Cutaneous immunopathology of androgenetic alopecia

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Male pattern baldness is assumed to result from a combination of normal serum concentrations of androgen and an appropriate genetic background. To study whether inflammation contributes to the development of androgenetic alopecia, direct immunofluorescence and dermatopathologic studies were performed on biopsy specimens from bald scalp of patients, with specimens from uninvolved scalp of these patients or from scalp of volunteers who were not bald serving as controls. Granular deposits of Immunoglobulin M or C3 (or both) were found at the basement membrane in 25 (96%) of 26 study patients and 1 (12%) of 8 control subjects. Granular C3 was also deposited on eccrine myoepithelial cells in 8 (31%) of 26 study patients, but no control subjects. Porphyrins were found in the pilosebaceous canal in 15 (58%) of 26 study subjects and in 1 (12%) of 8 control subjects.

These results support an inflammatory pathogenesis of androgenetic alopecia. *Propionibacterium acnes* is known to

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produce porphyrins. Ultraviolet radiation may excite microbiologic porphyrins that could activate C3 and, subsequently, the complement cascade producing inflammatory mediators.

(Key words: Androgenetic alopecia, male pattern baldness, hair loss, dermatology)

Androgenetic alopecia (AGA), also called male pattern baldness, is assumed to result from a combination of normal androgen levels and a genetic predisposition.¹ The diagnosis is established on the basis of history, especially family incidence, and the Hamilton² and Ludwig³ hair loss patterns. The histopathologic findings in AGA are widely known.^{4,5} They include a decrease in the diameter of the hair and hair follicle and a decrease in length of the hair root. The sebaceous glands are enlarged.

Further, inflammation has been thought to be minor or absent. Inflammation may play a role in the formation of Arao-Perkins bodies, small clusters of elastic tissue in the neck of the papilla resulting from repeated cycles of hair growth. These elastic fibers are clumped in the catagen stage and remain at the lowest point to which the papilla extended in the anagen stage. In AGA, the next anagen hair is slightly shorter than the one before, and its Arao-Perkins body will remain somewhat above the first body. Subsequently, these

Table 1
Distribution of Patients According to Hamilton and Ludwig Classifications (n = 26)

Classification	No. of patients
Hamilton² (men)	
Types I and II	4
Types III and IV	12
Types V and VI	3
Ludwig³ (women)	
Grade I	4
Grade II	3

elastic clumps may be aligned vertically, within the collapsed fibrous root sheath.⁶

Androgenetic alopecia results in a transformation of terminal follicles to vellus follicles, which in later stages become atrophic,⁷ and may disappear with dermal scarring.⁸ Dys-trophic hair follicles also are found frequently in long-standing advanced alopecia, particularly in aged people,⁸ but a lack of degenerative changes of the follicles or abnormalities of the surrounding tissue has been reported.⁹ The thinning of the hair shaft in AGA is thought to result from a diminution of the size of the hair follicles.

Thus, the process of androgenetic balding appears to be an insidious process resulting in hair follicle loss and scalp scarring. The dermatopathologic finding of an inflammatory component, which is believed to be characteristic of AGA by some, but not appreciated by others,⁹ suggests that inflammation may also be involved. To see if these changes of AGA may be a product of inflammation as well as hormonal and genetic predisposition, we have investigated the immunopathology of the scalp of patients with AGA. This article reports our preliminary findings, specifically focusing on histopathology and direct immunofluorescence.

Materials and methods

Patient selection

Twenty-six patients were entered into the study at random from a clinic population seeking diagnosis or treatment (or both) of hair loss (Table 1). The control group consisted of two persons free of

alopecia and six patients with androgenetic alopecia. Biopsy specimens were taken from the margin of involved scalp in patients and from uninvolved scalp (either from the vertex in the patients free of alopecia or from occipital scalp of patients with AGA) to serve as controls. The demographic characteristics of participants are given in Table 2.

Patient and specimen evaluation

The patients were evaluated by history and physical examination. The patients were clinically well and free of scalp disease. Eight of the 26 study patients and two of the eight control subjects had screening laboratory examination to exclude occult systemic disease. Laboratory tests included complete blood cell count, chemistry profile, serum thyroxine determination, erythrocyte sedimentation rate, urinalysis, serum protein electrophoresis, and tests for antinuclear antibody and rheumatoid factor.

