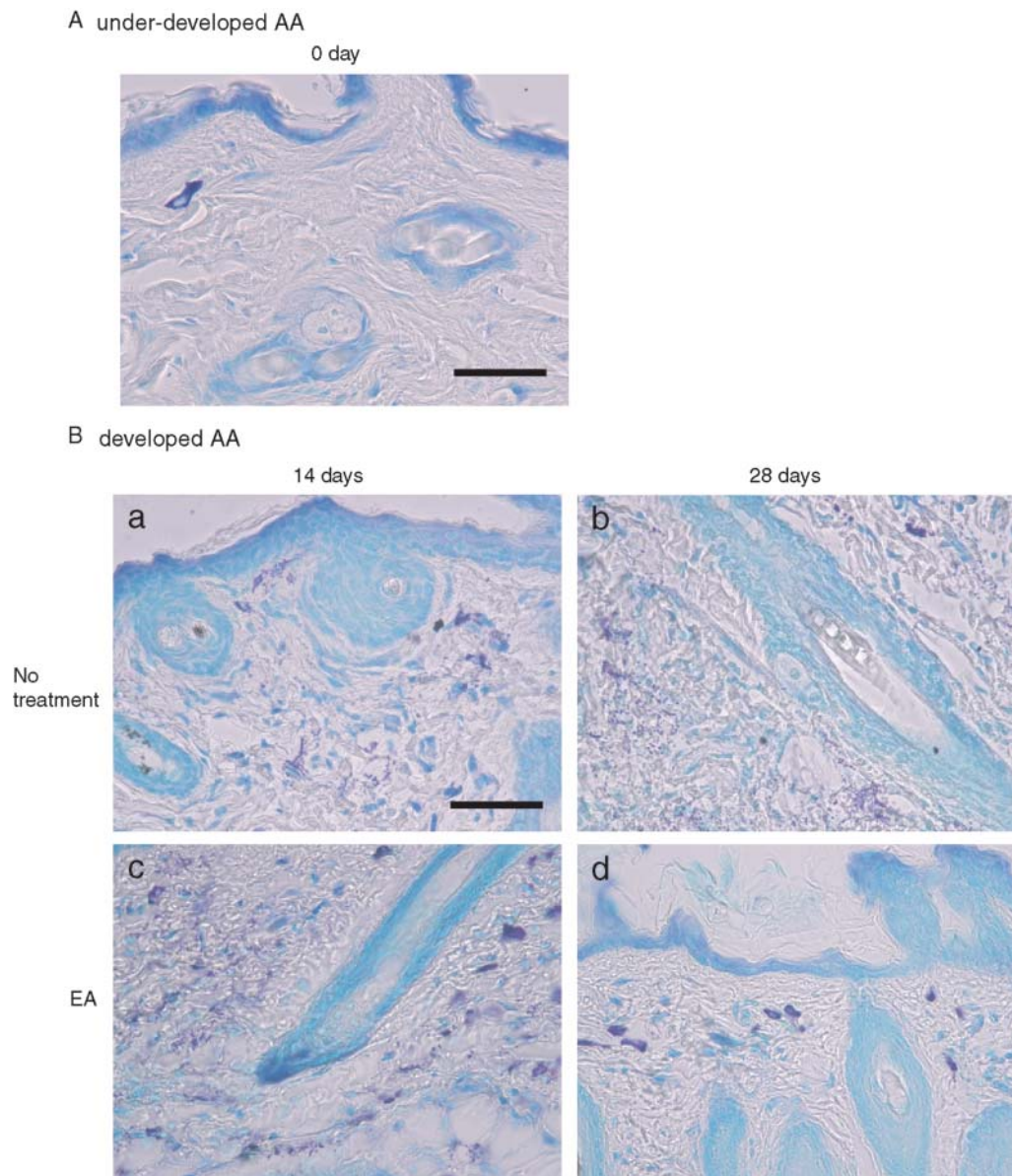


## Anti-inflammatory effect of electroacupuncture in the C3H/HeJ mouse model of alopecia areata

Accumulating experimental evidence has indicated that electroacupuncture (EA) stimulation may enhance immune function in several animal models of inflammatory diseases.<sup>1 2</sup> However, there are few clinical data on EA stimulation for autoimmune diseases and the mechanisms underlying the therapeutic effect of EA stimulation for autoimmune diseases remain unclear.

