant from: "Progetto Sanità" 1996–1997, Fondazione Cassa di Risparmio di Verona, Vicenza, Belluno ed Ancona and by a grant from Ministero Ricerca Scientifica e Tecnologica (40%). Fabio Poli is acknowledged for technical assistance.

REFERENCES

1. CHRISTOPHERS, E., and G. KRUEGER. Psoriasis. *In* FITZPATRICK, T. B., EISEN, A. C., WOLFF, K., FREEDBERG, E. M., AUSTEN, K. F. (editors), 1986. Dermatology in general medicine. McGraw-Hill Book Company, New York, 461–491.
2. BRAUN-FALCO, O. and E. CHRISTOPHERS. 1974. Structural aspects of initial psoriasis lesions. *Arch. Derm. Forsch.* **251**:95–110.
3. WAHBA, A., H. COHEN, M. BAR-ELI, and R. GALLILY. 1978. Neutrophil chemotaxis in psoriasis. *Acta Derm. Venereol.* **71**:186–188.
4. MICHAELSSON, G. 1980. Increased chemotactic activity of neutrophil leukocytes in psoriasis. *Br. J. Dermatol.* **103**:351–356.
5. FRAKI, J., L. JAKOI, A. DAVIES, R. LEFKOWITZ, R. SNYDERMAN, and G. LAZARUS. 1983. Polymorphonuclear leukocyte chemotaxis, β-adrenergic receptors and proteolytic enzymes of PMN leukocytes in the peripheral blood from psoriatic patients. *J. Invest. Derm.* **81**:254–257.
6. TERNOWITZ, T., and K. THESTRUP-PEDERSEN. 1985. Neutrophil and monocyte chemotaxis in pustolosis palmo-plantaris and pustular psoriasis. *Br. J. Dermatol.* **113**:507–513.
7. COBLE, B. I., G. BRIHEIM, C. DAHLGREN, and L. MOLIN. 1988. Function of exudate neutrophils from skin in psoriasis. *Int. Archs. Allergy Appl. Immun.* **85**:398–403.
8. LINDGREN, S., and O. STENDAHL. 1976. Phagocytic activity of polymorphonuclear leukocytes in generalized pustular psoriasis. *Acta Derm. Venereol.* **56**:229–233.
9. COBLE, B. I., C. DAHLGREN, L. MOLIN, and O. STENDAHL. 1987. Neutrophil function in psoriasis: Effects of retinoids. *Acta Derm. Venereol.* **67**:481–490.
10. LUNDIN, A., L. HAKANSSON, R. HALLGREN, G. MICHAELSSON, and P. VENGE. 1983. Studies on the phagocytic activity of the granulocytes in psoriasis and palmoplantar pustolosis. *Br. J. Dermatol.* **109**:539–547.
11. SEDGWICK, J., P. BERGSTRESSER, and E. HURD. 1981. Increased superoxide generation by normal granulocytes incubated in sera from patients with psoriasis. *J. Invest. Dermatol.* **76**:158–163.
12. SCHOPF, R., and E. STRAUSSFELD. 1985. Stimulus dependent increased generation of oxygen intermediates in monocytes and polymorphonuclear leukocytes in psoriasis. *J. Invest. Dermatol.* **84**:73–76.
13. GEERDINK, J., P. TROOST, J. SCHALKWIYK, L. JOOSTEN, and P. MIER. 1985. The "metabolic burst" in polymorphonuclear leukocytes from patients with quiescent psoriasis. *Br. J. Dermatol.* **112**:387–392.

