

# Quantitative and Ultrastructural Analysis of Inflammatory Infiltrates in Male Pattern Alopecia

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**In order to determine whether lymphocytic inflammation around the lower infundibula in male pattern alopecia is incidental or a general phenomenon, we performed morphometric and ultrastructural analysis of inflammatory infiltrates in the transitional zones of the vertex and occipital hairy scalps of 19 patients with male pattern alopecia. Six normal subjects served as controls. The number of inflammatory infiltrates around the follicular infundibula of the alopecic vertices and non-alopecic occiputs of male pattern alopecia patients was significantly greater than the corresponding control value. The number of mast cells in the widened fibrous tracts in the vertices of male pattern alopecia patients was significantly greater than those in the adventitial fibrotic sheaths of control subjects and the non-alopecic occiputs of male pattern alopecia patients. These data support the idea that the inflammatory process may be, at least in part, responsible for the development of male pattern alopecia.**

**Key words:** bulge; stem cell; mast cells; adventitial sheaths.

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The expression of male pattern alopecia (MPA), also called premature baldness or androgenetic alopecia, requires an exaggerated androgenic influence on hair follicles in genetically predisposed individuals (1). The histological findings of MPA have usually been described as being mainly a non-inflammatory process associated with increased numbers of miniature hair follicles with underlying fibrous tracts (2). These observations concentrated on alopecic skin, a terminal stage of MPA. However, a recent immunopathological study of hair-bearing transitional and alopecic scalp regions in 4 MPA sufferers (3) showed the presence of activated T-lymphocytes, which were concentrated around the putative stem cell region "bulge" (4), a zone directly beneath the orifice of the sebaceous duct. The idea that immune mechanisms may contribute to the pathogenesis of MPA was based on preliminary evidence of the efficacy of oral and topical cyclosporine in this disease (5). In order to determine whether lymphocytic inflammation around the lower infundibula in MPA is incidental or a general phenomenon and elucidate its significance, we performed morphometric analysis of biopsy specimens from the vertices (transitional zones in MPA sufferers) and occiputs (hairy zone) of 19 patients with MPA and 6 normal subjects. After opening the key code, we characterized further the ultrastructure of immune cells in MPA patients.

## MATERIALS AND METHODS

### Patients

Nineteen patients with MPA, showing no more than 50-mm circles of hair loss on their vertices (Hamilton Scale III-vertex or IV only), and 6 normal subjects participated in the study. All the subjects were male and their ages ranged from 18 to 49 years. Subjects who had dermatological disorders of the scalp, such as seborrheic dermatitis and atopic eczema, were excluded. Institutional Review Board approval of the protocol and written informed consent from the subjects were obtained.

### Biopsy

Two 6-mm punch biopsy specimens were taken (1 each from the occiput and vertex area of the scalp) from each subject. The vertex biopsy specimen was taken from the balding area between the hairy and balding areas of the scalp and occipital biopsy specimen was taken from the hairy area of each MPA patient, because MPA affects the frontal region and vertex of the scalp, but typically preserves the occipital fringe (1).

### Histopathology and morphometric analysis

For light microscopy, half of each specimen was fixed with 10% v/v formalin, dehydrated in a graded ethanol series, embedded in JB4 (glycolmethacrylate) and 2- $\mu$ m-thick sections were stained with 0.5% w/v toluidine blue. Morphometric analysis was performed by researchers who did not know the group and biopsy site before opening the key code utilizing a Microcomp image analysis system made by Southern Micro Instruments (Atlanta, GA, USA) with a colour camera (model DXC-325, Sony, Tokyo, Japan) mounted on an Olympus BH-2 research microscope (Tokyo, Japan). The number of inflammatory infiltrates in a 0.1-mm<sup>2</sup> area around the lower portion of follicular infundibula and the number of mast cells in a 0.01-mm<sup>2</sup> area of the adventitial fibrotic sheath or fibrous tract representing sclerotic sheath remnants were counted. The data were expressed as mean  $\pm$  SD. Statistical analysis was performed using Student's *t*-test (two-tailed) in the data with equal SDs. When the difference between the two SDs is significant, Mann-Whitney non-parametric test (two-tailed) was used. The differences were considered significant at  $p < 0.01$ .

