

A Study of Immunologic Difference Between Responders and Non-responders to Diphencyprone in Patients with Alopecia Areata

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Background: The pathogenesis of alopecia areata is still unknown, however autoimmune mechanism is strongly suggested. The topical immunotherapy using potent sensitizer has been used as new therapeutic modality. By this method in one half and to one third of the patients, hair growth is observed.

Objective: To evaluate the immunological profile between responders and non-responders to diphencyprone (DPCP) topical immunotherapy in alopecia areata patients.

Methods: After sensitization, DPCP was applied to the patients' scalp weekly for three months. Before and after treatment the therapeutic effect was evaluated by clinical observation by following items: complete baldness, baldness+vellus, baldness+terminal hair and normal hair. Peripheral T cell and T cell subsets, B cell and delayed hypersensitivity with various antigens were evaluated before and after treatment.

Results: The immunologic difference between responders and non-responders was not statistically different.

Conclusion: It is suggested that no major immunologic difference was observed between responders and non-responders before and after DPCP topical immunotherapy. Local mechanism seems to be related in the response to immunotherapy.

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Key Words: Alopecia areata, Immunotherapy, Immune parameter, Diphencyprone

The pathogenesis of alopecia areata is still unknown, however autoimmune mechanism has been suggested. There have been some contradictory reports of the immunologic parameters about alopecia areata. For example, there has been a report of no major disturbance of humoral immunity except elevation of organ specific auto-antibodies¹ and reports of change of lymphocytes subset; increase, decrease or normal²⁻⁵. There is also a report of anergy to delayed skin tests in certain cases of alopecia areata¹.

Several treatment modalities for alopecia areata have been suggested, however the prognosis is not so good in cases with extensive alopecia. Recently, immunotherapy has been tried using DNCB, squaric acid dibutylester, diphencyprone for the treatment of extensive alopecia. However, DNCB is discouraged due to mutagenicity and squaric acid dibutylester also due to high cost and instability. Diphencyprone (DPCP) seems to be superior to both agents due to lack of the disadvantages mentioned above and there are reports of its use in severe alopecia areata⁶.

Only about 1/2 to 1/3 of the treated cases were responsive to the DPCP treatment⁷⁻⁹ which suggests that there exist some heterogeneity of immunologic profile in cases with alopecia areata.

The aim of this study is know whether there is some difference of immunologic parameters between DPCP responders and non-responders in

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cases with moderate to severe alopecia areata before and after immunologic treatment. As immune parameters we used skin tests for delayed hypersensitivity and peripheral T cells, T cell subsets and B cells.

MATERIAL AND METHODS

Subjects Eighteen patients who visited our hair clinic were included in this study. Eight were female and 10 were male. Among the subtypes of alopecia, patients with alopecia areata were 8, patients with alopecia totalis were 3 and patients with alopecia universalis were 7. The mean age was 20.5 and distributed between 5 and 57 years (Table 1).

Table 1. Clinical characteristic of patients

Male: Female	10:8
Subtypes*	
AA	8
AT	3
AU	7
Age (mean)	20.5

Sensitization and challenge 2,3-diphenylcyclopropanone-1 (DPCP) was dissolved in acetone and solutions of 0.0001%-2% concentrations were made. For sensitization, 0.1 cc 0.1% DPCP solution was applied on the skin of upper left arm circled by a plastic ring of 2 cm diameter and it was dried up by a hair dryer. Sensitization was checked after two weeks by observation of skin inflammation on the application site. If there was no evidence of flare up sign one more sensitization have been performed and further checked with 0.0001% DPCP patch test. 0.0001-0.1% DPCP solution were applied to the patient's scalp every week and concentrations were adjusted which can sustain persistent itching of 2-3 days and can produces mild inflammation.

Evaluation of treatment effect The degree of alopecia was evaluated by the visual inspection of the area percentage of complete baldness, baldness with vellus hair, baldness with terminal hair (under 1cm) and normal (over 1cm length of terminal hair). It was estimated before and 3 months after treatment. We regard responders as the cases

of increased normal hair population (over 1cm length of terminal hair) and other were regarded as non-responders.

Immune parameters Before DPCP sensitization peripheral T cell subsets (T3, T4, T8) and B cell were checked, and recall antigens test for delayed hypersensitivity were performed with multitest CMI kit (Pasteur, Merieux, France). Three months after treatment the same immune parameters were repeated.

Statistics Wilcoxon rank sum test and Wilcoxon signed-rank test were used for the statistic evaluation.

RESULTS

Eight out of 18 were responders and 10 were non-responders. In responders the mean normal area was increased from 26.6% to 51.0%, however in non-responders the mean change was not marked between before (19%) and after (18%) treatment (Table 2,3).

Before treatment, the total T cell was 70.3% in responders and 67.8% in non-responders, the helper T cell was 39.5% in responders and 35.4% in non-responders, suppressor T cell was 31.1% in responders and 34.0% in non responders, and the B cell result 9.7% in responders and 7.7% in non-responders which did not show statistically significant difference. The multi-test CMI result showed 11.5 in responders and 10.7 in nonresponders which were not significantly different (Table 4).

After treatment, the total T cell was 69.4% in responders and 65.4% in non-responders, the helper T cell was 37.6% in responders and 33.7% in non-responders, and the suppressor T cell was 32.9% in responders and 31.8% in non-responders, and the B cell result was 9.4% in responders and 9.1% in non-responders which were not statistically different each other. The multi-test CMI result after treatment was 12.9 in responders and 11.3 in non-responders which was not significantly different (Table 5).

