No Basophil Infiltration in Alopecia Areata Irrespective of the Intensity of Eosinophil Infiltration

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Alopecia areata (AA) is an organ-specific autoimmune disease characterized by T-cell infiltrates and cytokine production around anagen-stage hair follicles. Although AA has traditionally been considered a T-helper (Th) 1 cytokine-mediated disease, association of AA with atopic dermatitis (AD), allergic rhinoconjunctivitis or asthma, all of which are mediated by Th2 cytokines, has been reported in 10–60% of patients (1, 2). In addition, it has been shown that not only Th1, but also Th2, cytokine responses are involved in an animal model of AA (3).

Basophils play a critical role in the development of IgE-mediated chronic allergic reactions by functioning as initiator cells (4, 5). In addition, basophils promote Th2 skewing by means of antigen presentation in an IgE-independent manner, and dendritic cells are not necessary in this process (6). Basophil infiltration into the tissues has been demonstrated in a number of human skin diseases, and is generally detected in skin diseases where eosinophils are present (4, 5).

Eosinophil infiltration into the skin lesions at any stage is a useful diagnostic feature of AA (7, 8). However, it is not known whether basophils infiltrate into AA skin lesions.

MATERIALS AND METHODS

Skin biopsy samples from the lesions of 28 patients with AA were collected after obtaining informed consent. The samples were separated into 2 groups based on histological observation of the intensity of eosinophil infiltration: 14 patients with dense eosinophil infiltration (2 men, 12 women; mean age 29.9 years) and 14 patients with no eosinophil infiltration (4 men, 10 women; mean age 29.1 years). Deparaffinized sections were immersed in 0.4 mg/ml proteinase K for 5 min for antigen retrieval. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide for 30 min. Staining for basophils with 2D7 (BioLegend, San Diego, CA, USA) and BB1 antibodies (kindly provided by Dr A.F. Walls, Immunopharmacology Group, University of Southampton, Southampton, UK) were performed for each groups. Slides were incubated with biotinylated horse anti-mouse serum, and then incubated with avidin-biotin-peroxidase complex (ABC-AP; Vector Laboratories, Burlingame, CA). Coloring reaction was performed with alkaline phosphatase substrate kit I (Vector) and nuclei were counterstained with haematoxylin.

RESULTS

In the group with eosinophil infiltration, the number of eosinophils counted in 3 high-power fields (× 40 objective) for each patient was 20.5 ± 19.4 (mean ± standard deviation) per field. Fig. 1 a and b show an example. Seven out of 14 patients with dense eosinophil infiltration had either urticaria, AD, rhinoconjunctivitis, food allergy, or an itchy sensation on the lesions, while only one patient with no eosinophil infiltration had AD. Staining for basophils revealed neither 2D7 antibody-positive nor BB1-positive cells in this group (Fig. 1 c,

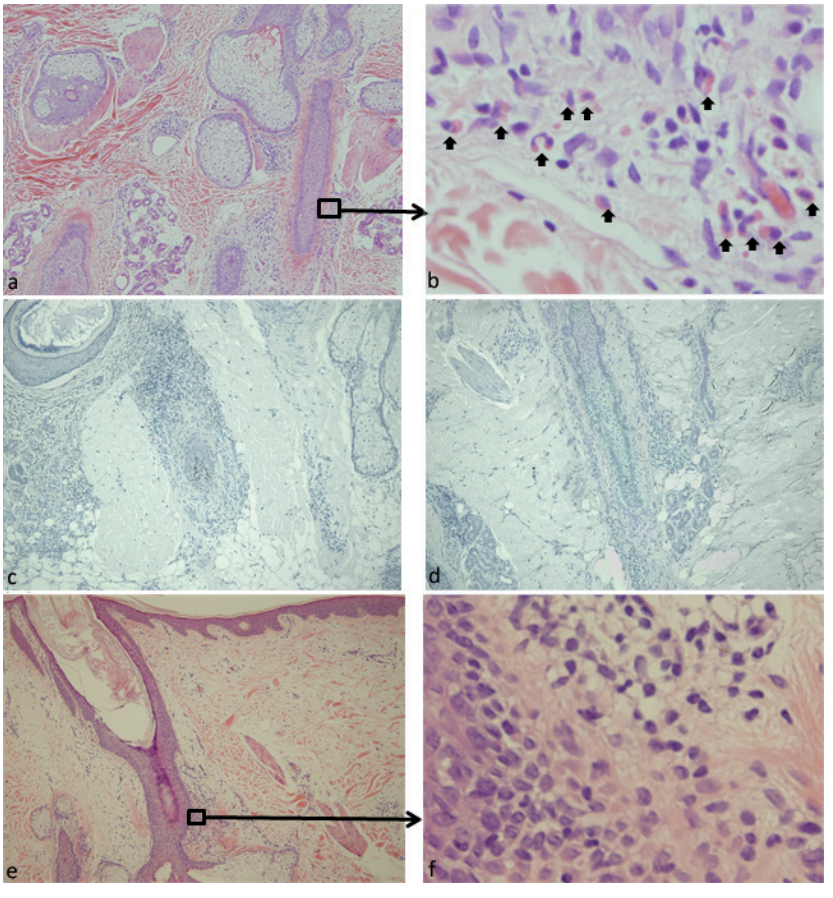

Fig. 1. Histopathological investigations of alopecia areata lesions. Numerous eosinophils (*arrows*) were present in a peribulbar lymphocytic infiltration of a patient in the dense eosinophil infiltration group (haematoxylin-eosin stain; original magnification (a) × 10, (b) × 40). In the same patient no basophils were observed when stained with (c) 2D7 or (d) BB1 antibodies. No eosinophils were observed in a patient in the group without eosinophil infiltration (H&E stain; original magnification (e) ×10, (f) ×40. No basophil were detected either (data not shown).

d). In the other group neither eosinophils (Fig. 1 e and f) nor basophils (data not shown) were present. In other words, basophils were not detected, regardless of the infiltration of eosinophils. Quality control of these assays was assured by staining of a positive control obtained from the skin lesions in lepromatous leprosy (9).

DISCUSSION

Basophil infiltration into the skin is thought to be accompanied by eosinophil infiltration (4). Marked basophil recruitment into the follicular and sebaceous regions was seen, together with eosinophil recruitment in eosinophilic pustular folliculitis (EPF), while no basophils were observed in the skin lesions of neutrophilic folliculitis (5). This is because both basophils and eosinophils express common chemokine receptors, such as CCR3 and CRTH2, and, in Th2-predominant inflammation, co-accumulation may occur via stimulation of these receptors (4). On the other hand, the number of basophils and the ratio of tissue basophils/eosinophils vary among skin diseases. The ratio of basophils to eosinophils is approximately one in EPF, prurigo, long-lasting urticaria, bullous pemphigoid, and greater than one in Henoch-Schönlein purpura (4). The typical characteristics of diseases associated with basophil infiltration are not yet fully understood. Although basophil infiltration into the skin is assumed to be accompanied by eosinophil infiltration, we did not detect basophils in AA lesions regardless of the infiltration of eosinophils.

Our observations indicate that the involvement of basophils in the pathogenesis of AA is limited, and that unknown, but separate, mechanisms are engaged in inducing infiltration of basophils and eosinophils.

The authors declare no conflicts of interest.

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