### Electron microscopy

The other half of each specimen was fixed immediately with 4% v/v glutaraldehyde in cacodylate buffer. Generally, ultrastructural analysis without the knowledge of grouping is difficult. Therefore, after opening the key code, 5 specimens from the vertices of MPA patients and 1 from the vertex of a normal subject were selected, post-fixed with 2% v/v osmium tetroxide in cacodylate buffer for 2 h at room temperature, dehydrated with a graded ethanol series and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and bismuth and observed using a Hitachi H-7000 electron microscope.

## RESULTS

### Histopathology

The biopsy specimens from the vertices (transitional zones between the alopecic and non-alopecic scalps) of the MPA

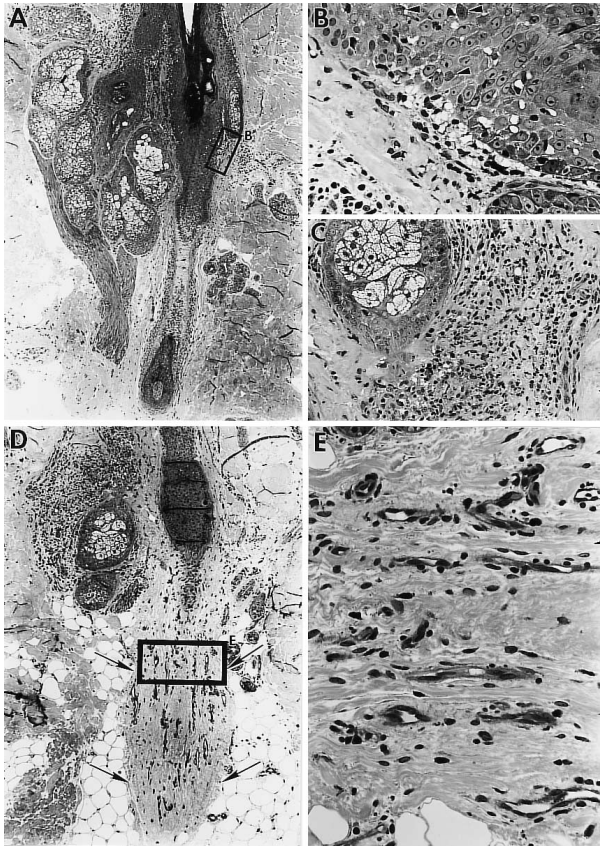


Fig. 1. Histopathology of the transitional zone between the alopecic and non-alopecic scalp of a patient with MPA. Inflammatory infiltrates consisting predominantly of lymphoid cells were observed consistently around the lower portion of the infundibulum, isthmus and sebaceous glands (A)  $\times 13$ ; (B)  $\times 92$ ; (C)  $\times 57$ . Hydropic degeneration of basal cells, sparse dyskeratotic cells (arrowheads) and exocytosis of lymphoid cells were observed in the follicular epithelium of the lower infundibulum (B). A widened fibrous tract (arrows), the residua of previous cycling elements extended down the subcutis (D,  $\times 13$ ). Panel E depicts a high-power view of the rectangular area shown in (D). The fibrous tract contained a number of fibroblasts, granule-filled mast cells and microvessels, which were occasionally dilated (E,  $\times 76$ ).

patients showed an apparent reduction in the percentage of hair in the anagen phase to approximately 50% of that in the hairy scalp areas of the same patients. Patchy inflammatory infiltrates consisting predominantly of lymphoid cells were observed around the lower portion of the infundibulum, isthmus and/or sebaceous glands in all specimens of this group, although their degrees varied (Fig. 1A–D). Occasional hydropic degeneration of basal cells, sparse dyskeratotic cells and exocytosis of lymphoid cells were observed in the infundibular follicular epithelium (Fig. 1B). No inflammatory infiltrates were observed around the majority of the bulbs. At alopecic sites, most of the hair follicles had been replaced by empty fibrous tracts, the residua of previous cycling elements, extending down the subcutis (Fig. 1D). These fibrous tracts appeared different from the collapsed sheaths of normal telogen follicles. The tracts were much broader than adventitial fibrotic sheaths and contained a number of fibroblasts, granule-filled mast cells and microvessels and, occasionally, they were dilated (Fig. 1E).