Mast cells are the central players in allergic inflammation, and it has recently been reported that mast cells are involved in autoimmune diseases and chronic inflammation.<sup>3 4</sup> Significant increases in mast cell degranulation were observed in these autoimmune diseases. Furthermore, severe mast cell degranulation and the accumulation of inflammatory cells around the anagen (growth phase) hair follicles were observed in autoimmune diseases such as the mouse model for alopecia areata (AA).<sup>5</sup> This self-attack of the hair follicle cells by inflammatory cells changes the hair

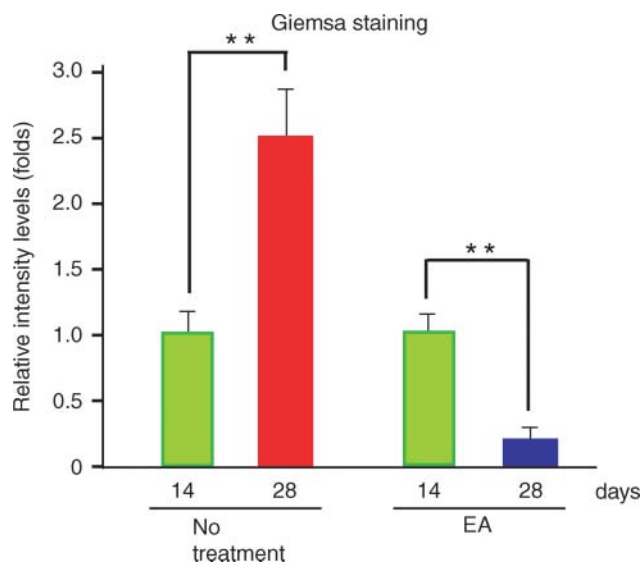


**Figure 1** Giemsa staining of the dermis around the hair follicle in C3H/HeJ mice. (A) Giemsa staining of the dermis in mice with underdeveloped alopecia areata (AA) (0 day). (B) (a,b) No electroacupuncture (EA) stimulation control group. Extensive degranulation of mast cells around the hair follicle was observed in mice that spontaneously developed AA (a, 14 days; b, 28 days). (c,d) EA stimulation group. Before EA stimulation (c), Giemsa staining of the dermis in mice that spontaneously developed AA (14 days). After 14 days of EA stimulation (28 days, d), degranulation of mast cells around the hair follicle cells was reduced significantly in the dermis of mice in which AA developed spontaneously. Scale bar: 50  $\mu$ m.

matrix cell phase to the telogen phase and results in hair loss.<sup>6</sup> A previous report indicated that acupuncture helps to improve hair loss and reduces the dosage of drugs required by patients with AA.<sup>7</sup> These reports suggest that the main pathological change in AA is the degranulation of mast cells in the dermis. In this study we used the C3H/HeJ mouse model of AA, in which AA develops spontaneously in female mice.<sup>8</sup>

ST36 *Zusanli* is the most commonly used acupuncture point for the purpose of immune strengthening and immune regulation in oriental medical clinics and is used to treat autoimmune diseases.<sup>9</sup> We applied EA stimulation at the ST36 point but not in the area of hair loss in C3H/HeJ mice in which AA was developing. During a 14-day period the 0.25 $\times$ 30 mm acupuncture needles were inserted perpendicularly as deep as 3–4 mm to the

right of ST36 and electrical stimulation of 1 Hz, 0.7 mA for 30 min was performed 8 times per day using a stimulator (Electrostimulator N-401, Ito Chotanpa). The stimulated skin was dissected and post-fixed in 10% paraformaldehyde for 1 day. Paraffin-embedded 7  $\mu$ m sections were then stained with Giemsa stain. Visual inspection and recording of images were performed using a Keyence BZ-9000 microscope.



**Figure 2** Quantification of the results shown in figure 1B. Results are expressed as mean±SEM of three independent experiments. Approximately 10 mice were counted for each sample. Asterisks indicate a significant increase or decrease in the rate of degranulation of mast cells around the hair follicle. \*\*p<0.01 (Student t test). EA, electroacupuncture.

After 14 days, extensive degranulation of mast cells around the hair follicle was observed in mice that spontaneously developed AA (14 days) compared with those with underdeveloped AA (0 day) (figure 1A, underdeveloped AA; 1Ba, c, developed AA). After 14 days of no treatment (28 days), the degranulation of mast cells around the hair follicle was increased significantly in the dermis of mice that spontaneously developed AA (figures 1Ba,b and 2, no treatment group; approximately 2.5-fold degranulation levels). By contrast, we first observed that the degranulation levels were significantly reduced by one-fifth after 14 days of EA stimulation (figures 1Bc,d and 2, EA group). Furthermore, EA helped to improve hair loss in the AA model mice. However, elucidation of the functional role of EA stimulation on the development of AA remains a primary goal of future research.

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