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Lack of response to laser comb in spontaneous and graft induced alopecia areata in C3H/HeJ mice

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spontaneous alopecia areata; skin graft-induced alopecia areata; laser comb treatment; autoimmune disease

To the Editor:

Alopecia areata (AA), the second most common form of hair loss in humans, is characterized by waxing and waning focal hair loss. Alopecic foci can expand to encompass the entire head (alopecia totalis) or body (alopecia universalis, AU). This cell mediated autoimmune disease is characterized histologically by the “swarm of bees”, CD4⁺ and CD8⁺ T cells, NK cells, and fewer numbers of other types of inflammatory cells in and around actively growing, anagen stage, hair follicles. Treatment of persistent, moderate to severe AA is palliative at best, often with a poor outcome (Harris *et al.*, 2010). Investigation of AA in humans and nonhuman animal models, primarily C3H/HeJ mice (Sundberg *et al.*, 1994), has provided new insights. Spontaneous AA in C3H/HeJ mice is sporadic, low frequency, and most commonly affects older female mice. Full thickness skin grafts of AA donor skin to young unaffected, immunocompetent, histocompatible recipients provides a highly reproducible model (McElwee *et al.*, 1998) useful in preclinical trials (Sun *et al.*, 2008).

Heat shock was used to induce a type of AA in C3H/HeJ mice (Wikramanayake *et al.*, 2010) and used to determine if a commercially available laser comb induced hair regrowth (Wikramanayake *et al.*, 2012). The laser comb received a 501(k) clearance as a device safe for marketing by the United States Food and Drug Administration for the treatment of male pattern baldness (Leavitt *et al.*, 2009). Laser comb treatment induced cosmetic regrowth of the very small, focal areas of heat shock induced AA after 6 weeks. To test if the laser comb would induce regrowth of hair in older C3H/HeJ mice with spontaneous or graft-induced AU, a similar treatment regimen was used.

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All protocols were approved by The Jackson Laboratory Animal Care and Use Committee. Only C3H/HeJ female mice (JR# 659, The Jackson Laboratory, Bar Harbor, ME) were used. Retired breeders were obtained and aged until approximately 20% developed spontaneous AU. Twelve of these spontaneous AU mice were used directly. Others were used as donors for full thickness skin grafts to female recipients using protocols previously described in detail (McElwee *et al.*, 1998). Twelve grafted mice were aged until they developed AU. Each group was divided into cohorts of 6 mice each and treated. One set of spontaneous and one of graft induced AU were held manually and their ventral abdominal area was exposed for 20 seconds to the laser comb (Hairmax Lux 9, professional 12, laser comb, Lexington International, Boca Raton, FL, wave length 655nm, beam diameter <5 mm, divergence 57 mrad three times per week for 6 weeks). At 6 weeks no change in the pelage was noted so treatments were continued an additional 6 weeks (12 weeks total). The second group of spontaneous or graft-induced mice with AA were treated in the same manner but the laser was not turned off (controls). After 12 weeks mice were euthanized by CO₂ asphyxiation, ventral skin collected, fixed in Fekete's acid alcohol formalin, processed routinely for histology, 5 Lm sections stained with hematoxylin and eosin, and slides examined.

Mice treated with the laser on or off and examined weekly for 6 weeks and then continued for 6 more weeks had no gross (Fig. 1a) or histological differences in skin for either the spontaneous or graft-induced AA mice. Histologic features of AA were confirmed in donor mice and in all 24 mice used in this study (Fig. 1b). There was mild epidermal acanthosis with moderate to marked infiltrates of mostly lymphocytes and fewer granulocytes in and around late anagen stage hair follicles with disrupted outer root sheath architecture. All 24 mice in this study had diffuse AA with generalized hair loss and only remnants of hair whether or not the laser comb was turned on or off (Table 1).

Failure to reproduce the earlier laser comb study to induce regrowth of heat shock-induced AA in dorsal skin of C3H/HeJ mice (Wikramanayake *et al.*, 2012) may be due to the heat-induced lesions being very small, as seen in early stage AA, that also may wax and wane in human AA. In the initial laser comb study the AA lesions were assumed to regrow as only laser treated mice responded. These results are in contrast to the current studies using AU mice rather than early onset, less severely affected AA mice. Alternatively, treating dorsal skin in the initial report versus ventral skin in this study may be an issue. However, dorsal and ventral lesions in both spontaneous and graft/induced lesions respond similarly in most other studies, so this seems unlikely (King *et al.*, 2008).