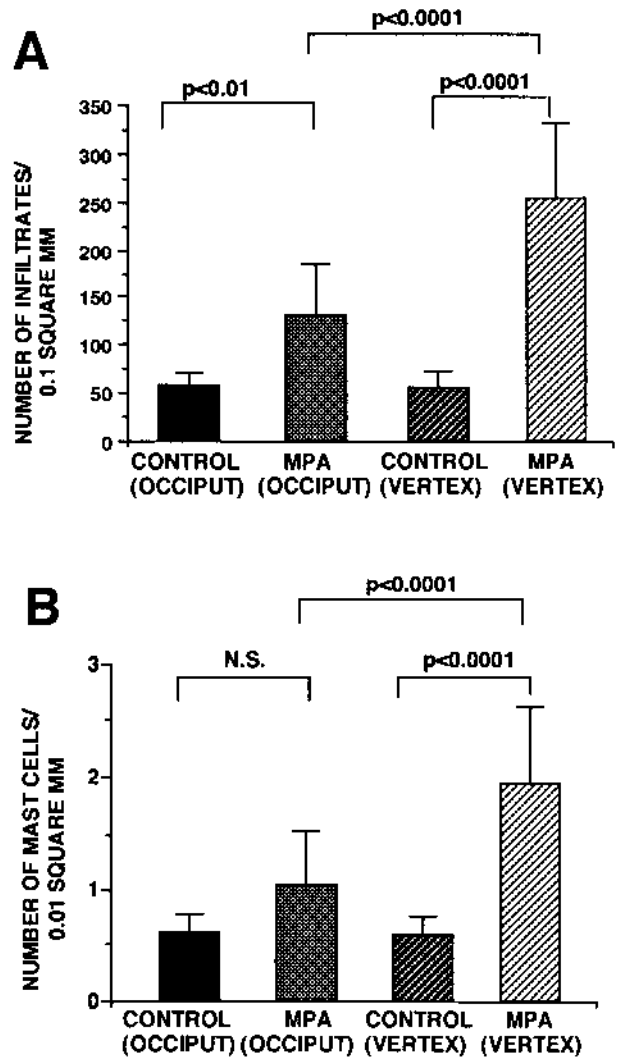


Fig. 2. Morphometric analysis. (A) Mean  $\pm$  SD of inflammatory infiltrates around the lower portion of follicular infundibula in the occiputs and vertices of male pattern alopecia patients and control subjects. (B) Mean  $\pm$  SD of mast cells in the widened fibrous tracts in the occiputs and vertices of male pattern alopecia patients and control subjects.