Handling of specimens

Two 4-mm punch biopsy specimens were obtained from each patient. One specimen was sent for hematoxylin and eosin as well as elastic tissue staining, and the other was used for immunopathologic study. Biopsy specimens were sectioned vertically. The follicular cycle, stage, and absolute and average number per section were recorded. Inflammation was graded as +1 (10 to 20 perifollicular lymphocytes) to +4 (more than 100 perifollicular lymphocytes).

The biopsy specimens for study by direct immunofluorescence were prepared according to the following procedure after being transported in Michel's medium. Biopsy tissues were washed in three 10-minute cycles to remove transport medium. They were then frozen in liquid nitrogen at 30°C, and sectioned at 4 μ m. All tissue sections were processed immediately without fixation. Tissue sections were covered with fluorescein isothiocyanate conjugates of the monospecific goat anti-human antibodies immunoglobulins M (IgM), G (IgG), and A (IgG); C3; and fibrinogen (Sanofi Diagnostics Pasteur Inc, Chaska, Minn), which were diluted to 30 to 35 μ g/mL with 5 mmol/L of sodium barbital buffer, pH 7.5, containing 0.2 mmol/L of calcium chloride, 0.8 mmol/L of magnesium chloride, 0.7 mol/L of sodium chloride, and 0.5% Tween 20 (Fisher Scientific, Fairlawn, NJ).

These tissue sections were then incubated for 20 minutes at room temperature in a moisture chamber. Conjugates were rinsed from slides with the same buffer and washed for 10 minutes. Coverslips

Figure 1. Granular deposits of C3 at basement membrane of dermoepidermal junction.

Figure 2. Granular deposits of IgM at the basement membrane of hair follicle.

Figure 3. Negative control IgM with autofluorescence of proteins in secretory coils of eccrine glands.

Figure 4. Negative control with C3. No reactivity at basement membrane of dermoepidermal junction.

Figure 5. Porphyrins fluoresce within pilosebaceous canal.

(Funding for color printing provided by the Department of Dermatology, Grandview Hospital Medical Center, Dayton, Ohio.)

Table 2
Demographic Features of Participants

Group	Age, yr		Sex		Race	
	Range	Average	Male	Female	White	Black
Study patients (n = 26)	19–58	35	19	7	24	2
Control subjects (n = 8)	30–62	40	7	1	7	1

were mounted with the use of buffered glycerin with 0.1% paraphenylenediamine (modified technique of Beutner¹⁰). Slides were examined by means of a Leitz Ortholux-2 epiillumination microscope (Leica, Malvern, Pa) with a 150-watt xenon light source, 25-mm ($\times 250$) and 50-mm ($\times 500$) water immersion lenses, and an H3 filter. All specimens were studied without information about the patients.

The immunofluorescence was graded as +/- (doubtful) to 4+ (strong). Only scored results of 1+ or greater were considered positive. We believe this technique is specific enough that a result of +1 is positive. There is no nonspecific binding of these conjugates, but some tissues do exhibit autofluorescence.

Results

Dermatopathologic findings

The average number of follicles in patients with AGA was three per 4-mm specimen, with a range of zero to six (Table 3). In comparison, the average number of follicles for control subjects was five per 4-mm specimen, with a range of four to seven. A lymphatic infiltrate is a more prominent feature in study patients than in control patients. In study patients, perifollicular lymphocytic inflammation was seen and, in some cases, a perivascular lymphocytic infiltrate was noted. In six patients, prominent perisebaceous gland inflammation was observed. Scarring (perifollicular fibrosis) in a mild to moderate degree was seen in most of the study patients and in none of the control subjects. Only one control subject had *Demodex sp* as well as bacteria, *Pityrosporum ovale*, and inflammation.