14. TAKEMATSU, H., and H. TAGAMI. 1993. Quantification of chemotactic peptides (C5a anaphylatoxin and IL-8) in psoriatic lesional skin. *Arch. Dermatol.* **129**:74–80.
15. SCHRÖDER, J. M., H. GREGORY, J. YOUNG, and E. CHRISTOPHERS. 1992. Neutrophil-activating proteins in psoriasis. *J. Invest. Dermatol.* **98**:241–247.
16. ANTTILA, H. S. I., S. REITAMO, P. ERKKO, M. CESKA, B. MOSER, and M. BAGGIOLINI. 1992. Interleukin-8 immunoreactive in the skin of healthy subjects and patients with palmoplantar pustolosis and psoriasis. *J. Invest. Dermatol.* **98**:96–101.
17. GILLITZER, R., U. RITTER, U. SPANDAU, M. GOEBELER, and E. B. BRÖCKER. 1996. Differential expression of GRO-α and IL-8 mRNA in psoriasis: A model for neutrophil migration and accumulation in vivo. *J. Invest. Dermatol.* **107**:778–782.
18. TERNOWITZ, T. 1986. Monocyte and neutrophil chemotaxis in psoriasis. Relation to the clinical status and the type of psoriasis. *J. Am. Acad. Dermatol.* **15**:1191–1199.
19. LEIBOVICI, V., M. STATTER, L. WEINRAUCH, E. TZFONI, and Y. MATZNER. 1990. Effect of zinc therapy on neutrophil chemotaxis in psoriasis. *Isr. J. Med. Sci.* **26**:306–309.
20. SENN, H. J., and W. F. JUNGI. 1975. Neutrophil migration in health and disease. *Semin. Hematol.* **12**:27–45.
21. BIASI, D., L. M. BAMBARA, A. CARLETTO, M. CARAFFI, M. C. SERRA, S. CHIRUMBOLO, and P. BELLAVITE. 1993. Factor-specific changes in oxidative burst response of human neutrophils in skin window exudates. *Inflammation* **17**:13–23.
22. BIASI, D., L. M. BAMBARA, A. CARLETTO, P. CARAMASCHI, M. C. SERRA, C. SANTONASTASO, P. GUZZO, and P. BELLAVITE. 1993. Molecular mechanisms of priming: exudate human neutrophils are primed to fMET-LEU-PHE but have normal intracellular calcium and cAMP responses. *Bulletin of Molecular Biology and Medicine* **18**:225–235.
23. BELLAVITE, P., A. CARLETTO, D. BIASI, P. CARAMASCHI, F. POLI, F. SUTTORA, and L. M. BAMBARA. 1994. Studies of skin window exudate human neutrophils. Complex patterns of adherence to serum-coated surfaces in dependence on fMLP doses. *Inflammation* **18**:575–587.
24. BELLAVITE, P., P. GUARINI, D. BIASI, A. CARLETTO, M. T. TREVISAN, P. CARAMASCHI, L. M. BAMBARA, and R. CORROCHER. 1995. Correlations between the intensity of fMLP-dependent respiratory burst and cellular fatty acid composition in human neutrophils. *Br. J. Haematol.* **89**:271–276.
25. BIASI, D., A. CARLETTO, C. DELL'AGNOLA, P. CARAMASCHI, F. MONTESANTI, G. ZAVATERI, S. ZEMINIAN, BELLAVITE, P., and BAMBARA, L. M. 1996. Neutrophil migration, oxidative metabolism and adhesion in elderly and young subjects. *Inflammation* **20**:673–681.
26. BIASI, D., A. CARLETTO, P. CARAMASCHI, P. BELLAVITE, G. ANDRIOLI, M. CARAFFI, M. L. PACOR, and L. M. BAMBARA. 1995. Neutrophil functions, seronegative spondylarthropathies and HLA B27: study on 43 patients. *Clin. Exp. Rheumatol.* **13**:623–627.
27. CARLETTO, A., D. BIASI, L. M. BAMBARA, P. CARAMASCHI, M. L. BONAZZI, P. LUSSIGNOLI, G. ANDRIOLI, and P. BELLAVITE. 1997. Studies of skin-window exudate human neutrophils: increased resistance to pentoxifylline of the respiratory burst in primed cells. *Inflammation* **21**:191–203.
28. CARLETTO, A., P. BELLAVITE, P. GUARINI, D. BIASI, S. CHIRUMBOLO, P. CARAMASCHI, L. M. BAMBARA, and R. CORROCHER. 1996. Changes of fatty acid composition and oxidative metabolism of human neutrophils migrated into the inflammatory exudate. *Inflammation* **20**:123–137.
29. BELLAVITE, P., S. CHIRUMBOLO, C. MANSOLDO, G. GANDINI, and P. DRI. A simultaneous assay for oxidative metabolism and adhesion of human neutrophils. Evidence for correlations and dissociation of the two responses. *J. Leukocyte Biol.* **51**:329–333.
30. METCALF, J. A., J. I. GALLIN, W. M. NAUSEEF, and R. K. ROOT. 1986. Laboratory manual of neutrophil function. Raven Press, New York.
31. BAZZONI, F., M. A. CASSATELLA, F. ROSSI, M. CESKA, B. DEWALD, and M. BAGGIOLINI.

1991. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide-1/inteleukin-8. *J. Exp. Med.* **173:**771–774.

32. BRIHEIM, G., O. STENDHAL, B. I. COBLE, and C. DAHLGREN. 1988. Exudate polymorphonuclear leukocytes isolated from chambers are primed for enhanced response to subsequent stimulation with the chemoattractant fMet-Leu-Phe and C3 opsonized yeast particles. *Inflammation* **12:**141–152.
33. KEAT, A. C. S., B. THOMAS, J. J. DIXEY, M. OSBORN, C. SONNEX, and D. TAYLOR-ROBINSON. 1987. Chlamydia trachomatis and reactive arthritis: the missing link. *Lancet* **i:**72–74.
34. GRANFORS, S. K., and A. TOIVANEN. 1986. IgA-anti-yersinia antibodies in yersinia triggered reactive arthritis. *Ann. Rheum. Dis.* **45:**561–565.
35. LEUNG, D. Y. M., P. WALSH, R. GIORNO, and D. A. NORRIS. 1993. A potential role for superantigens in the phatogenesis of psoriasis. *J. Invest. Dermatol.* **100:**225–228.