**CLINICAL REPORT**

**Failure to Detect Clonality in Eosinophilic Pustular Folliculitis with Follicular Mucinosis**

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Eosinophilic pustular folliculitis is characterized by an eosinophil-rich inflammatory follicular and perifollicular infiltrate primarily centred at the level of the follicular isthmus and sebaceous duct. Follicular mucinosis has been observed in lesions of eosinophilic pustular folliculitis. Clinical and histological features of eosinophilic pustular folliculitis with follicular mucinosis and alopecia mucinosa are very similar. Alopecia mucinosa may be a clonal T-cell dermatosis. A monoclonal re-arrangement of the T-cell receptor gene was detected in about half of the cases in alopecia mucinosa. To investigate T-cell clonality in a series of eosinophilic pustular folliculitis with follicular mucinosis, we performed heteroduplex analysis of re-arranged T-cell receptor \( \gamma \) gene in seven cases of eosinophilic pustular folliculitis with follicular mucinosis. All cases were negative for heteroduplex-PCR analysis. The failure to demonstrate clonality may be consistent with a reactive nature of eosinophilic pustular folliculitis with follicular mucinosis. Key words: eosinophilic pustular folliculitis with follicular mucinosis; clonality.

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Eosinophilic pustular folliculitis (EPF) is a chronic inflammatory disorder of unknown aetiology (1–4). The disease is more prevalent in people of Asian descent (1, 2). Although the clinical presentation can be diverse, the histological pattern of EPF is diagnostic (5–7). It is characterized histologically by a follicular and perifollicular inflammatory infiltrate containing abundant eosinophils (5, 6). Follicular mucinosis is an unusual finding in reports of EPF, but has been described in several cases (2, 6–9). The histological features of EPF with follicular mucinosis and alopecia mucinosa (AM) are very similar (2, 6–9). In addition, the clinical appearance of EPF may resemble that of alopecia mucinosa (10, 11). This raises the question of whether EPF with follicular mucinosis is actually a variant of AM. We performed heteroduplex analysis of re-arranged T-cell receptor (TCR) \( \gamma \) gene for clonality assessment in paraffin-embedded skin biopsy samples of seven cases of EPF with follicular mucinosis.

**MATERIALS AND METHODS**

**Patient samples**

Fifteen cases of EPF were diagnosed during 1990–2003 at Asan Medical Center, Korea. Among these cases, a diagnosis of EPF with follicular mucinosis was made in 7 cases on the basis of the following features: 1) eosinophil-rich follicular and perifollicular infiltrate centred on the isthmus and/or sebaceous gland, often associated with infundibular infiltrate (Fig. 1a); 2) mucin deposits were found in pilosebaceous units by alcian blue staining (Fig. 1b).

Follicular lymphoma cells of thyroid gland and intestinal T-cell lymphoma cells were used as negative and positive controls, respectively.

**DNA extraction**

Genomic DNA from formalin-fixed paraffin-embedded blocks was extracted in the following manner. Ten 10-mm sections were cut from each block, deparaffinized in xylene, rinsed with 95% ethanol, dried, resuspended in tris-EDTA (10 mM Tris-hydrochloric acid and 1 mM EDTA, pH 7.8) and incubated with proteinase K (Sigma-Aldrich, St Louis, MO, USA) at 37°C overnight. Samples were heated for 10 min at 95°C (to inactivate proteinase K), and supernatants were phenol-chloroform extracted and ethanol precipitated. Dried DNA was resuspended in sterile water and used for the polymerase chain reaction (PCR).

**Heteroduplex-PCR for TCR**

**PCR primers and PCR reaction conditions.** Primers used for amplifying the TCR \( \gamma \) gene V segments were as follows: V2: 5'-CTTCCTGCGAGATGACTCTACATACTCCAAGGTTG-3'; V3: 5'-CTTCCTGCGAGATGACTCTACATACTCCAAGGTTG-3'; V4: 5'-CTTCCTGCGAGATGACTCTACATACTCCAAGGTTG-3'; V5: 5'-GGNACTGCAAGGAAGGAATCTGGCATTCATCG-3'. For the J segment, the following three primers were included: JGT12: 5'-AAGTGTTCATCAGCCCAA-3'; jgt3: 5'-AGTTACTATGAGCTACGATCGG-3'; jgt4: 5'-TGGATTTGATCGTTTGGCC-3'. The PCR was performed under the following conditions: 1.0 μg of genomic DNA was amplified with primers in the 25 μl of reaction volume containing 10 pmol of each primer, and 1 unit of Taq polymerase, using one cycle of 94°C for 5 min, 45 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by one cycle of 72°C for 10 min.

