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IMMUNOFLUORESCENCE STUDIES ON COMPLEMENT COMPONENTS IN THE HAIR FOLLICLES OF NORMAL SCALP AND OF SCALP AFFECTED BY ALOPECIA AREATA

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Abstract. The deposition of complement components (Clq, C4 C3, C5 and C9) and properdin in scalp hair follicles was examined by the immunofluorescene method in patients with alopecia areata and in normal subjects. C3, C5 and C9 were found deposited there. The complement deposition was most frequent in the case of C3 and less frequent in C9 and C5. There was no difference regarding deposition between normal subjects and patients with alopecia areata. No deposition of Clq, C4 or properdin was observed in the hair follicles of the scalp. These findings suggest a relationship between the hair cycle and the activity of C3 and its late complement components in the scalp.

Key words: Hair follicle; Complement components; Immunofluorescence study

We have previously reported the deposition of C3, a complement component, in the hair follicles of the scalp (2). Bystryn et al. (1), having observed the deposition of C3 in affected hair follicles of patients with alopecia areata or male pattern alopecia, stated their belief that this phenomenon is involved in the pathogenesis of alopecia. Our study, however, revealed deposition of C3 in the hair follicles of normal subjects, too. Furthermore, since the behavior of C3 deposition reflected the morphological state of hair follicles in each stage of the hair cycle, a relationship between the hair cycle and C3 was suggested (2).

The exact role of C3 in the hair follicle of the scalp is still completely unknown. This prompted us to undertake the present study which evaluated the deposition of complement components other than C3 in the hair follicles of the scalp. If deposition of other complement components could be confirmed, this would lend strong support to the theory of a relationship between hair follicles of the scalp and complement components.

In the present study an attempt was made to examine by the immunofluorescence method the deposition of Clq, C4, C3, C5, C9 and properdin in the hair follicles of the scalps affected by alopecia areata and in normal controls. The study succeeded in demonstrating the deposition of C3, C5 and C9 and suggested a relationship existing between the hair cycle and the activity of C3 and its late complement components.

MATERIALS AND METHODS

Normal hair follicles and follicles affected by alopecia areata were obtained. Normal hair follicles were collected from 8 patients during resection of benign tumors of the scalp. Of these 8 patients, 3 were female and 5 were male. Their ages ranged from 9 to 65 years; 4 of these cases have been reported previously (2). Of 15 patients with alopecia areata, 5 were female and 10 were male. Their ages ranged from 14 to 40 years; 9 of these patients have been described previously (2). Biopsy material was taken from the margin of the lesion in each alopecia areata patient. The details of the direct immunofluorescence method are as described in our previous study (2).

Indirect immunofluorescence method

The deposition of C9 and properdin was examined by the following technique. Cryostat sections were washed in phosphate-buffered saline (PBS), pH 7.2 for 15–20 min and then incubated with unlabeled rabbit anti-human C9 or unlabeled rabbit anti-human properdin. This was performed in a moist chamber at room temperature for approximately 1 h. After further washing in PBS for 20 min the sections were incubated again, this time with fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG under the above conditions for 30 min. The cultures were then washed in PBS for 20 min, and sealed in buffered glycerine before examination by fluorescence microscopy (Olympus, type BHL, Japan).

Conjugates

All conjugates used except anti-human properdin were obtained from commercial sources. FITC-labeled rabbit

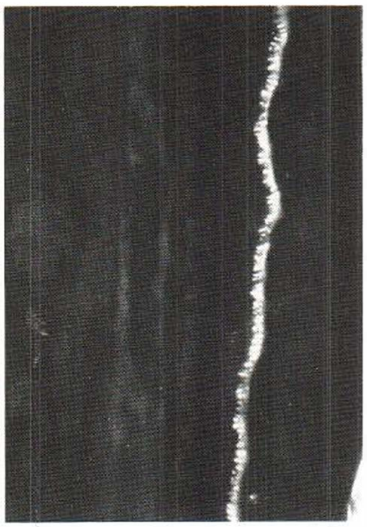

Fig. 1. IF staining of anagen follicle in normal scalp, showing linear deposition of C9 in connective tissue sheath of the internal layer (×234).

anti-human complement was available for Clq, C3, C4 and C5. The characteristics of these antisera are summarized in Table I, along with those of FITC-labeled goat anti-rabbit IgG. The unlabeled rabbit anti-human C9 was prepared by Behringwerke (West Germany), and the unlabeled rabbit anti-human properdin was furnished by Dr Konno (3) at the Department of Biochemistry, Hokkaido University School of Medicine, Japan.

The specificity of each of these antisera was ascertained by immuno-electrophoresis, Ouchterlony's method. A blocking test with unlabeled antisera was also performed. In this test it was determined that fluorescence was extinguished or was attenuated with combinations of complement components of the same class, whereas it neither disappeared nor was attenuated with certain other complement components.

