Acta Derm Venereol (Stockh) 1986; 66: 93–97

Presence of Chemotactic Peptides Other Than C5a Anaphylatoxin in Scales of Psoriasis and Sterile Pustular Dermatoses

HIDEAKI TAKEMATSU,[1] TADASHI TERUI,[1] KYOKO OHKOHCHI,[1] HACHIRO TAGAMI,[1] RYUJI SUZUKI[2] and KATSUO KUMAGAI[2]

[1]*Department of Dermatology, Tohoku University School of Medicine and* [2]*Department of Microbiology, Tohoku University School of Dentistry, Sendai, Japan*

Takematsu H, Terui T, Ohkohchi K, Tagami H, Suzuki R, Kumagai K. Possible presence of chemotactic peptides other than C5a anaphylatoxin in scales of psoriasis and sterile pustular dermatosis. Acta Derm Venereol (Stockh) 1986: 66: 93–97.

A unique leukocyte chemotactic factor of around 12 kD molecular weight has been suggested to be responsible for the mechanisms involved in transepidermal migration of leukocytes observed in psoriasis and related sterile pustular dermatoses. Although we confirmed with radioimmunoassay the presence of a C5-cleavage product in the chemotactic fractions from psoriatic scale extract eluted by gel filtration HPLC, the neutrophil chemotactic activity demonstrated in the peak fraction was only partially inhibited by rabbit antiserum to human C5a, whereas that noted in the peak fraction, prepared from zymosan activated serum, was totally abrogated by the same treatment. Similarly the chemotactic fraction prepared from the scale extracts of other related pustular dermatoses were only partially inhibited with anti-C5a antiserum. These findings suggest that chemotactic peptides other than C5a also play a role in the transepidermal migration of leukocytes in these dermatoses. *Key words: Chemotactic factor.* (Received August 14, 1985.)

Hachiro Tagami, Department of Dermatology, Tohoku University School of Medicine, 1-1 Seiryomachi, Sendai 980, Japan.

The formation of sterile subcorneal pustules characterizes certain dermatoses such as psoriasis, pustular psoriasis, pustulosis palmaris et plantaris (PPP), and eosinophilic pustular folliculitis (EPF). We have demonstrated the presence of a unique leukocyte chemotactic factor with molecular weight (MW) of around 12 KD in psoriatic scale extracts, for which we coined a term psoriatic leukotactic factor (PLF). PLF consists of chemotactic peptides, whose activity is totally abolished by trypsin digestion, being eluted in the region of cytochrome c marker by gel filtration. It is thought to contain a C5-cleavage product based on partial reduction of chemotactic activity by treatment with anti-C5 antiserum (2). A similar chemotactic factor can be found in the scale extracts from PPP (3), SPD (4), and EPF (5). By radioimmunoassay we have recently confirmed the presence of chemotactic C5a anaphylatoxin together with non-chemotactic C3a and C4a anaphylatoxins in the scale extracts from these dermatoses (6). However, we have not elucidated yet whether the chemotactic activity of PLF is solely due to C5 cleavage products or whether other chemotactic peptides with similar molecular weight, e.g. interleukin 1 (IL 1) or epidermal cell-derived thymocyte activating factor (ETAF) (7, 8), are also responsible for its chemotactic activity. Since anti-C5a antiserum has become available for us recently, the present study was designed to determine whether the chemotactic activity of PLF can be suppressed by the action of the anti-C5a antiserum in the same way as that noted with zymosan-activated serum, whose chemotactic activity is almost solely due to that of C5a.

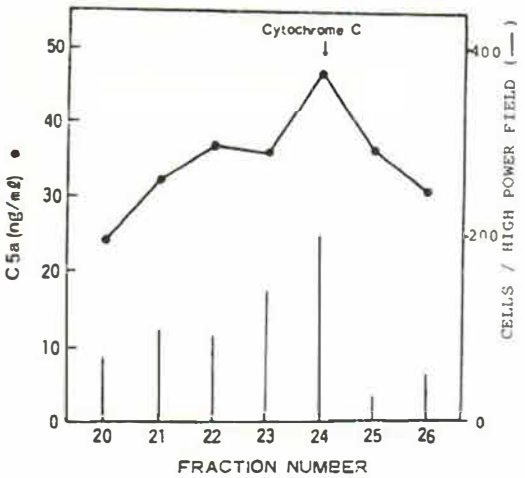

Fig. 1. Gel filtration HPLC elution profile of psoriatic scale extract near the molecular marker of cytochrome c. Each fraction was tested for C5a level with radioimmunoassay (closed circle) and for neutrophil chemotactic activity (bar).

MATERIALS AND METHODS

Extracts of stratum corneum

Water-soluble components were extracted in phosphate-buffered saline (PBS; pH, 7.4) containing streptomycin (100 μg/ml) from stratum corneum samples collected from four patients with psoriasis vulgaris, two with pustular psoriasis, two with PPP, two with SPD and one with EPF with palmoplantar pustular lesions as described earlier (1).