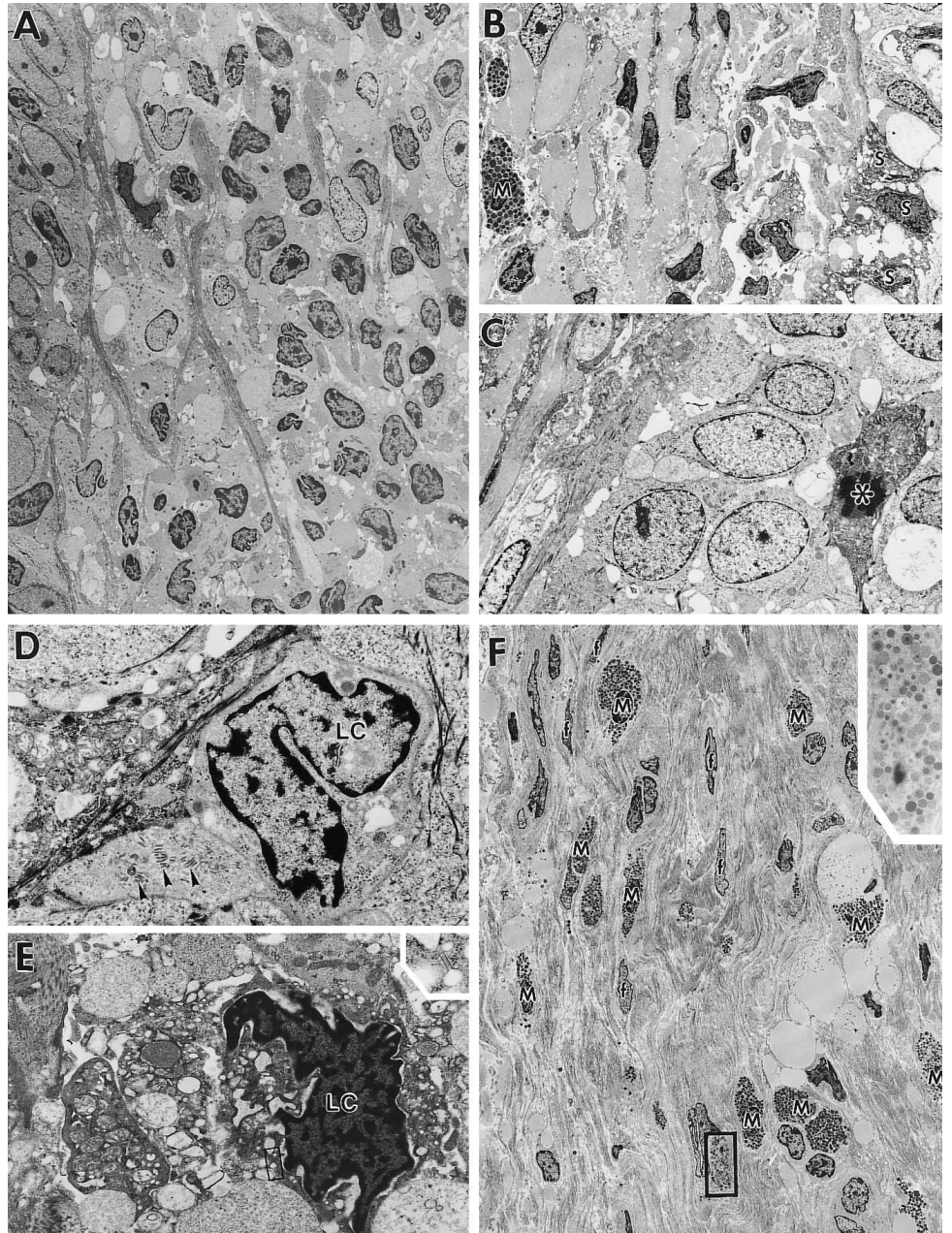
The biopsy specimens from the occipital, non-alopecic, scalps of the MPA patients revealed occasional inflammatory infiltrates around the lower infundibular portion, isthmus and/or sebaceous glands, although there were fewer than in the vertex. In some cases, a few fibrous tracts extended from follicles that were involute and becoming miniaturized.

Occasional spotty, small foci of minimal lymphocytic infiltrates were observed around venules in the upper dermis and perifollicularly at about the level of the sebaceous ducts in the biopsy specimens from the normal subjects.

#### Morphometric analysis

The number of inflammatory infiltrates around the lower infundibular portion in the vertices of MPA patients was significantly greater than the corresponding control value (Mann–Whitney test;  $p < 0.0001$ ) and that in the non-alopecic occiputs of MPA patients (Student's *t*-test;  $p < 0.0001$ ).

Fig. 3. Ultrastructure of the transitional zone between the alopecic and non-alopecic scalp of a patient with MPA. Dense inflammatory infiltrates consisting of lymphocytes and macrophages were observed around the middle portion of the follicular epithelium (A,  $\times 900$ ). Follicular epithelium showing shrunken basal cells with vacuolar degeneration (S) (B  $\times 1,180$ ) and a small number of dyskeratotic cells (asterisk in C,  $\times 2,300$ ). Marked hyperplasia of Langerhans' cells (LC), characterized by dendritic morphology and Birbeck granules (arrowheads) was observed within follicles (D,  $\times 6,250$ ) and adjacent adventitial sheaths at the lower infundibulum (E,  $\times 5,800$ , rectangular area, inset right upper corner shows a Birbeck granule,  $\times 9,870$ ). Increased numbers of fully-granulated mast cells (M) and occasional degranulating mast cells (rectangular area, inset right upper corner  $\times 2,160$ ) were embedded in the widened fibrous tract. Some mast cells (M) were intimately associated with fibroblasts (f) (F  $\times 900$ ).



Interestingly, the number of infiltrates in the non-alopecic occiputs of MPA patients was significantly greater than the corresponding control value (Mann-Whitney test;  $p < 0.01$ ; Fig. 2A). The number of mast cells in the widened fibrous tracts in the vertices of MPA patients was significantly greater than those in the adventitial fibrotic sheaths of control subjects (Mann-Whitney test;  $p < 0.0001$ ) and non-alopecic occiputs of MPA patients (Student's *t*-test;  $p < 0.0001$ ; Fig. 2B).

