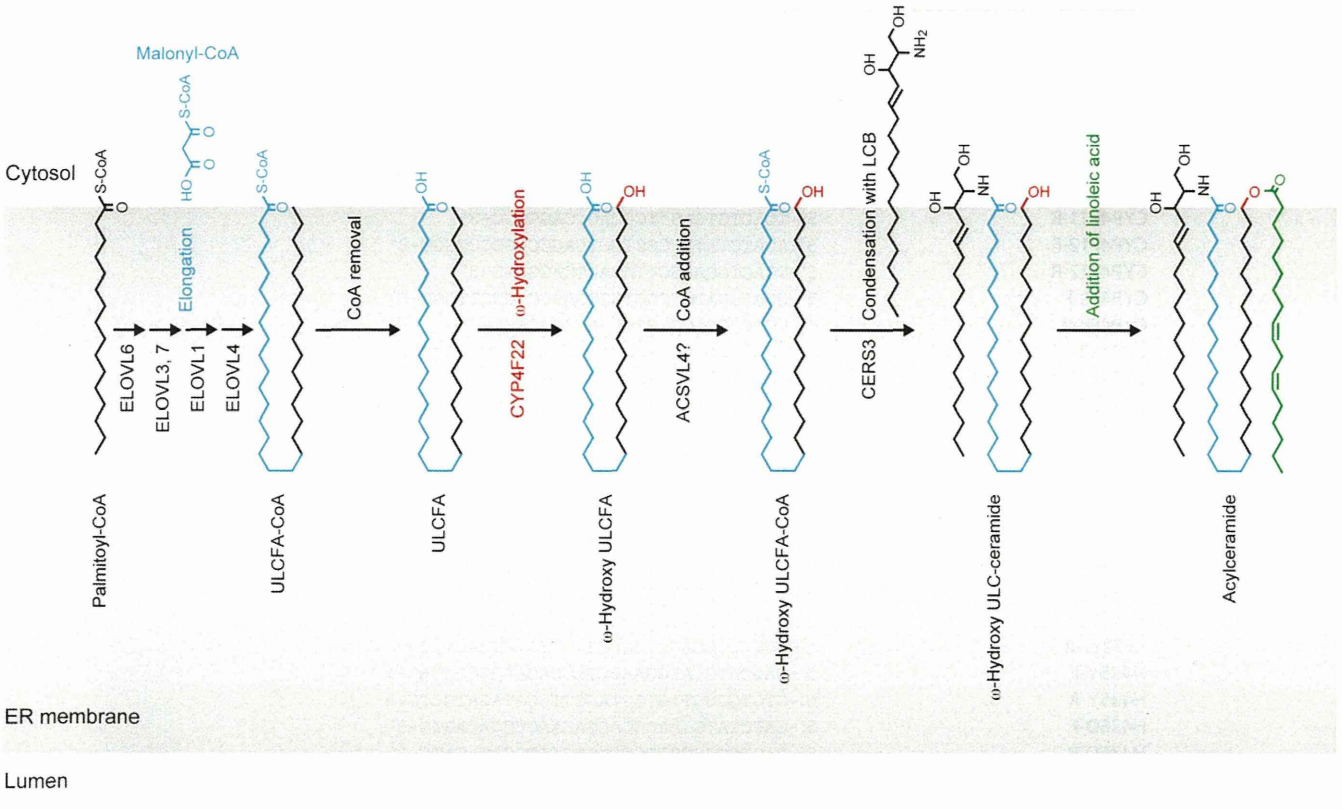

Fig. S6. Working model for the acylceramide synthesis in the ER. Palmitoyl-CoA is elongated to ULCFA-CoA on the cytosolic side of the ER membrane. During FA elongation, ULCFA (C30–C36) portions of ULCFA-CoAs may be bent in the cytosolic leaflet of the ER membrane. After conversion of ULCFA-CoA to ULCFA, the ω-carbon of ULCFA is hydroxylated by CYP4F22. ω-Hydroxy-ULCFA then is converted to ω-hydroxy-ULCFA-CoA by acyl-CoA synthetase, followed by synthesis of ω-hydroxyceramide by CERS3. Finally, an acyltransferase catalyzes the formation of an ester bond between linoleic acid and the ω-hydroxy group of ω-hydroxyceramide, producing acylceramide.

