Demonstration of neutrophil chemotactic anaphylatoxins in human dandruff

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Summary. In contrast to scales collected from the scalps of nine healthy individuals where a few parakeratotic cells are observable, a large number of parakeratotic cells associated with some infiltrated polymorphonuclear leukocytes (PMNLs) were found in the scales obtained from 11 individuals complaining of dandruff. Therefore, we determined the neutrophil chemotactic properties of the water-soluble extracts of dandruff scales and normal control scalp scales. Aqueous extracts fractionated by Sephadex G-75 showed a potent chemotactic activity only in the fractious of the dandruff patients that eluted with cytochrome C marker (cyt C; molecular weight, 12 kDa). It was comparatively stable to heat but was greatly inhibited by the addition of anti-C5 antiserum. Radioimmunoassay demonstrated that, although small amounts of C5a and C4a anaphylatoxins were demonstratable even in the extracts of normal scalp, they were found in significantly increased amounts in the extracts of dandruff. Moreover, there was a significantly positive correlation between C5a and C4a concentrations in these extracts. These results suggest that classical complement pathway activation with resultant production of C5a anaphylatoxin is involved in the migration of PMNLs into the lesional skin of dandruff.

Key words: Dandruff — Anaphylatoxins — C5a — Chemotaxis

Kligman [8] defined dandruff as excessive, clinically noninflammatory scaling of scalp. Its clinical features are, however, sometimes indistinguishable from those of mild cases of seborrheic dermatitis or psoriasis [12]. Moreover, histologically, infiltrating leukocytes were often seen in the stratum corneum (SC) in addition to a large number of parakeratotic cells [8]. These histologic features are also characteristically found in such inflammatory skin diseases as seborrheic dermatitis and psoriasis [10]. Therefore, we can speculate that dandruff is a mild form of these dermatoses, and that the infiltration of leukocytes may be related to the excessive scaling commonly found in these dermatoses.

Transepidermal migration of leukocytes is thought to be essential to psoriasis [5]. Tagami et al. [15] reported the presence of potently chemotactic peptides with a molecular weight around 12 kDa in the psoriatic scales. Recent studies have disclosed the presence of several peptide chemotactic factors which include C5 fragments [4, 11], IL-1 like substances [4], and anionic neutrophil-activating peptide (ANAP) [11]. In order to determine whether, like in psoriasis, C5 fragments are also demonstrable in dandruff, we have attempted to characterize the chemotactic activity in water-soluble extracts of dandruff scales by gel filtration. After confirmation of the presence of chemotactic C5 complement cleavage products, we have further assessed concentrations of complement components C5a and C4a in the scale extracts by radioimmunoassay (RIA).

Materials and methods

Inclusion criteria

Since there is no generally accepted criteria for clinical diagnosis of dandruff, we at first selected nonpsoriatic individuals with subjective complaints of dandruff. We further chose those whose complaints were objectively substantiated by quantitative measurement of dandruff scales. The quantitative measurement of dandruff scales was conducted by brushing the scalp for 75 s using a special device equipped with a brush and a suction apparatus. The amount of scales collected by this method on 1 day after shampooing of the head was reproducible in each subject. In this study, we selected as dandruff subjects those with an amount of scalp scales above 10 mg. The amount of scales was less than 3 mg in normal control subjects.
Subjects
Scales of the scalp were collected by brushing of the whole scalp from 11 male volunteers without any subjective or objective signs of dandruff. Their ages were between 24 and 39 years. They were permitted use only of a shampoo which contained no known antidandruff agents, but were prohibited use of other cosmetic products.

Histologic study
After delipidization with acetone, each individual’s scales of the scalp were fixed in formalin and embedded in paraffin. The prepared sections were stained with hematoxylin and eosin (H&E).

Preparation of scale extracts
Water-soluble substances were extracted with 0.15 M phosphate buffered saline (PBS; pH 7.4) from the collected scalp scales. Each scale was suspended in PBS of 20 times the original dry weight, and homogenized in a glass homogenizer. The homogenates were centrifuged at 10,000 g for 15 min at 4°C.

Molecular sieve chromatography
Two extracts each of dandruff scales and normal scalp scales were fractionated by gel filtration on Sephadex G-75 (1.5 cm ID × 27.0 cm) using PBS as an eluting medium. The extracts of 0.5 ml were applied to the column, and fractions of 2 ml were collected. The content of protein was monitored by absorbance at 280 nm. Bovine serum albumin (BSA), cytochrome C (cyt C), and vitamin B₁₂ (V. B₁₂) were used as molecular markers.

Chemotactic assay
Chemotactic activity and random migration of guinea pig PMNLs were measured by using a modified Boyden chamber with 3 μm polycarbonate filter (Nucleopore, Corp., Calif., USA) [3]. Guinea pig PMNLs were used to measure the chemotactic activity in this study after confirming that they show a chemotactic response comparable to that of human peripheral blood neutrophils. Briefly, fractionated samples or a positive control, formyl-met-leu-phe (FMLP) 1 × 10⁻⁶ M, was placed in the bottom compartment, while the PMNL suspension (1 × 10⁶ cells/ml) was applied in the top compartment, and the chambers were incubated at 37°C for 40 min under 5% CO₂. After incubation, the filter was fixed in methanol, and stained with Gimsa’s solution. PMNLs which had migrated completely through the filter were counted at ten fields randomly. Chemotactic activity was expressed as an average number of PMNLs per high power field (× 400).

Antiserum
Goat anti-serum to human C5 and to human C3a were purchased from Cappel Laboratories (Westchester, USA).

Radioimmunoassay
C₅a and C₅a des arg, C₄a and C₄a des arg in the extracts (described collectively as C₅a and C₄a respectively in this paper) were quantified according to the procedure of Wander and Hugli [22]. The assay kits for C₅a and C₄a were purchased from