#### Electron microscopy

The specimens obtained from the transitional areas of the hairy and balding scalps of MPA patients revealed dense inflammatory infiltrates, consisting of lymphocytes and macrophages, around the middle portion of the follicular epithelium (Fig. 3A), which showed vacuolar degeneration of

basal cells and widening of the intercellular spaces (Fig. 3B). A few dyskeratotic cells containing homogeneously aggregated nuclear chromatin, cytoplasmic vacuoles and abnormally condensed tonofilaments were encountered (Fig. 3C). Marked hyperplasia of Langerhans' cells, characterized by dendritic morphology and Birbeck granules, was observed within follicles (Fig. 3D) and adjacent adventitial sheaths at the lower infundibulum where lymphocytic infiltration was prominent (Fig. 3E). Large numbers of fully-granulated mast cells and occasional degranulating mast cells were embedded in the widened fibrous tracts and some mast cells were intimately associated with fibroblasts (Fig. 3F).

#### DISCUSSION

The results of this study indicate that lymphocytic inflammation around the lower portions of the follicular infundibula in

MPA patients was not incidental, but a general phenomenon. The number of inflammatory infiltrates around the infundibula in the vertices of MPA patients was significantly greater than the corresponding control value ( $p < 0.0001$ ). Interestingly, the number of infiltrates in the non-alopecic occiputs of MPA patients was significantly greater than that in the occiputs of control subjects ( $p < 0.01$ ), whereas it was lower than the value for vertices of MPA patients. These data suggest that lymphocytic infiltration around the lower infundibulum is a specific histological feature of MPA and may not merely represent the expression of actinic damage, seborrheic dermatitis, porphyrin elaboration by follicular bacteria and/or the result of applying grooming agents to the scalp. Our results, however, could be the secondary phenomenon and raise questions concerning the primary factors that induce follicular inflammation.

In our histological and ultrastructural study, a lichenoid tissue reaction characterized by hydropic degeneration of basal cells, sparse dyskeratotic cells and exocytosis of lymphocytes was observed near the putative stem cell region "bulge" (4). Other types of permanent alopecia associated with lichen planopilaris (6), lupus erythematosus (7) and the recently described postmenopausal frontal fibrosing alopecia (8) also involve inflammation and similar epithelial injury at the same infundibular level. In these disorders, stem-cell injury elicited by lymphocytes may result in permanent hair loss, although we found no direct evidence of cytotoxicity to the stem-cell population.

An increased number of mast cells in the widened fibrous tracts in the vertices of MPA patients was also a general event. The ultrastructural study showed occasional mast cells undergoing degranulation. Mast cell products, such as histamine (9) and fibroblast growth factor (FGF) (10), have been implicated in the induction of aberrant fibrosis in skin diseases. Quantitative studies revealed increased numbers of mast cells in rapidly progressive scleroderma (11), porphyria cutanea tarda (12), hypertrophic scars (13) and chronic graft-vs.-host disease (14). In MPA, mast cell factors may contribute to the aberrant fibrosis in the adventitial sheath, leaving behind non-trichogenic superficial portions of infundibular epithelium and underlying fibrous tracts formerly occupied by intact follicles. Mast cell granules contain cytokines such as tumour necrosis factor- $\alpha$ , which is capable of inducing endothelial leukocyte adhesion molecule-1 (ELAM-1) on the microvascular endothelium and trafficking effector lymphocytes into the skin (15).

Our ultrastructural study may suggest increased numbers of Langerhans' cells within follicles and adjacent adventitial sheaths at the lower infundibulum in the vertex biopsy specimens of MPA patients. This result parallels the immunohistochemical distribution of CD1a-positive dendritic cells in the transitional zones of MPA sufferers (3). The follicular infundibulum of the normal follicle has been shown to be richly endowed with CD1a- and HLA-DR-positive dendritic cells (16). Recent data indicated that the infundibulum is an early target of allergic contact dermatitis (17) and acute graft-vs.-host-disease (18). Thus, the hair follicle appears to participate actively in cutaneous immune responses.

In conclusion, our quantitative analysis and ultrastructural

observations of MPA patients support the idea that the inflammatory process may be, at least in part, responsible for the development of male pattern alopecia.

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