Table S1. Ceramide levels in the stratum corneum

	Ceramide level, ng/μg protein			
Ceramide class	Control	Patient	Mother	Father
NDS	1.10	0.82	2.63	2.17
NS	1.94	3.58	5.16	3.34
NH	3.21	1.82	6.55	4.27
NP	5.07	2.06	12.77	9.07
ADS	0.64	0.46	0.81	0.90
AS	1.36	4.20	3.18	2.61
AH	2.64	2.94	4.53	4.88
AP	2.59	2.33	4.57	7.51
EOS	0.41	0.02	0.61	0.44
EOH	0.37	0.02	0.65	0.45
EOP	0.10	0.01	0.27	0.24
Total	19.44	18.26	41.73	35.86

Control, wild type; patient, R243H/D380T fs2X; patient's mother, WT/D380T fs2X; patient's father, WT/R243H.

Table S2. Primers used in this study

Primer	Sequence
CYP4F2-F	5′-GGATCCATGTCCCAGCTGAGCCTGTCCTGG-3′
CYP4F2-R	5′-TCAGCTCAGGGGCTCCACCCGCAGC-3′
CYP4F3-F	5′-AGATCTATGCCACAGCTGAGCCTGTCCTCGC-3′
CYP4F3-R	5′-TCAGCTCAGGGGCTCCACCCGCAGC-3′
CYP4F11-F	5′-AGATCTATGCCGCAGCTGAGCCTGTCCTGGC-3′
CYP4F11-R	5′-TCACTGTGAGTTCGCACCCAGGGGC-3′
CYP4F12-F	5′-AGATCTATGTCGCTGCTGAGCCTGCCCTGGC-3′
CYP4F12-R	5′-TCACTGCAAGCTTACATTCAGGGGC-3′
CYP4F8-F	5′-GGATCCATGTCGCTGCTGAGCCTGTCTTGGC-3′
CYP4F8-R	5′-TCAGCCCAGGGGTTCTACTCGCAGC-3′
CYP4F22-F	5′-GGATCCATGCTGCCCATCACAGACCGCCTGC-3′
CYP4F22-R	5′-TCAGGCCCGCGGAGGCAGCGGCTCC-3′
CERS2-F	5′-TGTCGACATGCTCCAGACCTTGTATGATTACTTC-3′
CERS2-R	5′-TCAGTCATTCTTACGATGGGTTGTATTG-3′
CERS3-F	5′-GGATCCATGTTTTGGACGTTTAAAGAATGGTTC-3′
CERS3-R	5′-CTAATGGCCATGCTGGCCATTGGGAAT-3′
F59L-F	5′-GCGGCTGCGCTGCTTGCCCCAGCCTCCCCGG-3′
F59L-R	5′-CCGGGGAGGCTGGGGCAAGCAGCGCAGCCGC-3′
R243H-F	5′-GCTGTCTGTCCGGCACCAGTATCGCTTGCAC-3′
R243H-R	5′-GTGCAAGCGATACTGGTGCCGGACAGACAGC-3′
R372W-F	5′-GAAGTCATGAAAGGCTGGGAGCTGGAGGAGC-3′
R372W-R	5′-GCTCCTCCAGCTCCCAGCCTTTCATGACTTC-3′
H435Y-F	5′-CAGCATCTATGGAACCTACCACAACCCCACAG-3′
H435Y-R	5′-CTGTGGGGTTGTGGTAGGTTCCATAGATGCTG-3′
H436D-F	5′-CATCTATGGAACCCACGACAACCCCACAGTG-3′
H436D-R	5′-CACTGTGGGGTTGTCGTGGGTTCCATAGATG-3′
D380T fsX2-F	5′-GAGCTGGAGGAGCTGGAGTGGACGATCTGACTCAGCTGCCC-3′
D380T fsX2-R	5′-GGGCAGCTGAGTCAGATCGTCCACTCCAGCTCCTCCAGCTC-3′
T-E85/K-F	5′-GAGGCGGGCCTTCAAGATGAGGGATCCAAGAAGGTACTGGACAACATG-3′
T-E85/K-R	5′-CATGTTGTCCAGTACCTTCTTGGATCCCTCATCTTGAAGGCCCGCCTC-3′
T-H155/R-F	5′-GGTGACAAGTGGAGCCGGCACGGATCCCGTCGCCTGCTGACACCCGCC-3′
T-H155/R-R	5′-GGCGGGTGTCAGCAGGCGACGGGATCCGTGCCGGCTCCACTTGTCACC-3′
T-A285/L-F	5′-CCAGGAACGGCGGCGGGCAGGATCCCTGCGTCAGCAGGGGGCCGAG-3′
T-A285/L-R	5′-CTCGGCCCCCTGCTGACGCAGGGATCCTGCCCGCCGCCGTTCCTGG-3′
T-C361/R-F	5′-CCGGAATACCAGGAGAAATGCGGATCCCGAGAAGAGATTCAGGAAGTC-3′
T-C361/R-R	5′-GACTTCCTGAATCTCTTCTCGGGATCCGCATTTCTCCTGGTATTCCGG-3′
T-D455/N-F	5′-CCCTACCGCTTTGACCCGGACGGATCCAACCCACAGCAGCGCTCTCC-3′
T-D455/N-R	5′-GGAGAGCGCTGCTGTGGGTTGGATCCGTCCGGGTCAAAGCGGTAGGG-3′
T-R508/K-F	5′-CGAACGCGCAAGGTGCGGCGGGGATCCAAGCCGGAGCTCATACTGCGC-3′
T-R508/K-R	5′-GCGCAGTATGAGCTCCGGCTTGGATCCCCGCCGCACCTTGCGCGTTCG-3′

