

Influence of Th2 cells on hair cycle/growth after repeated cutaneous application of hapten

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Summary

Exposure to contact allergens in order to produce allergic contact dermatitis (ACD) seems to induce hair cycle/growth, but the mechanism of this remains unclear. In the current study, we investigated this mechanism and found that repeated application of hapten induced production of interleukin (IL)-4 in lymph-node immune cells. In addition, hair growth was induced in mice after the adoptive transfer of T-helper (Th)2 cells that had been purified from mice exposed to repeated cutaneous application of hapten. These findings lead us to speculate that Th2 cells that are repeatedly hapten-sensitized are recruited to hapten-challenged skin areas, and thus stimulate the production of IL-4 in the vicinity of the hair follicles, which influences hair cycle/growth. Our results may provide fundamental insights into the mechanism of contact hypersensitivity-induced hair cycle/growth.

It has been reported that producing allergic contact dermatitis (ACD) using hapten stimulates hair growth.¹ Although the modification of immune responses in ACD is considered to be related to the induction of hair growth,² its precise underlying mechanism remains unclear. In this study, we sought to address this issue using contact hypersensitivity (CHS) in a murine model of ACD.^{3,4}

Report

To examine the association between CHS and hair growth, 7-week-old female BALB/c mice were sensitized with 50 µL of 2,4,6-trinitro-1-chlorobenzene (TNCB; 1% solution in acetone) on the shaved abdominal skin, and divided into three groups. The 'acute'

CHS group was resensitized at the same site on days 9 and 15 (Fig. 1a), while the 'chronic' CHS group were resensitized every 3 days for 2 weeks (days 0–15) (Fig. 1a). The third group was not given any further treatment. The weight of hair is considered to be a useful method to evaluate hair growth,^{5,6} thus we weighed the hair (mg/cm²) 1 week after the final TNCB treatment. Repeated application of TNCB in the chronic group induced hair growth to a greater degree compared with that in either the untreated control group or the acute group (Fig. 1b,c).

The skin of the mice that received repeated TNCB applications (chronic group) showed hyperkeratosis, dermal lymphocytic infiltration, and more hair follicles in anagen compared with either the acute or control group (Fig. 1d). Because the composition of peribulbar mononuclear cells is modulated in skin lesions of human and murine CHS, we hypothesized that TNCB-sensitized lymphocytes may produce cytokines that promote or affect hair growth.

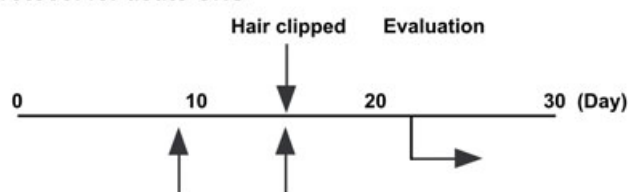
We examined the production by lymphocytes from lymph nodes (LNs) of representative T-helper (Th)1

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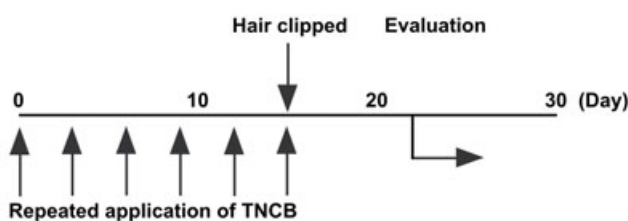
Conflict of interest: none declared.

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(a) Protocol for acute CHS



Protocol for chronic CHS



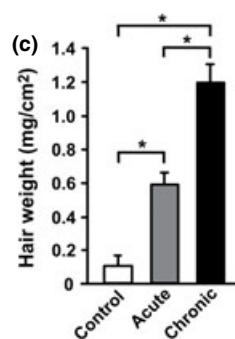
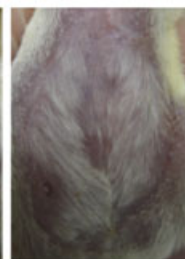
(b) Control



Acute

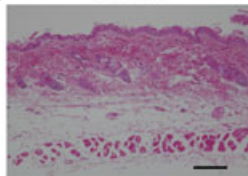


Chronic

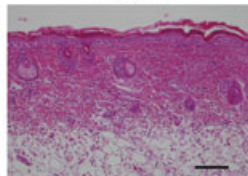


(d)

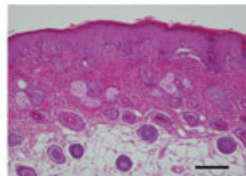
Control



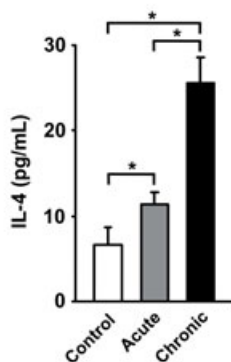
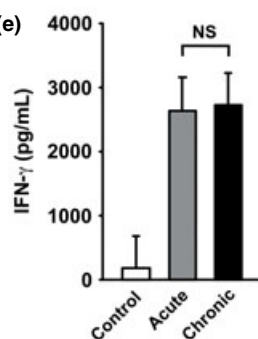
Acute