An alternative issue is whether heat shock/induced AA is representative of human AA. The initial report showed gross and histologic evidence that it was indeed quite similar to the established mouse models (Wikramanayake *et al.*, 2010). However, in the subsequent laser comb study, the gross lesion illustrated was totally different. While the authors stated that they confirmed histologic lesions of AA in two mice prior to treatment, their descriptions and figures after treatment show no evidence of AA. Rather, their sham treated mice (laser turned off) show normal mouse skin in telogen, the common, persistent stage found in adult mouse skin. The representative mouse treated with the laser on had normal hair follicles in late anagen (lacking clear evidence of inflammation in and around the follicles) (Wikramanayake *et al.*, 2012) which is found although infrequently in adult mice (Sundberg

and Silva, 2012). Alopecia in small foci in C3H/HeJ mice can be due to AA but also is not less commonly due to grooming behavior abnormalities (barbering, a form of trichotilomania). The laser comb, as used in the original study, potentially could induce anagen activation in the heat shock induced AA of the normally quiescent mouse telogen follicles.

In summary, laser comb treatment did not induce hair growth in C3H/HeJ mice with extensive AA in well/established spontaneous arising or full thickness skin graft mouse models of human AA. More work on the heat shock/induced AA model needs to be done to define its value in preclinical trials.

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Abbreviations

AA	alopecia areata
AU	alopecia universalis

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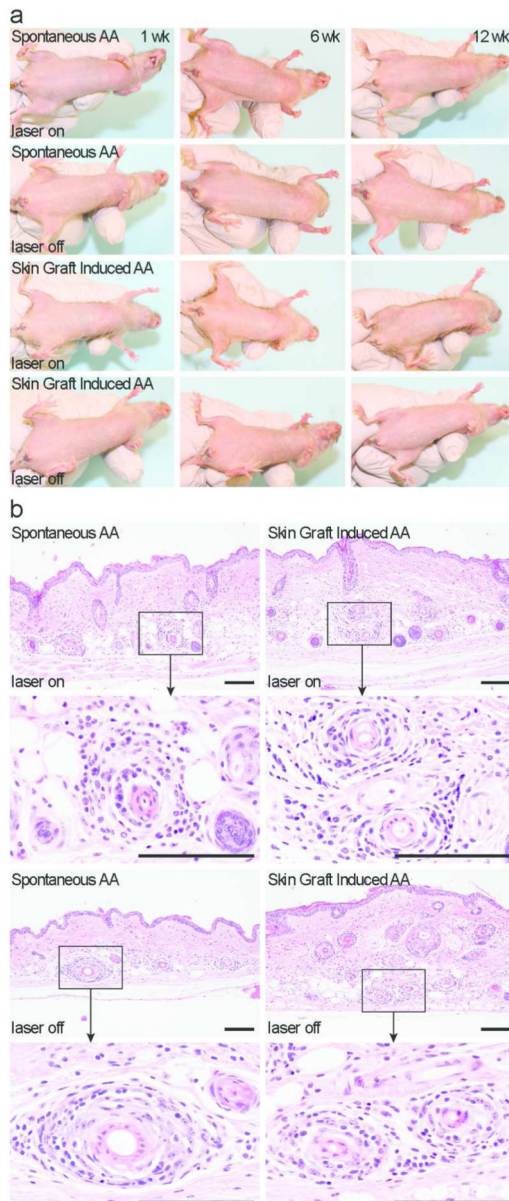


Figure 1. Response of mice with alopecia universalis to a laser comb

(a) All mice, whether they had spontaneous or full thickness skin graft induced alopecia areata, had diffuse alopecia with sparse defective hair shafts. Treatment with the laser comb turned on or turned off had no effect. Mice were examined at 6 and 12 weeks after treatment with no improvement in the alopecia. (b) Microscopic evaluation of all 24 mice had classical histological features of alopecia areata in C3H/HeJ mice. Two representative mice from each group are illustrated. There were lymphocytes in and around anagen stage hair follicles with various degrees of follicular dystrophy resulting in damaged hair shafts that broke off at the skin surface. Boxed areas are enlarged to illustrate the details. Bar = 100 μ m.

Table 1

Groups of 6 mice with spontaneous or full thickness skin graft-induced alopecia areata were treated with the laser comb for a total of 12 weeks. There were no differences between those treated with the comb turned on (test) or off (controls). All mice were confirmed to have alopecia areata by histopathology.

Test Group	6 weeks Alopecia	12 weeks Alopecia	Alopecia Areata Histopathology
Laser ON			
Spontaneous AA	6/6	6/6	6/6
Graft-induced AA	6/6	6/6	6/6
Laser OFF			
Spontaneous AA	6/6	6/6	6/6
Graft-induced AA	6/6	6/6	6/6