Table S3. Selected *m/z* values for ceramide and FA species in MS analysis

Lipid species	Precursor ion, Q1	Product ion, Q3	Collision energy, V
d18:1/C16:0 Cer	520.2, 538.2	264.2	20
d18:1/C18:0 Cer	548.2, 566.2	264.2	20
d18:1/C20:0 Cer	576.3, 594.3	264.2	20
d18:1/C22:0 Cer	604.3, 622.3	264.2	25
d18:1/C24:1 Cer	630.3, 648.3	264.2	25
d18:1/C24:0 Cer	632.3, 650.3	264.2	30
d18:1/C26:1 Cer	658.4, 676.4	264.2	30
d18:1/C26:0 Cer	660.4, 678.4	264.2	30
d18:1/C28:1 Cer	686.4, 704.4	264.2	30
d18:1/C28:0 Cer	688.4, 706.4	264.2	30
d18:1/C30:1 Cer	714.4, 732.4	264.2	35
d18:1/C30:0 Cer	716.4, 734.4	264.2	35
d18:1/C32:1 Cer	742.5, 760.5	264.2	35
d18:1/C32:0 Cer	744.5, 762.5	264.2	40
d18:1/C34:1 Cer	770.5, 788.5	264.2	40
d18:1/C34:0 Cer	772.5, 790.5	264.2	40
d18:1/C36:1 Cer	798.5, 816.5	264.2	45
d18:1/C36:0 Cer	800.5, 818.5	264.2	45
d18:1/ωhC24:0 Cer	648.3, 666.3	264.2	30
d18:1/ωhC26:1 Cer	674.4, 692.4	264.2	30
d18:1/ωhC26:0 Cer	676.4 694.4	264.2	30
d18:1/ωhC28:1 Cer	702.4, 720.4	264.2	30
d18:1/ωhC28:0 Cer	704.2, 722.4	264.2	30
d18:1/ωhC30:1 Cer	730.4, 748.4	264.2	35
d18:1/ωhC30:0 Cer	732.4, 750.4	264.2	35
d18:1/ωhC32:1 Cer	758.5, 776.5	264.2	35
d18:1/ωhC32:0 Cer	760.5, 778.5	264.2	40
d18:1/ωhC34:1 Cer	786.5, 804.5	264.2	40
d18:1/ωhC34:0 Cer	788.5, 806.5	264.2	40
d18:1/ωhC36:1 Cer	814.5, 832.5	264.2	40
d18:1/ωhC36:0 Cer	816.5, 834.5	264.2	40
ωhC26:1 FA	577.2	238.9	55
ωhC26:0 FA	579.2	238.9	55
ωhC28:1 FA	605.2	238.9	55
ωhC28:0 FA	607.2	238.9	55
ωhC30:1 FA	633.2	238.9	60
ωhC30:0 FA	635.2	238.9	60
ωhC32:1 FA	661.2	238.9	60
ωhC32:0 FA	663.2	238.9	60
ωhC34:1 FA	689.2	238.9	60
ωhC34:0 FA	691.2	238.9	60
ωhC36:1 FA	717.2	238.9	60
ωhC36:0 FA	719.2	238.9	60

