Acta Dermatovener (Stockholm) 60: 33–37, 1980

IMMUNOFLUORESCENT STUDIES OF COMPLEMENT C3 IN THE HAIR FOLLICLES OF NORMAL SCALP AND OF SCALP AFFECTED BY ALOPECIA AREATA

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Abstract. The deposition of complement C3 in the hair follicles of scalps of 4 normal subjects and of the affected parts of 9 patients with alopecia areata was found by the direct immunofluorescent method. No deposition of immunoglobulins (IgG, IgA, IgM, IgD and IgE) was found. In the normal scalps, complement C3 was found to be deposited in the hyaline membrane of the hair bulb and in the internal layer of connective tissue sheath at the lower about 2/3 transient portion of anagen follicles, and in the thickened hyaline membrane of the catagen follicles. The deposition of complement C3 was also found in the anagen and catagen-like hair follicles even in the patients with alopecia areata, which was similar to the finding in the normal scalps. From the fact that the deposition of complement C3 was found chiefly in the transient portion of the hair follicles of the normal scalps, and that the behavior of deposition of complement C3 reflected the morphological state of hair follicles in each stage of the hair cycle, even in alopecia areata, complement C3 may be intimately related to the hair cycle, even though its significance is as yet unknown.

Key words: Hair follicle; Alopecia areata; Complement C3; Immunoglobulin; C3 deposition; Immunofluorescence

The etiology of alopecia areata has not yet been clarified, even though several hypotheses have been suggested. Alopecia areata is known to be complicated frequently with autoimmune-related diseases and to be related to immunological abnormality. From the fact that lymphocytic infiltration around and/or within the hair matrix has been histologically demonstrated (7, 11, 12) and that recently a decrease in the number of circulating T-lymphocytes was demonstrated in a patient with alopecia areata (4, 6), attention has been called to its relation to cell-mediated immunity. On the other hand, many investigators have denied the involvement of humoral immunity in patients with alopecia areata, e.g., immunoglobulin deposits in the hair follicles of the affected areas and autoantibodies to the hair follicle (3, 5, 8).

The complement components in patients with alopecia areata were studied by Berrens et al. (2), who found no abnormalities in the sera. However, nothing appears to have been reported on the positive deposition of the complement components in the hair follicles (9).

In the present study, the authors made an immunohistochemical investigation of the deposition of complement C3 in patients with alopecia areata and found that the complement was deposited in the hair follicles. A similar finding was also forthcoming in the hair follicles of normal human scalps, which proved that the deposition of complement C3 in the hair follicles was not characteristic of alopecia areata. These appear to be interesting findings suggestive of a relationship between the hair cycle and complement components. Details of the findings are presented in this paper.

MATERIALS AND METHODS

Normal scalp specimens were available from 4 patients who underwent resection of benign skin tumors of the scalp. They comprised 2 women and 2 men, aged 21–65 years. Table I shows the age, sex and biopsied site of each patient, together with the results.

Nine patients with alopecia areata were selected, comprising 3 women and 6 men aged 20–40 years. Table II shows the clinical findings in them, together with results. Biopsy was performed in the margin of the lesion of each patient.

The tissue specimen obtained from each of these patients was frozen with dry ice–acetone immediately after biopsy, and stored at −70°C until use. The specimen was sectioned at 4–5 μm thickness with the cryostat before use, and the sections were dried in air for 15–30 min. Part of each section was fixed in 99.5% ethyl alcohol at −20°C. The direct immunofluorescent method was performed on these unfixed or fixed sections. The sections were washed with phosphate-buffered saline (PBS), pH 7.2, for 15 min, and incubated with the conjugate in a moist chamber at room temperature for 30 min. The sections were then washed with PBS for 15 min, sealed with buffered

Table I. *Immunofluorescent findings (C3) in normal scalps.*
ND=not done

Case no.	Sex	Age (y.)	Biopsy site	Deposition of complement C3	
				Anagen follicles	Catagen follicles
1	F	21	lt-temporal	+	+
2	F	26	Parietal	+	+
3	M	37	lt-occipital	+	+
4	M	65	lt-temporal	+	ND

glycerol, and observed under the fluorescence microscope (the Olympus, type BHF). The sections observed were then stained with hematoxylin and eosin, to observe the histology of hair follicle under the light microscope.

Conjugates

Fluorescein isothiocyanate (FITC)-labeled rabbit antihuman complement C3, available commercially from two suppliers, was used (Medical and Biological Labs., Japan, and Dakopatts, Denmark). Table III shows the characteristics of these antisera. Their specificity was proved by immunoelectrophoresis, absorption test with zymosan (Sigma Chemical Co., St. Louis, Mo.) and blocking test using the unlabeled rabbit antihuman complement C3 (Dakopatts, Denmark). The deposition of immunoglobulins (FITC-labeled rabbit antihuman IgG, IgA and IgM; Dakopatts, Denmark, and IgD and IgE; Behringwerke, West Germany) was also studied as controls.

