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.[1 2] 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.[3 4] 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).[5] This self-attack of the hair follicle cells by inflammatory cells changes the hair

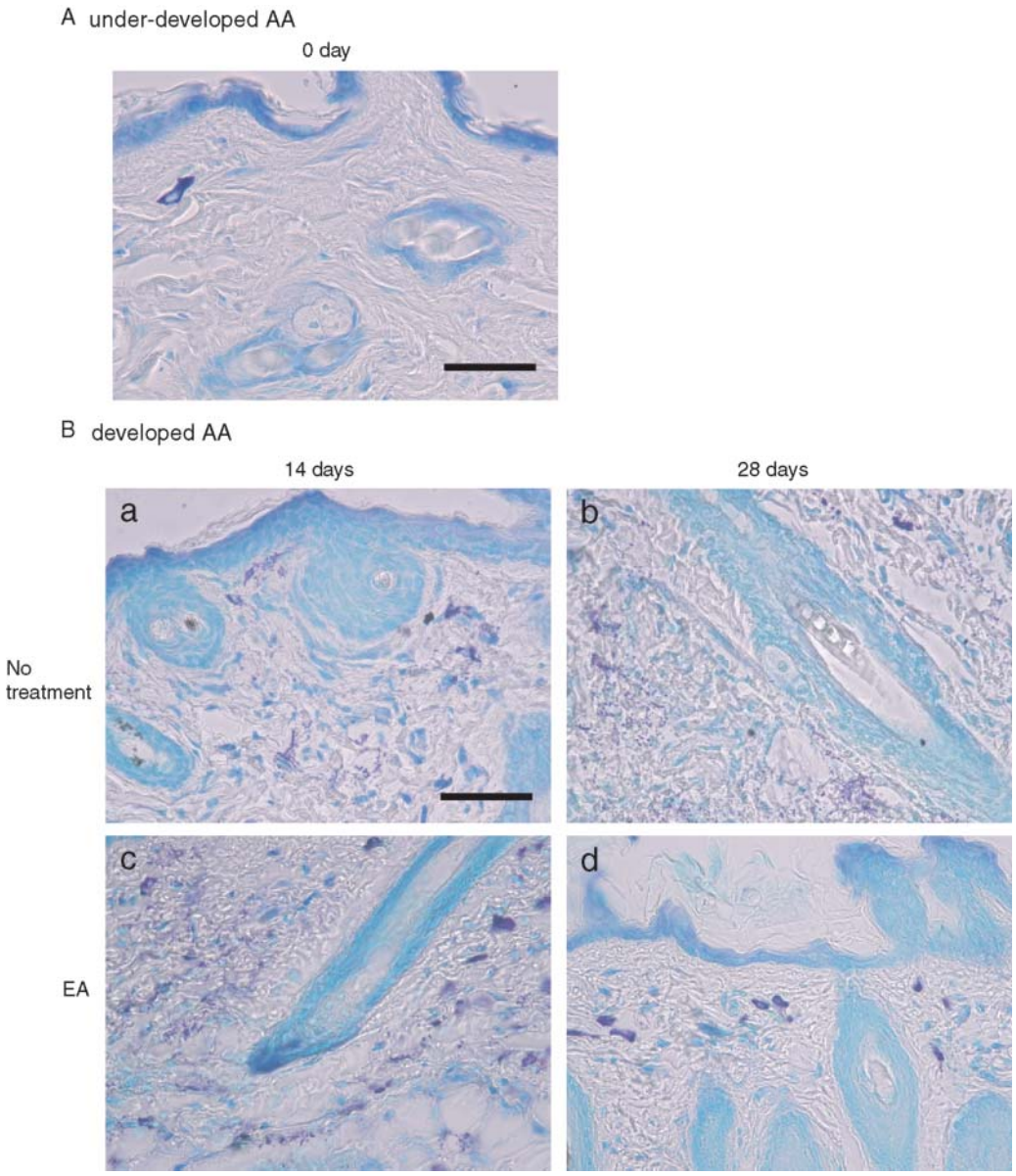

Figure 1 Giemsa staining of the dermis around the hair follicle in C3H/HeJ mice. (A) Giemsa staining of the dermis in mice with underdeveloped alopecia areata (AA) (0 day). (B) (a,b) No electroacupuncture (EA) stimulation control group. Extensive degranulation of mast cells around the hair follicle was 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 μm.

matrix cell phase to the telogen phase and results in hair loss.[6] A previous report indicated that acupuncture helps to improve hair loss and reduces the dosage of drugs required by patients with AA.[7] 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.[8]

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.[9] 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 0.25×30 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 μm sections were then stained with Giemsa stain. Visual inspection and recording of images were performed using a Keyence BZ-9000 microscope.