Cer, ceramide; ωh, ω-hydroxy.

Table S4. Selected *m/z* values for ceramide species in the MS analysis of the stratum corneum of human subjects

m/z value	Ceramide species		
570.5	NDS C32		
584.5	NDS C33	NH C32	AS C32
598.5	NDS C34	NH C33	AS C33
612.5	NDS C35	NH C34	AS C34
626.5	NDS C36	NH C35	AS C35
640.5	NDS C37	NH C36	AS C36
654.5	NDS C38	NH C37	AS C37
668.6	NDS C39	NH C38	AS C38
682.6	NDS C40	NH C39	AS C39
696.6	NDS C41	NH C40	AS C40
710.6	NDS C42	NH C41	AS C41
724.6	NDS C43	NH C42	AS C42
738.6	NDS C44	NH C43	AS C43
752.6	NDS C45	NH C44	AS C44
766.7	NDS C46	NH C45	AS C45
780.7	NDS C47	NH C46	AS C46
794.7	NDS C48	NH C47	AS C47
808.7	NDS C49	NH C48	AS C48
822.7	NDS C50	NH C49	AS C49
836.7	NDS C51	NH C50	AS C50
850.7	NDS C52	NH C51	AS C51
864.8	NDS C53	NH C52	AS C52
878.8	NDS C54	NH C53	AS C53
892.8		NH C54	AS C54
602.5	AP C32		
616.5	AP C33		
630.5	AP C34		
644.5	AP C35		
658.5	AP C36		
672.6	AP C37		
686.6	AP C38		
700.6	AP C39		
714.6	AP C40		
728.6	AP C41		
742.6	AP C42		
756.6	AP C43		
770.7	AP C44		
784.7	AP C45		
798.7	AP C46		
812.7	AP C47		
826.7	AP C48		
840.7	AP C49		
854.7	AP C50		
868.8	AP C51		
882.8	AP C52		
896.8	AP C53		
910.8	AP C54		
568.5	NS C32		
582.5	NS C33		
596.5	NS C34		
610.5	NS C35		
624.5	NS C36		
638.5	NS C37		
652.5	NS C38		
666.6	NS C39		
680.6	NS C40		
694.6	NS C41		
708.6	NS C42		
722.6	NS C43		
736.6	NS C44		
750.6	NS C45		
764.7	NS C46		

Table S4. Cont.