RESULTS

Deposition of complements C3, C5 and C9 was found in hair follicles in normal scalps and in those of patients with alopecia areata. The incidence of deposition in the hair follicle was highest for C3, followed by C9 and then C5. In this regard, there was no overt difference between normal scalps and those of affected patients. No deposition of Clq, C4 or properdin was detected in either normal or affected hair follicles.

Findings in normal scalp

In normal scalp there was deposition of C3 in both anagen phase and catagen phase follicles. In anagen follicles, C3 deposition was found mainly in the hair bulb and the transient portion of the hair follicle, whereas in catagen follicles rich deposition of C3 was observed in the portion of thickened hyaline membrane. These features agree with findings reported previously (2). All anagen follicles were positive for C3 in 8 cases. Catagen follicles are very rare in the normal scalp; it is necessary prepare quite a number of specimens in order to obtain them. Such catagen follicles were present in only 4 cases and all revealed C3 deposition.

The search for deposition of C5 in anagen follicles proved positive in 3 of 7 cases examined, but

Table I. *Characteristics of FITC-labeled antisera*

	FITC content (μg/ml)	Protein content (mg/ml)	F/P molar ratio
Anti-human Clq			
(Behringwerde)[a]	45.2	23.7	1.0
Anti-human C4			
(Med. & Biol. Labs.[b]	13.9	4.1	1.4
Behringwerke)	63.5	25.1	1.0
Anti-human C3			
(Med. & Bio. Labs	14.2	4.8	1.2
Dakopatts)[c]	12.2	2.4	2.1
Anti-human C5			
(Med. & Bio. Labs.)	10.6	4.1	1.1
Anti-rabbit IgG			
(Med. & Bio. Labs.)	36.6	10.0	1.5

[a] West Germany. [b] Japan. [c] Denmark.

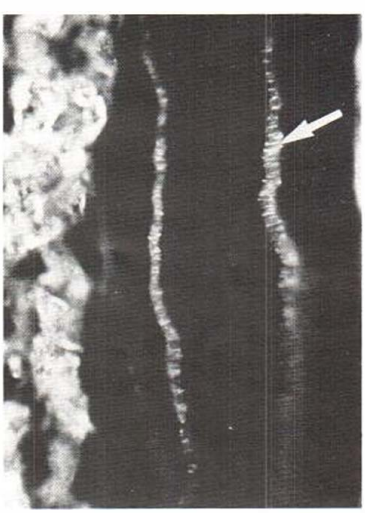

Fig. 2. IF staining of catagen follicle in normal scalp, showing heavy deposition (arrow) of C9 in thickened hyaline membrane (×117).

Table II. *Immunofluorescence findings in hair follicles of normal subjects (8 cases)*

	C1q	C4	Properdin	C3	C5	C9
Anagen	0/7[a]	0/7	0/3	8/8	3/7	4/5
Catagen	0/5	0/5	0/1	4/4	0/3	1/1

[a] Number of positive cases/number of cases examined.

none was found in the hair bulb. In the transient portion of the hair follicle there was deposition of C5 in the internal layer (annular) of the connective tissue sheath and its pattern was the same as for C3. Catagen follicles were identified in 3 cases but none showed C5 deposition.

In anagen follicles deposition of C9 was found in 4 of 5 cases, and at the same sites as C3 (Fig. 1). Catagen follicles were found in only 1 of 5 cases despite of the collection of many specimens. In this instance there was marked deposition of C9 in the corresponding portion of the thickened hyaline membrane (Fig. 2).

None of the hair follicles in the scalp examined proved positive for Clq, C4, or properdin, but the number fo cases (3 cases) studied was limited. These results are summarized in Table II.

Findings in scalps affected by alopecia areata

In this disease hair follicles often undergo catagen-like changes. The behavior of C3 deposition in such follicles resembled that in catagen follicles in the normal scalp, with prominent deposition in the portion of thickened hyaline membrane. There was C3 deposition in anagen-like follicles as well in anagen follicles of the normal scalp. With respect to site and pattern of deposition, there was no difference between the two. Deposits of C3 were present in all anagen-like follicles (9 cases) and catagen-like follicles (13 cases).

Of the affected follicles, anagen-like ones were positive for C5 deposition in 2 of 12 cases. The site of deposition was the internal layer (annular) of the connective tissue sheath in the transient portion of the hair follicle, but none was seen in the hair bulb. In catagen-like follicles there was no deposition of C5 in the thickened hyaline membrane, while anagen-like follicles deposition of C9 was found in 7 of 9 cases (Fig. 3) and in catagen-like follicles in 8 of 13 cases (Fig. 4). No deposition of C9 was demonstrated in the hair bulb of anagen-like follicles, although the other results of the immunofluorescence study were similar to those for anagen follicles in normal scalp.