RESULTS

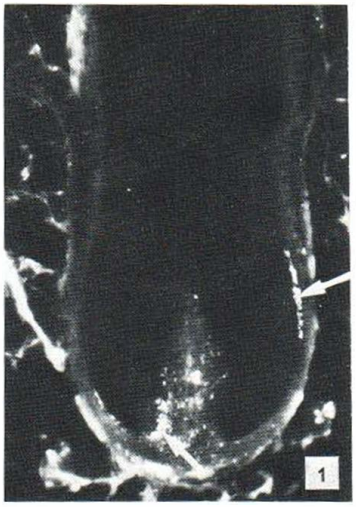
The deposition of complement C3 was found in the anagen and catagen hair follicles of the normal scalps and chiefly in the hair follicles presenting catagen-like changes in alopecia areata. There was no difference in the deposition of complement C3 between the conjugates available from the two suppliers. No deposition of immunoglobulins was found in the hair follicles of the affected area of alopecia areata or of the normal scalps.

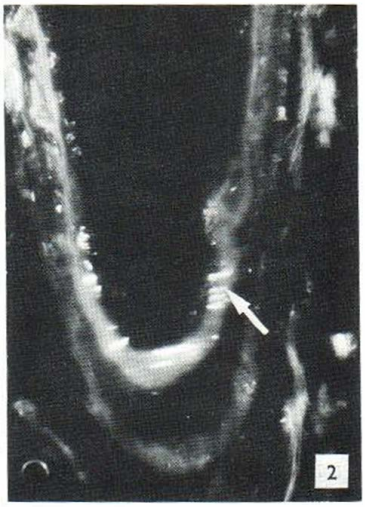
Findings in the normal scalp

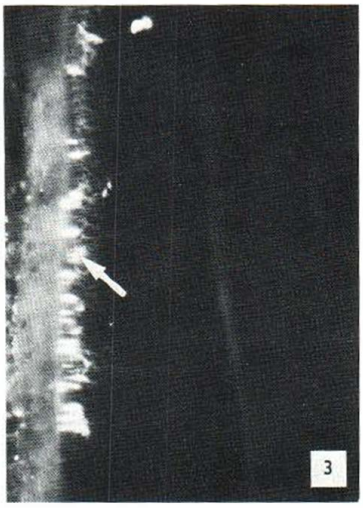
The granular or reticular pattern of complement C3 was found to be deposited in part of what appeared to be the hyaline membrane of the hair bulb of anagen hair follicles. The deposition was found even in the dermal papillae on some sections, but there was no deposition in the upper portion of dermal papillae (Fig. 1). The deposition of complement C3 was also discovered in the internal layer (annular) of the connective tissue sheath at the lower about $\frac{2}{3}$ transient portion of the hair follicle, which was linear and perpendicular to the long axis of the follicle, running along with the collagenous

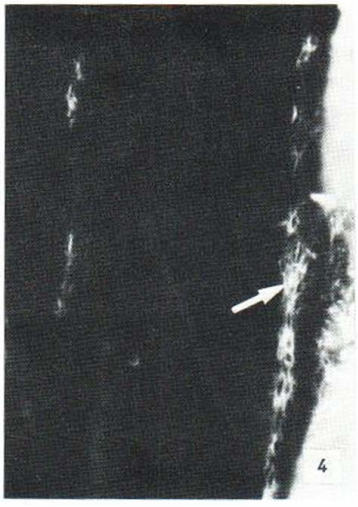
Table II. *Immunofluorescence finding (C3) in alopecia areata*
ND=not done

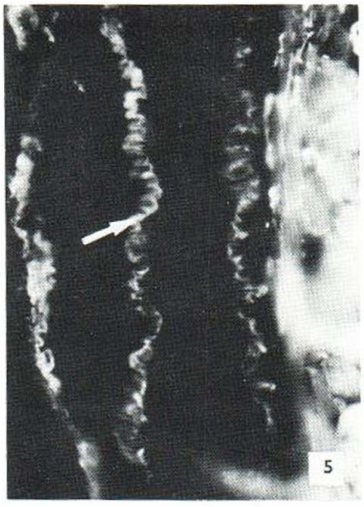
Case no.	Sex	Age (y.)	Diagnosis	Duration	Deposition of complement C3	
					Anagen follicle	Catagen-like follicle
5	M	23	Alopecia areata (single patch)	1 day	+	+
6	M	20		4 months	ND	+
7	M	24		3 months	ND	+
8	F	25	Alopeicia areata	1 year	+	+
9	M	37	(multiple patches)	9 months	+	+
10	M	40		1 month	+	+
11	F	20		2 months	ND	+
12	F	25	Alopecia totalis	5 months	+	+
13	M	25	Alopecia universalis	3 months	+	+











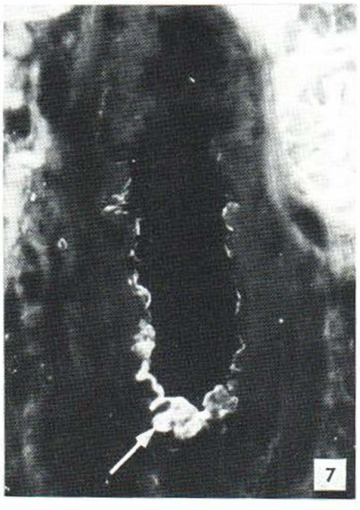


Fig. 1. IF staining of the anagen follicle in normal scalp, showing granular deposition (*arrows*) of C3 in the hyaline membrane of hair bulb (case 2, ×117).