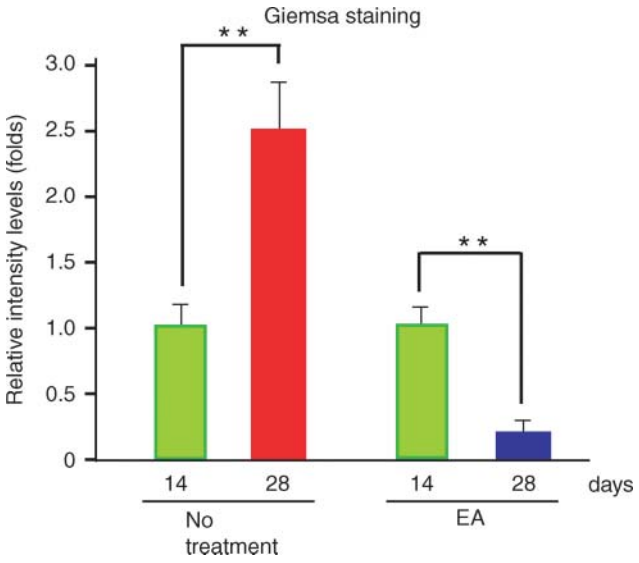

Figure 2 Quantification of the results shown in figure 1B. Results are expressed as mean±SEM of three independent experiments. Approximately 10 mice were counted for each sample. Asterisks indicate a significant increase or decrease in the rate of degranulation of mast cells around the hair follicle. **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.

Tameyasu Maeda,[1] Manabu Taniguchi,[1] Shinsuke Matsuzaki,[2] Kenta Shingaki,[1] Shigeyuki Kanazawa,[3] Shingo Miyata[1,4]

[1]Department of Anatomy and Neuroscience, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan
[2]Department of Child Development and Molecular Brain Science, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Suita, Osaka, Japan
[3]Department of Plastic Surgery, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan
[4]Division of Molecular Brain Science, Research Institute of Traditional Asian Medicine, Kinki University, Osaka-sayama, Osaka, Japan

Correspondence to Dr Shingo Miyata, Division of Molecular Brain Science, Research Institute of Traditional Asian Medicine, Kinki University, 337-2, Ohno-higashi, Osaka-sayama, Osaka 589-8511, Japan; smiyata@med.kindai.ac.jpTM and MT contributed equally.

Acknowledgements We thank Ms Y Ohashi and Ms E Moriya for technical assistance and Dr M Tohyama for valuable comments.

Contributors TM and MT contributed to the conclusions drawn from figures 1 and 2 and to the writing of the manuscript. SMa, KS and SK performed immunohistochemistry. SMi provided intellectual input, funding, coordination of the project and contributed to the writing of the manuscript.

Funding This work was supported in part by the Global COE Program from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Competing interests None.

Ethics approval This work was approved by the Institutional Animal Care and Use Committee of Osaka University (No. 19-038-0).

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Maeda T, Taniguchi M, Matsuzaki S, *et al*. *Acupunct Med* 2013;**31**:117–119.

Received 29 August 2012
Revised 4 October 2012
Accepted 12 October 2012
Published Online First 27 October 2012

Acupunct Med 2013;**31**:117–119.
doi:10.1136/acupmed-2012-010240

REFERENCES

1 Liu XY, Zhou HF, Pan YL, *et al*. Electro-acupuncture stimulation protects dopaminergic neurons from inflammation-mediated damage in medial forebrain bundle-transected rats. *Exp Neurol* 2004;189:189–96.
2 Yim YK, Lee H, Hong KE, *et al*. Electro-acupuncture at acupoint ST36 reduces inflammation and regulates immune activity in collagen-induced arthritic mice. *Evid Based Complement Alternat Med* 2007;4:51–7.
3 Walker ME, Hatfield JK, Brown MA. New insights into the role of mast cells in autoimmunity: evidence for a common mechanism of action? *Biochim Biophys Acta* 2012;1822:57–65.
4 Amin K. The role of mast cells in allergic inflammation. *Respir Med* 2012;106:9–14.
5 Siebenhaar F, Sharov AA, Peters EM, *et al*. Substance P as an immunomodulatory neuropeptide in a mouse model for autoimmune hair loss (alopecia areata). *J Invest Dermatol* 2007;127:1489–97.
6 McDonagh AJ, Messenger AG. Alopecia areata. *Clin Dermatol* 2001;19:141–7.
7 Ge S. Treatment of alopecia areata with acupuncture. *J Tradit Chin Med* 1990;10:199–200.
8 McElwee KJ, Freyschmidt-Paul P, Sundberg JP, *et al*. The pathogenesis of alopecia areata in rodent models. *J Investig Dermatol Symp Proc* 2003;8:6–11.
9 Liu YM, Liu XJ, Bai SS, *et al*. The effect of electroacupuncture on T cell responses in rats with experimental autoimmune encephalitis. *J Neuroimmunol* 2010;220:25–33.

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

Tameyasu Maeda, Manabu Taniguchi, Shinsuke Matsuzaki, Kenta Shingaki, Shigeyuki Kanazawa and Shingo Miyata

Acupunct Med 2013 31: 117-119 originally published online October 27, 2012
doi: 10.1136/acupmed-2012-010240