Chronic



(e)



(f)

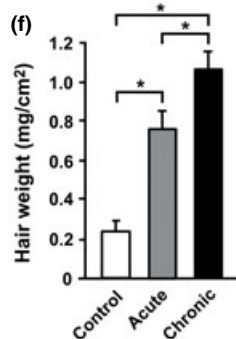


Figure 1 (a) Protocol for acute and chronic contact hypersensitivity (CHS). Mice were sensitized and elicited with applications of 2,4,6-trinitro-1-chlorobenzene (TNCB) on the shaved abdominal skin either on days 9 and 15 (acute CHS) or by six repeated applications of TNCB every 3 days (chronic CHS). Hair growth was then evaluated. (b) Clinical findings of hair growth in untreated (control), acute and chronic CHS groups. (c) Hair weight as an indicator of hair growth in untreated (control), acute and chronic CHS groups. (d) Histological findings of the skin. Skin samples were excised from mice. Scale bar = 100 μ m. (e) Cytokine levels. Immune lymph-node (LN) cells were cultured for 72 h, and the supernatants subjected to cytometric bead array analysis to quantify IFN- γ and IL-4. (f) Hair weight of the mice adoptively transferred with LN cells from untreated (control), acute and chronic groups are depicted. Data are presented as means $\pm$ SEM. * P < 0.01. NS, not significant.

and Th2 cytokines, namely, interferon (IFN)- γ and interleukin (IL)-4, respectively, after TNCB application. Single-cell suspensions were prepared from skin

draining the inguinal and axillary LNs 5 days after the final application of TNCB to the abdomen, and 10^6 LN cells were stimulated with anti-CD3 monoclo-

nal antibody for 72 h before supernatants were collected for measurement of the amounts of IFN- γ and IL-4, using a cytometric bead array system (BD Biosciences, Franklin Lakes, NJ, USA). The amount of IL-4 in the chronic group was much greater than that in the control and acute groups, whereas the level of IFN- γ production was comparable across acute and chronic groups (Fig. 1e).

We then evaluated whether the sensitized T cells modulate hair growth in the acute and chronic CHS models. Using a cell separator, (autoMACS; Miltenyi Biotec, Tokyo, Japan), CD90+ cells were sorted from the inguinal and axillary LNs of each group of mice, and were intravenously transferred into naive BALB/c mice. The recipients were single-challenged with TNCB, and hair weight was evaluated as a marker of hair growth 3 weeks later. In the mice that received CD90+ cells, hair growth was enhanced in the chronic group compared with the acute or the untreated control groups (Fig. 1f).

In this study, we found that the draining LN cells from the chronic CHS group produced more IL-4 and enhanced the hair cycle/growth of naive mice. Therefore, we speculate that repeated sensitization with hapten induces Th2 cells that in turn migrate to TNCB-challenged skin sites and produce IL-4 in the vicinity of the hair follicles, stimulating hair elongation and maintenance of the anagen phase by an undetermined mechanism. Our results may provide fundamental insight into the induction of hair cycle/growth by the local cytokine environment or chronic ACD. Because atopic dermatitis is mediated by Th2 cytokines,⁷ it would be of interest to examine whether hair cycle/growth is enhanced in patients with chronic contact or atopic dermatitis. Our studies may shed some light on the mechanisms underlying the induction of hair cycle/growth by chronic CHS.

Learning points

- Repeated application of hapten enhances hair growth in the murine contact hypersensitivity model.
- IL-4 but not IFN- γ production was increased in the chronic group compared with the control and acute groups.
- IL-4 may influence the hair cycle in chronic contact hypersensitivity.

References

- 1 Li LF, Fiedler VC, Kumar R. The potential role of skin protein kinase C isoforms alpha and delta in mouse hair growth induced by diphencyprone-allergic contact dermatitis. *J Dermatol* 1999; **26**: 98–105.
- 2 Hofmann U, Tokura Y, Ruckert R *et al.* The anagen hair cycle induces systemic immunosuppression of contact hypersensitivity in mice. *Cell Immunol* 1998; **184**: 65–73.
- 3 Egawa G, Kabashima K. Skin as a peripheral lymphoid organ: revisiting the concept of skin-associated lymphoid tissues. *J Invest Dermatol* 2011; **131**: 2178–85.
- 4 Honda T, Egawa G, Grabbe S *et al.* Update of immune events in the murine contact hypersensitivity model: toward the understanding of allergic contact dermatitis. *J Invest Dermatol* 2013; **133**: 303–15.
- 5 Price VH, Menefee E, Strauss PC. Changes in hair weight and hair count in men with androgenetic alopecia, after application of 5% and 2% topical minoxidil, placebo, or no treatment. *J Am Acad Dermatol* 1999; **41**: 717–21.
- 6 Buhl AE, Waldon DJ, Conrad SJ *et al.* Potassium channel conductance: a mechanism affecting hair growth both *in vitro* and *in vivo*. *J Invest Dermatol* 1992; **98**: 315–19.
- 7 Kabashima K. New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity. *J Dermatol Sci* 2013; **70**: 3–11.