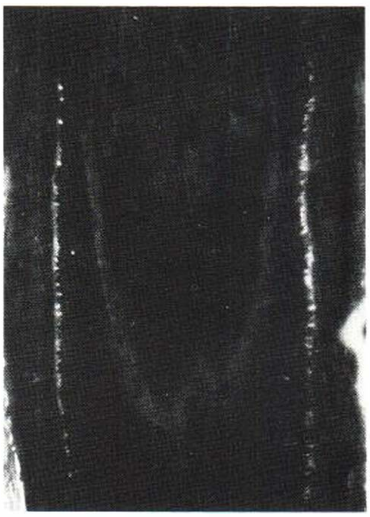

Fig. 3. IF staining of anagen-like follicle in a lesion of alopecia areata, showing linear deposition of C9 in the connective tissue sheath of the internal layer (×117).

No deposition of Clq, C4 or properdin was observed in hair follicles of patients with alopecia areata.

All these results are summarized in Table III.

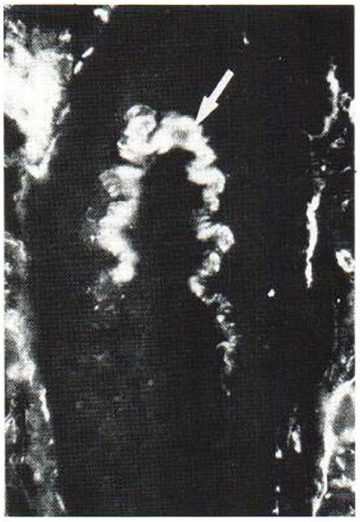

Fig. 4. IF staining of catagen-like follicle in a lesion of alopecia areata, showing heavy deposition (arrow) of C9 in the thickened hyaline membrane (×117).

Table III. *Immunofluorescence findings in hair follicles of patients with alopecia areata (15 cases)*

	C1q	C4	Properdin	C3	C5	C9
Anagen-like	0/12[a]	0/9	0/5	9/9	2/12	7/9
Catagen-like	0/15	0/12	0/6	13/13	0/13	8/13

[a] Number of positive cases/number of cases examined.

DISCUSSION

Our present study revealed that both C5 and C9, as well as C3, are present in the scalp hair follicle. There was no deposition of C5 or C9 in the hair bulb, nor deposition of C5 in the thickened hyaline membrane of catagen or catagen-like follicles. Other findings of C5 and C9 by immunofluorescence technique were much the same as those of C3. No definite difference existed between normal hair follicles and those of patients with alopecia areata.

C3 deposition in scalp hair follicles was reported by Bystryn et al. (1) as well as in our previous study (2). They used the immunofluorescence method to study patients with alopecia areata, alopecia totalis and male pattern alopecia and found frequent C3 deposition in alopecia areata. They suggested that C3 may be involved in the pathogenesis of alopecia areata, since the site of preferential accumulation of C3 in the lower segment of hair follicles in alopecia areata is similar to that of lymphoid cell accumulation.

By contrast, our previous study (2) did not reveal any difference between the normal scalp and that affected by alopecia areata as regards immunofluorescence features. In the present study, the results obtained were the same. Hence it seems unlikely that deposition of complement components in scalp hair follicles is involved in the pathogenesis of alopecia areata. As regards functional aspects, however, some difference might exist between complement components deposited in hair follicles of normal scalp and those in hair follicles of scalp affected by alopecia. This consideration led us to examine C3 as well as other complement components, but no significant difference could be detected between the two.

It is well known that there are two pathways leading to activation of the various components of the complement sequence—the classical and the alternative. Our present study was undertaken partly to elucidate these pathways, but failed to demonstrate deposition of C1q, C4 or properdin in the hair follicle. On the other hand, the fact that C3, C5 and C9 were detected suggests a relationship between the hair follicle and the activity of the complement cascade after C3.

Late acting complement components C5–C9 are immunologically important because of their known ability to damage cell membranes. Mayer (4) gave the following explanation of the mechanism of membrane damage. C5–C9 aggregate in an annular fashion on the surface of cell membranes, forming themselves into a doughnut shape. This formation finds its way to the lipid bilayer of the cell membrane, permitting efflux of ions and influx of water molecules, resulting in cell rupture. This phenomenon takes place at biologic membranes which are structurally characterized by protein molecules floating in lipid bilayer (5). Deposition of C5 or C9, by contrast, occurs in the connective tissue sheath of the hair follicle. C9 also becomes deposited in the thickened hyaline membrane. Whether complement components cause damage to connective tissue remains unknown. It is conceivable that C5–C9 affect the connective tissue of the hair follicle, and through this mechanism participate in the conversion of anagen follicles to catagen follicles.

The role of complement components in the hair follicle is poorly understood. However, our present study has shown that they are present both in normal scalp and in the hair follicles of patients with alopecia areata. Complement components often become deposited in the transient portion of the hair follicle. The pattern of deposition varies in a consistent manner according to the morphology of the hair follicle. These results suggest a relationship between the hair cycle and the activity of complement components C3–C9.

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