In responders as well as in non-responders all immune parameters were not statistically different each other between before and after treatment (Table 4,5).

Table 2. Clinical assessment in responsive group

	Pretreatment				Posttreatment			
	CB	B+V	B+T	N	CB	B+V	B+T	N
1	0	60	10	30	0	10	20	70
2	60	10	10	20	30	30	10	20
3	0	15	15	70	0	0	5	95
4	99	0	0	1	25	25	10	40
5	0	25	25	50	0	10	20	70
6	10	40	20	30	5	10	5	80
7	15	15	20	50	5	15	10	70
8	50	30	5	15	30	5	5	60
Mean	24.4	19.5	10.5	26.6	9.5	10.5	8.5	51.0

CB: complete baldness, B+V: baldness+vellus hair

B+T: baldness+terminal hair, N: normal

Table 3. Clinical assessment in non-responsive group

	Pretreatment				Posttreatment			
	CB	B+V	B+T	N	CB	B+V	B+T	N
1	100	0	0	0	100	0	0	0
2	100	0	0	0	99	1	0	0
3	15	15	0	70	15	15	10	60
4	0	30	10	60	0	35	10	60
5	50	20	30	0	90	10	0	0
6	100	0	0	0	100	0	0	0
7	70	30	0	0	70	30	0	0
8	70	25	5	0	100	0	0	0
9	5	30	5	60	5	20	15	60
10	30	60	10	0	50	30	20	0
Mean	54.0	21.0	6.0	19.0	62.9	14.1	5.5	18.0

CB: complete baldness, B+V: baldness+vellus hair

B+T: baldness+terminal hair, N: normal

Table 4. Pretreatment immunologic profile

T ₃		T ₄		T ₈		B		CMI	
R*	NR*	R*	NR*	R*	NR*	R*	NR*	R*	NR*
76	65	46	43	31	25	14	4	12.5	10
64	65	36	25	31	47	10	8	10	13.5
64	58	23	41	38	19	16	12	7.5	16.5
77	70	35	37	41	35	4	3	14.5	8
75	80	49	32	28	49	10	9	7	11
72	73	44	44	30	32	3	8	13	2
82	69	48	42	32	24	11	10	15	15
52	69	35	36	18	36			12.5	3
	66		30		33				19
	63		24		40				9
70.3	67.8	39.5	35.4	31.1	34.0	9.7	7.7	11.5	10.7

*: P>0.05, statistically not significant

R: responders

NR: non-responders

Table 5. Pretreatment immunologic profile

T ₃		T ₄		T _s		B		CMI	
R*	NR*	R*	NR*	R*	NR*	R*	NR*	R*	NR*
79	63	33	42	49	20	5	10	3	2
68	69	43	33	28	39	18	10	36	7
64	67	22	34	45	31	12	10	2	23.5
80	65	39	37	42	25	6	7	17	11
82	70	68	36	17	37	14	11	12	11
55	66	31	36	21	29	7	9	7	12
73	65	35	32	40	32	4	7	15.5	13.5
54	74	30	24	21	47			11	9.5
	67		44		27				10
	48		19		31				13
69.4	65.4	37.6	33.7	32.9	31.8	9.4	9.1	12.9	11.3

*: P>0.05, statistically not significant

R: responders

NR: non-responders

DISCUSSION

In the pathogenesis of alopecia areata, there have been some suggestions that immune mechanism may play a major role¹⁰. For example, 1) alopecia areata sometimes associated with other autoimmune diseases such as thyroiditis, vitiligo and myasthenia gravis¹⁰. 2) There have been reports of peripheral blood lymphocytes changes¹. 3) Helper T cells^{11, 12} and immune complex¹⁰ and Langerhans cells¹³ are infiltrated around hair follicles, and MHC class I and II can be expressed which are not observed in ordinary hair follicles¹⁴. 4) Cases with alopecia areata frequently respond to immunotherapies. 5) And there were reports of observation of thyroglobulin and parietal cell antibodies although humoral immunity is not regarded so important¹.

There have been contradictory reports about peripheral lymphocytes change in alopecia areata. Hordinsky et al² reported the suppressor T cell population is increased in alopecia areata, while, Ledesma et al³ reported it is decreased. Todes-Taylor et al⁴ reported there was no difference in total T cell, and T cell subsets in alopecia compared with control and Baadsgaard¹⁵ reported similar result. Our data did not show any marked change in T cell, T cell subsets and B cell in alopecia areata.

Lowy¹⁵ reported no total T cell change in alopecia areata patients during isopronosine treat-

ment, while van Neste¹⁶ reported change of lymphocytes subset during DNCB treatment. Our result showed that there were no difference in immune parameters of alopecia areata before and after treatment either in responders or in non-responders. Therefore, in the mechanism of hair growth by immunotherapy a local effect is important than a systemic effect which has been already suggested by Monk¹⁷. The mechanism has been also suspected by T cell subset change around the affected hair follicles^{18, 19} and antigenic competition theory²⁰ by non specific antigens.

There was a report of anergy of delayed hypersensitivity in alopecia areata patients¹, however the multi-test CMI result of our study did not show any change before and after treatment in both responders and non-responders similar to other authors²¹. This is opposite to Brocker's recent observation²² that delayed hypersensitivity decrease during local immunotherapy.

Hordinsky et al² reported B cell is decreased in alopecia areata, however our result showed that in B cell, no marked change was observed before and after immunotherapy as other authors²¹.

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