Immunopathologic findings

Immunoreactants were found in the scalp of

25 (96%) of 26 study patients (Figs 1 and 2). In these patients, IgM or C3 (or both) was deposited at the basement membrane of follicles, sebaceous glands, or the dermoepidermal junction. Immunoglobulins G and A and fibrinogen were found infrequently (Table 4). Only 1 (12%) of the 8 control specimens displayed immunoreactivity; the specimen was from the uninvolved scalp of a patient with AGA. In this specimen, C3 was present at the basement membrane of the sebaceous gland. (Figure 3 is a negative control IgM with autofluorescence, and Figure 4 is a negative control with C3.) Finding porphyrins in the pilosebaceous canal, around the hair shaft, and in the sebaceous duct was unexpected. Discovered by their natural fluorescence, porphyrins were found in 15 (58%) of 26 study patients and in 1 (12%) of 8 of the control specimens (Fig 5). *Pityrosporum ovale* was found in the pilosebaceous canal and on the stratum corneum by direct microscopic examination of specimens from 2 (8%) of the 26 study patients.

Laboratory findings

One patient with AGA had a mildly elevated leukocyte count (12800/mm³ [limits of normal, 7800 to 11800/mm³]), and erythrocyte sedimentation rate (42 mm/h [limits of normal, 0 to 20 mm/h]). He was clinically well. All other laboratory results in patients were within normal limits.

Discussion

Hamilton¹¹ was the first to demonstrate that adequate levels of androgens and an appropriate genetic background are necessary for the development of male pattern alopecia. He reported that alopecia did not develop in men

Table 3
Dermatopathologic Findings (Hematoxylin-Eosin–Stained Sections)
in Patients With Androgenetic Alopecia

Component/finding	Patients (n = 22)		Controls (n = 6)	
	No.	%	No.	%
• Follicles				
Anagen	43/80	54	30/31	97
Telogen	16/80	20	1/31	3
Catagen	2/80	2	...	...
Undeterminable	19/80	24	...	...
• Scarring*	19/22	86	0	0
• Inflammation†	22/22	100		66‡
• Bacteria §	8/22	36	1/6	17
• <i>Pityrosporum ovale</i> §	2/22	9	3/6//	50
• Parakeratosis	5/16¶	31	0	0
• Spongiosis	2/16¶	12	0	0
• Elastic tissue streamers	6/14#	43	1/6	17
• Arao-Perkins bodies	3/14#	21	0	0
• Actinic elastosis	2/22	9	...	...

* Scarring = perifollicular fibrosis.
† Ranges of inflammation: + 1 = 10 to 20 perifollicular lymphocytes; + 2 = 30 to 50 perifollicular lymphocytes; + 3 = 50 to 100 perifollicular lymphocytes; + 4 > 100 perifollicular lymphocytes.
‡ All had 0 to + 1.
§ Observed by direct observation of sections stained with hematoxylin and eosin.
// One had + 1 and two had + 2.
¶ Dermatopathologist commented on only 16 of 22 patients' sections.
Elastic tissue staining was done in 14 of 22 patients' sections. Seven of 14 were positive; two had both elastic tissue streamers and Arao-Perkins bodies.

with appropriate genetic background who were castrated before puberty. When he gave these same men male hormones, AGA developed. Further, Orentreich¹² showed that full-thickness punch grafts from occipital donor scalp could be transplanted to bald donor scalp. Although the response of the target organ to adequate androgen levels is of prime importance in the development of AGA,¹² other factors, such as inflammation, may be important in its pathogenesis.

Lattanand and Johnson,⁴ who examined 347 tissue specimens from 23 patients, found inflammation in nearly half of the specimens. Abell¹³ analyzed scalp biopsy specimens, sectioned transversely, from 693 patients with

AGA. Hair follicle size and hair shaft diameter, but not hair follicle density, were reduced. A decrease in anagen follicles and an increase in telogen follicles and telogen germinal units were noted. An inflammatory reaction was present in 75% of the specimens, and dermal fibrosis, in 25%. Follicular units had been destroyed by diffuse fibrosis in nearly 5%. Abell¹³ thought that "inflammation, although often insignificant, frequently leads to an inflammatory folliculitis and perifollicular fibrosis and usually to a destructive scarring process." Others¹⁴⁻¹⁸ have not associated significant inflammation with AGA.