Preparation of zymosan-activated serum (ZAS)

To make complement-activated serum, fresh normal human serum was mixed with zymosan (Sigma Chemical Co., St. Louis, Missouri) as described by Repo (9). After incubation of the mixture for 30 min at 37°C, zymosan particles were removed by centrifugation.

Gel filtration HPLC

The chemotactic fractions of the scale extracts and ZAS were separated by gel filtration-high performance liquid chromatography (HPLC) (LKB, Sweden) TSK-gel G20005W column 60 cm × 7 mm eluted with PBS at 1 ml/min. A half ml of each sample was applied to HPLC column and fraction of 0.5 ml were collected. These were tested for neutrophil chemotaxis assay and radioimmunoassay for C5a.

Neutrophil chemotactic assay

Guinea pig neutrophil chemotaxis was measured in a Blind Well Chamber (Labo Science, Tokyo, Japan) with a 3-μm Uni-Pore polycarbonate membrane (Bio-Rad Laboratories, Richmond, California) as reported elsewhere (5).

Radioimmunoassay for C5a

In radiommunoassay for human C5a and C5a des Arg, referred to here collectively as C5a, fractions eluted around the cytochrome c marker were measured as reported previously (6).

Antibody blocking experiments

Antibody blocking experiments were performed as previously performed by us (2). Briefly, 40 μl of rabbit anti-C5a antiserum (a gift of Dr T. N. Hugli, La Jolla, California) or nonimmune rabbit serum (Miles Scientific, Napeville, Illinois) were incubated at 37°C for 30 min with 1 ml of the peak chemotactic column fraction eluted near the cytochrome c marker. The antibody-treated fractions and nonimmune rabbit serum-treated control fractions were then assayed for neutrophil chemotactic activity.

IL 1 assay

IL 1 activity was assayed by measuring the mitogenic activity for the thymocyte population nonagglutinated by peanut agglutinin (PNA^- thymocyte) prepared from the thymocytes of male Balb/c mice

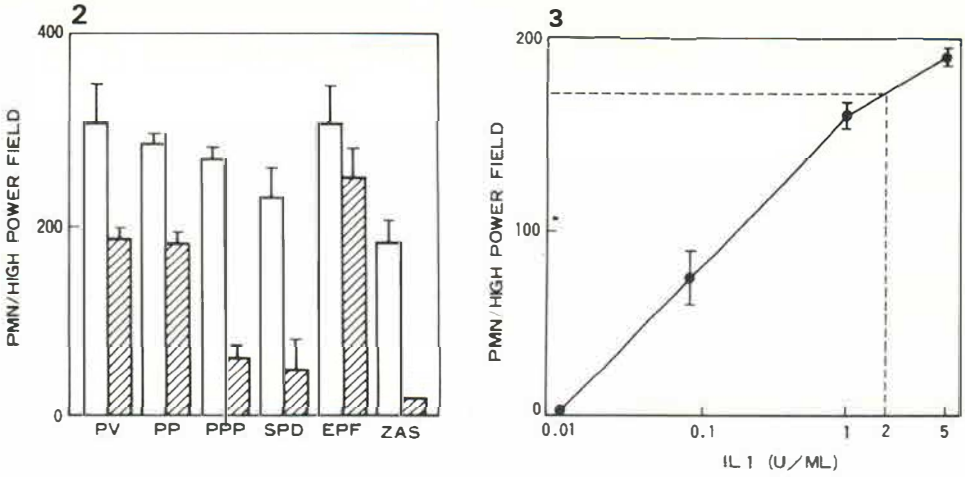

Fig. 2. Effect of rabbit anti-C5a antiserum on the chemotactic activity of the fraction eluted with the cytochrome c marker. Chemotactic activity of the fraction with the addition of anti-C5a antiserum (40 μl/ml) is shown by hatched bar and that with the addition of normal rabbit serum (40 μl/ml) by open bar. PV = psoriasis vulgaris, PP = pustular psoriasis, PPP = pustulosis palmaris et plantaris, SPD = subcorneal pustular dermatosis, EPF = eosinophilic pustular folliculitis, ZAS = zymosan-activated serum. Results are expressed as mean ± standard deviation of three experiments.

Fig. 3. Relationship between IL 1 level and neutrophil chemotactic activity. The dashed line shows the level of IL 1-like activity detected in the chemotactic fraction of psoriatic scale extract.

described by Conlon et al. (11) with a minor modification (12). Briefly, PNA^- thymocytes at 2×10^5 cells/well in 0.2 ml RPMI 1640 medium supplemented with fetal calf serum (FCS), 2-mercaptoethanol, L-glutamine and antibiotics were cultured at 37°C in 5% CO_2 with log_2-diluted samples (0.1 ml) in a 96 well microassay plate. After culturing for 48 h, the cells were pulsed with 0.5 μCi ^3H thymidine (TdR) for 16 h and harvested onto fiber. The radioactivity incorporated into the cells was counted by a liquid scintillation counter. Results are expressed as the mean and standard deviation of specific ^3H-TdR incorporation (cpm). The IL 1 activity is quantitated by probit analysis. The units of IL 1 are expressed in units (U) by comparing experimental probit data with that obtained with the use of a laboratory standard purified from the culture supernatants of a murine macrophage cell line $P388D_1$. One U of a standard IL 1 preparation is the concentration to stimulate 50% of the maximum ^3H-TdR incorporation uptake by PNA^- thymocytes, at a final dilution of ½ assays. A minimum concentration of IL 1 as low as 0.05 U/ml can be significantly assessed by probit analysis.