Fig. 2. IF staining of the anagen follicle in normal scalp, showing linear deposition (*arrow*) of C3 in the connective tissue sheath of the internal layer (case 2, ×117).

Fig. 3. IF staining of the anagen follicle in normal scalp, showing linear and intercellular deposition (*arrow*) of C3 in the outer root sheath (case 2, ×234).

Fig. 4. IF staining of the anagen follicle in normal scalp, showing intercellular deposition (*arrow*) of C3 in the outer root sheath at the level of the sebaceous gland (case 2, ×234).

Fig. 5. IF staining of the catagen follicle in normal scalp, showing heavy deposition (*arrow*) of C3 in the thickened hyaline membrane (case 3, ×117).

Fig. 6. IF staining of the catagen-like follicle in the lesion of alopecia areata, showing heavy deposition (*arrow*) of C3 in the thickened hyaline membrane (case 7, ×117).

Fig. 7. IF staining of the catagen-like follicle in the lesion of alopecia areata, showing partial deposition (*arrow*) of C3 in the thickened hyaline membrane (case 5, ×117).

Table III. *Characteristics of FITC-labeled anti-C3*

	FITC content (μg/ml)	Protein content (ng/ml)	F/P molar ratio
Med. & Bio. Labs.	14.2	4.77	1.2
Dakopatts	12.2	2.4	2.1

fibers (Fig. 2). The deposition was also found in the intercellular spaces of some outer root sheaths (Fig. 3). The deposition was scanty in the permanent portion of the hair follicles, but was found in the intercellular spaces of outer root sheaths at the level of the sebaceous glands in some patients (Fig. 4).

In hair follicles assuming the shape of a catagen follicle, the deposition of complement C3 was striking in the thickened hyaline membrane (Fig. 5). The catagen follicles were scanty in the normal scalp, but when many sections were prepared from them, the catagen follicles were found in 3 patients, and the deposition of complement C3 in the catagen follicles was found in all three.

The telogen follicle was found in only one patient, in whom no deposition of complement C3 was found. These findings are summarized in Table I.

Findings in scalps of patients with alopecia areata

Although the anagen hair follicles were scanty in the tissue specimens biopsied in this study, the anagen follicles in the area involved by a alopecia areata were all attended by many melanin granules in the dermal papillae or in the part adjacent to the hair bulb, and many matrix cells were also degenerated. The deposition of complement C3 in these hair follicles was similar to that in the anagen hair follicles in the normal scalp, there being no difference in the findings made by the immunofluorescent method.

On the other hand, in the hair follicles presenting catagen-like changes, as in the catagen hair follicles in the normal scalp, the deposition of complement C3 was striking in the thickened hyaline membrane (Fig. 6). This marked deposition of complement C3 in the hyaline membrane occurred in membrane that was lightly stained with eosin, but the deposition was scanty in the deeply stained hyaline membrane where it occurred only in part (Fig. 7). These findings are summarized in Table II.

DISCUSSION

The present study disclosed the occurrence of complement C3 in the hair follicles of the scalp. This paper may be the first to describe the discovery of complement C3 in the hair follicles of normal scalps and of scalps affected by alopecia areata.

What is the role of this complement C3 in the hair follicles? According to the authors' findings, there was no difference in the deposition of complement C3 in the hair follicles between normal scalps and the scalps affected by alopecia areata. Thus, this deposition cannot be considered to be related to the cause of this disease. Berrens et al., (2) found no difference in the serum complement components between patients with alopecia areata and normal subjects.

It would appear that complement C3 is closely related to the hair cycle, that the deposition of complement C3 in the hair follicles of the normal scalps occurred chiefly in the transient portion of the hair follicles, and that the behavior of the deposition was reflected in the morphological state of hair follicles in each stage of the hair cycle, even in alopecia areata.

Generally, the routes of activation of complements are divided into the classical and the alternative pathways, and also known to be related to the coagulation and fibrinolytic systems. Plasmin in the fibrinolytic system has also been considered to activate complement C1s (10) and C3 (13). It is not known by which of these pathways the deposition of complement C3 in the hair follicle takes. From the fact that the deposition of complement C3 in the hair follicles occurred chiefly in the connective tissue sheath surrounding the hair follicles and in the thickened hyaline membrane, the components of the connective tissues surrounding the hair follicles may be considered to be related to complement components.

It is well known that the connective tissues are close in the amino acid sequence of chemical structure to the first complement component, C1q. Recently, Al-Adnani et al. (1) found that the human skin fibroblasts have C1q-producing and secreting activities. These findings suggest the possibility that complement C3 found in the hair follicles results from the activation of complement C1q.

To clarify the significance of C3 deposition in the hair follicle, further studies of this problem are now in progress.

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Received June 1, 1979

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