Kligman⁹ in 1988 reported that substantial lymphohistiocytic infiltration is highly char-

Table 4
Immunopathologic Results: Number of Biopsy Specimens Positive
for Each Immunoreactant in Study Patients (n = 26)

Site	Immunoreactant					
	IgG	IgM	IgA	C3	Fibrin	Porphyryns
• Basement membrane						
Follicles	4	15	3	9	7	0
Sebaceous glands	5	12	0	12	4	0
Dermoepidermal junction	2	6	1	2	1	0
• Blood vessels	0	13	0	5	4	0
• Myoepithelial cells	0	0	0	8	0	0
• Cytoids	0	3	0	0	0	0
• Pilosebaceous canal	0	0	0	0	0	15

acteristic of male pattern baldness. He found that the inflammatory foci were restricted to perifollicular location around the sebaceous glands and infundibulum. Our dermatopathologic observations are in agreement with those of Abel and Kligman. The dermatopathologic finding of an inflammatory process supports in part the hypothesis of a role for inflammation in the pathogenesis of AGA.

Our immunopathologic findings also support a role for inflammation in the pathogenesis of AGA. Immunoglobulin M or C3 (or both) was detected at the basement membrane in 96% of study patients but in only 12% of control specimens taken from uninvolved scalp of a patient. No immunoreactants were found in any of the other control specimens.

The presence of porphyrins in the pilosebaceous canal of 58% of study patients and in that of 12% of the control subjects may support the thesis that porphyrins may play a role in AGA. Conversely, because 42% of the study patients did not have porphyrins, one must conclude either that porphyrins are not essential in the pathogenesis of AGA, or that our examination may not have been sensitive enough to find porphyrins that were present. We discovered that porphyrins are lost through diffusion from the preparations over time (days to weeks). We hypothesize that this loss is due to the water solubility of porphyrins. Perhaps those slides on which porphy-

rins were not found were not examined soon enough, or perhaps those porphyrins (if present) had diffused away more rapidly. More work needs to be done to answer these questions. Where porphyrins came from is another provocative question. One possible source is the resident microbiologic flora of the pilosebaceous canal.

Propionibacterium acnes has been shown to produce porphyrins.¹⁹ This species is capable of producing inflammation by three different mechanisms. First, *P. acnes* is able to activate complement via both classic and alternative pathways.²⁰ Second, this microorganism produces porphyrins. Porphyrins of *P. acnes* may not be equivalent to human porphyrins, but one can speculate that they would have similar biologic activity as a result of their water solubility and polar molecular configuration. Gigli²¹ and Lim²² and their respective associates have shown that human porphyrins activate complement in the presence of light. Lim and colleagues^{23,24} showed the production of a C5-dependent chemotactic factor. Microbiologic porphyrins may activate complement as do human porphyrins. Further, *P. acnes* may directly or indirectly produce chemotaxis for mononuclear cells, and cytotoxicity.²⁵

The presence of IgM implies that an antigen is present. *Propionibacterium acnes* may be an antigen source. Indeed, Saint-Leger and coworkers²⁶ proposed a pathogenesis for acne

vulgaris that may correlate with our hypothesis for the pathogenesis of AGA. They hypothesized that light-activated *P. acnes* porphyrins oxidize squalene to produce a toxic inflammatory reaction. Light exposure to the scalp may play an important role in initiating inflammation of AGA. Clinical observations of both Hamilton and Ludwig tend to support this hypothesis. Hamilton and Ludwig both observed that in earliest stage AGA in both men and women, the regions involved are those that we have noticed are exposed to sun. As observed clinically, alopecia always seems to start in the hairline part or the vertex whorl of the scalp (or both) in patients with AGA.

Conclusion

Our preliminary findings support the view that inflammation may play a role in AGA. The exact role is unknown, but we speculate that light striking the scalp at the vertex or at the hairline part may excite porphyrins produced by the resident flora, activating complement, and hence generating inflammatory immunoreactants. The inflammatory reaction then generated may exacerbate the degree of alopecia.

The data we presented here, although preliminary, support the view that inflammation may be a major component of AGA, in addition to appropriate genetic background and adequate androgen levels. This hypothesis, if true, has implications for therapeutic management of this extremely common disorder. The use of antimicrobials to decrease counts of *P. acnes* organisms or the reduction of sun exposure (or both) may lessen the extent of male pattern baldness.

Supported by AOA Bureau of Research grant No. 90-18-295.

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