Source of IL 1 and its partial purification

Partially purified human IL 1 was obtained as described by Kasahara et al. (10). In brief human peripheral blood mononuclear cells adhered on to plastic dishes were plated in 1% FCS-RPMI 1640 medium at a concentration of 5×10^6 cells/ml. The adherent cells were stimulated with 100 μg/ml of silica (Sigma Chemical Co., St. Louis, Missouri) at 37°C for 48 h. The culture supernatants were concentrated at ×100 with Amicon Diaflo Ultrafiltration Membrane System (YM10, Danvers, Massachusetts), and were dialysed against 0.15 M PBS, pH 7.4, containing 0.05% polyethylene glycol (PEG 6000, Sigma Chemical Co). This was applied on Ultrogel AcA54 column (2.5×90 cm) and was eluted with PBS. Active IL 1 fraction with peak MW of 14 kD was used as partially purified IL 1.

RESULTS

Presence of C5a in chemotactic fractions of psoriatic scale extract

Gel filtration HPLC revealed a peak of C5a eluted in the region of cytochrome c marker. However, the detectable C5a level did not closely parallel that of corresponding neutrophil chemotactic activity (Fig. 1).

Inhibition of neutrophil chemotactic activity by anti-C5a antiserum

As shown in Fig. 2, rabbit anti-C5a antiserum reduced the activity of the peak chemotactic fraction of ZAS eluted in the region of the cytochrome c marker nearly to a baseline level. By contrast, the treatment with anti-C5a antiserum produced 47, 37, 78, 79, and 19% reduction in chemotactic activity detected in similar fractions of the scale extracts from psoriasis vulgaris, pustular psoriasis, PPP, SPD, and EPF, respectively.

IL 1 activity

The ability of partially purified IL 1 to elicit PMN migration is expressed in Fig. 3. A dose-response curve was established for IL 1 in the range between 0.01 and 5 U/ml. The maximal chemotactic fraction of the scale extract from psoriasis vulgaris that eluted near the cytochrome c marker showed an IL 1 activity of 2 U/ml, which is expressed as a dashed line in Fig. 3.

DISCUSSION

By C5a radioimmunoassay we can detect peptides which carry the same antigenic portion as C5a des Arg irrespective of its biological function. The present investigation demonstrates the presence of C5a in PLF fraction using radioimmunoassay. However, there is some discrepancy between the C5a level and corresponding neutrophil chemotactic activity. Furthermore, with anti-C5a antiserum we attained only a limited inhibition of the chemotactic activity in the fraction containing PLF in contrast to complete abolition of the peak chemotactic activity detectable in the gel filtration fraction of ZAS. Hence we suspect the presence of coexisting chemotactic peptides with similar molecular weight other than C5a in PLF.

IL 1 is a macrophage-produced peptide that exerts a number of immunostimulatory and inflammatory effects. Epidermal keratinocytes have also been shown to produce ETAF, which has many similarities to IL 1, sharing similar molecular weight around 15 kD. Since they also have molecular weight similar to that of PLF and are chemotactic for neutrophils (7, 8), it is reasonable to presume their implication in the transepidermal leukocyte migration observed in psoriasis. Recently the presence of a chemotaxin biochemically resembling IL 1 (13) or chemokinetic polypeptides distinct from C5a (14) has been reported in psoriatic scales. In the present study we demonstrated IL 1 activity of 2 U/ml in the PLF fraction from the scale extract of psoriasis vulgaris. However, our recent mass screening for IL 1 activity in various crude horny tissue samples revealed that the IL 1 activity was not always detectable in scale extracts prepared from psoriasis and related pustular dermatoses, being much lower than that of control horny tissue extracts obtained from non-inflammatory skin, where transepidermal leukocyte migration does not occur (15). Therefore, although IL 1 activity is demonstrable in PLF, it is quite unlikely that IL 1 or ETAF constitutes a major chemotactic component in PLF. There are probably other undefined potent chemotactic peptides with molecular weight similar to that of C5a in PLF.

ACKNOWLEDGEMENT

The authors thank Mrs C. Kato and Y. Ito for excellent technical assistance. The present study was supported in part by Grant-in-aid for Scientific Research Nos 59480244 and 59770708 and by Grant-in-aid for Cancer Research No 60010007 from the Ministry of Education, Science and Culture of Japan.

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