**Heteroduplex analysis.** The PCR products were applied for heteroduplex analysis by incubating at 94°C for 5 min and subsequently cooled (to a lower temperature) to induce duplex formation. This renaturation step was performed at 4°C for 60 min. After duplex formation the heteroduplexes and/or homoduplexes were immediately loaded on 8%
non-denaturing polyacrylamide gels in 0.5% Tris-boric acid-EDTA buffer, run at room temperature, and visualized by ethidium bromide staining. Pstl-digested λDNA was used as a size marker.

RESULTS

There were three women and four men, ranging in age from 7 to 47 years at diagnosis (mean age, 31 years). All had predominantly annular follicular erythematous papules and pustules on the face (Fig. 2); four had symptoms of associated pruritus. Laboratory investigations showed peripheral eosinophilia in one case. None of the patients had systemic lymphoproliferative diseases or any signs of HIV infection. Several therapeutic options were used, including steroids (oral and topical), dapsone and naproxene. All the cases were negative for heteroduplex-PCR for TCRγ (Fig. 3).

DISCUSSION

EPF with follicular mucinosis has been present in a few reports (2, 6 – 9). In a series by Ishiguro et al. (2) Alcian blue or Nissl modified staining showed the accumulation of acid mucopolysaccharides in 7 of the 20 patients with EPF. Lee et al. (6) reported follicular mucinosis in 41% of EPF. Follicular mucinosis in association with EPF seems not uncommon, occurring in 7 of 15 cases of EPF in our series.

AM is a reaction pattern in the follicular epithelium characterized by a mucinous degeneration of the outer sheath of follicles and sebaceous glands, accompanied by an inflammatory infiltrate often containing eosinophils (12). AM has been described as occurring: 1) idiopathically; 2) in association with malignant lymphomas such as mycosis fungoides and Sezary syndrome; and 3) in a variety of unrelated conditions, which may be inflammatory, hamartomatous, hyperplastic or neoplastic (11 – 14).

Lee et al. (6) considered that EPF with follicular mucinosis tended to show more numerous eosinophils, less abundant mucin and eosinophilic infundibular pustules or sebaceous micro-abscesses rather than AM. However, in many cases, histological differentiation between AM and

Fig. 1. (a) Eosinophil-rich follicular and perifollicular infiltrate centred on the isthmus and/or sebaceous gland (H&E, ×200). (b) Alcian blue staining reveals follicular mucin deposition (×400).

Fig. 2. Annular follicular erythematous papules and pustules on the face.
EPF with follicular mucinosis can be difficult. The clinical appearances of both diseases look similar. Both can have chronic eruptions with multiple discrete papules, reminiscent of acne or indurated plaques (2, 10, 11). In addition, both may be associated with immunological dysfunction, lymphoma, leukoemia or haematological disease (13, 14). Responses to dapsone and indomethacin have been reported in both diseases (2, 13, 15). This raises the question of whether EPF with follicular mucinosis actually is a variant of AM. There are many previous observations describing a monoclonal population of T lymphocytes in cases of AM (10, 13, 16). Alopecia mucinosa may be a clonal T-cell dermatosis, similar to lymphomatoid papulosis and pityriasis lichenoides chronicum (13, 16). In one series, 71% (5/7) of patients with idiopathic AM had clonality as determined by Southern blot analysis (16). PCR analysis of idiopathic and lymphoma-associated AM could not help in differentiating the two types (13). A monoclonal re-arrangement of the TCRγ gene could be identified in 6/11 (55%) and 9/19 (47%) of idiopathic and lymphoma-associated AM, respectively, indicating that a monoclonal population of T lymphocytes can be detected in about half of the cases in both groups (13).

We performed clonality assessment in paraffin-embedded skin biopsy samples of seven cases of EPF with follicular mucinosis. To our knowledge, our analysis is the first to examine the clonality of EPF with follicular mucinosis. We used sensitive PCR amplification followed by heteroduplex analysis of the TCRγ gene. Molecular analysis of PCR-based TCRγ genes is frequently used to prove the clonality, but the main disadvantage is the risk of false-positive results due to background amplification of polyclonal reactive T lymphocytes. One of the methods that has been applied to solve this background amplification of PCR includes heteroduplex analysis. In our study, all the cases showed negative reaction in heteroduplex-PCR for TCRγ. The failure to demonstrated clonality may be consistent with the reactive nature of EPH with follicular mucinosis. Of course, the demonstration of a clonal re-arrangement is not synonymous with malignant neoplasia, but it does indicate an abnormal proliferation of cells that may progress to malignancy. The clonal lymphocytic expansion indicates a need for continued surveillance of patients. Heteroduplex-PCR may help to differentiate between doubtful cases of AM and EPF with follicular mucinosis and may help to determine the prolonged follow-up.

The nature of mucin in AM and EPF with follicular mucinosis and the cells that produce the mucin are still unknown. Several studies have suggested that mucin is produced by cells of the hair follicles and that T lymphocytes and eosinophils probably stimulate follicular keratinocytes to produce mucin (17, 18).

REFERENCES