m/z value	Ceramide species		
778.7	NS C47		
792.7	NS C48		
806.7	NS C49		
820.7	NS C50		
834.7	NS C51		
848.7	NS C52		
862.8	NS C53		
876.8	NS C54		
586.5	NP C32	ADS C32	
600.5	NP C33	ADS C33	AH C32
614.5	NP C34	ADS C34	AH C33
628.5	NP C35	ADS C35	AH C34
642.5	NP C36	ADS C36	AH C35
656.5	NP C37	ADS C37	AH C36
670.6	NP C38	ADS C38	AH C37
684.6	NP C39	ADS C39	AH C38
698.6	NP C40	ADS C40	AH C39
712.6	NP C41	ADS C41	AH C40
726.6	NP C42	ADS C42	AH C41
740.6	NP C43	ADS C43	AH C42
754.6	NP C44	ADS C44	AH C43
768.7	NP C45	ADS C45	AH C44
782.7	NP C46	ADS C46	AH C45
796.7	NP C47	ADS C47	AH C46
810.7	NP C48	ADS C48	AH C47
824.7	NP C49	ADS C49	AH C48
838.7	NP C50	ADS C50	AH C49
852.7	NP C51	ADS C51	AH C50
866.8	NP C52	ADS C52	AH C51
880.8	NP C53	ADS C53	AH C52
894.8	NP C54	ADS C54	AH C53
908.8			AH C54
1072.9	EODS C66		
1086.9	EODS C67	EOH C66	
1100.9	EODS C68	EOH C67	
1114.9	EODS C69	EOH C68	
1128.9	EODS C70	EOH C69	
1142.9	EODS C71	EOH C70	
1156.9	EODS C72	EOH C71	
1170.9		EOH C72	
1070.9	EOS C66		
1084.9	EOS C67		
1098.9	EOS C68		
1112.9	EOS C69		
1126.9	EOS C70		
1140.9	EOS C71		
1154.9	EOS C72		
1088.9	EOP C66		
1102.9	EOP C67		
1116.9	EOP C68		
1130.9	EOP C69		
1144.9	EOP C70		
1158.9	EOP C71		
1172.9	EOP C72		

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SHORT COMMUNICATION

Annular Elastolytic Giant Cell Granuloma Successfully Treated with Minocycline Hydrochloride

Ai Nanbu, Kazumitsu Sugiura*, Michihiro Kono, Yoshinao Muro and Masashi Akiyama

*Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku Nagoya 466-8550, Japan. *E-mail: kazusugi@med.nagoya-u.ac.jp*

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Annular elastolytic giant cell granuloma (AEGCG) is a rare granulomatous skin disease characterised by small papules that evolve into annular/polycyclic plaques. Plaques typically have a slightly raised border; the centre may be hypopigmented and/or atrophic. Lesions are usually located on sun-exposed areas such as the face and the neck (1, 2). Histologically, AEGCG is associated with multinucleated giant cell infiltrate, elastolysis, and elastophagocytosis, localised mainly in the mid-dermis. Elastophagocytosis is considered to be brought on by one or more triggers, which leads to the loss of elastic fibres (3); in AEGCG, the possible triggers include ultraviolet radiation, heat, or other unknown factors that induce a cellular immune response (4). AEGCG is often refractory to any treatment, and no standard therapy has been established (5). Here, we report a case of AEGCG that improved after 11 weeks of treatment with oral minocycline hydrochloride.

CASE REPORT

A 46-year-old Japanese man was referred to our hospital. He had a well-demarcated, annular, erythematous plaque with a raised border and slightly atrophic centre (Fig. 1a, b) on his left temple. The patient first noticed the lesion one year prior to his first visit to our hospital. Although he had been treated with a 7-week course of topical beclomethasone dipropionate at a previous hospital, the lesion progressed in size, ultimately measuring 5 cm in diameter. The patient did not complain of any pain or pruritus. Histopathology of a punch biopsy, which was taken from the elevated border, revealed an infiltrate of lymphocytes and macrophages that formed non-palisading granulomas in the upper to mid-dermis (Fig. 2a). Elastica van Gieson stain of the tissue revealed a marked reduction in elastic fibres, and evidence of phagocytosis of elastic fibres by multinucleated giant cells and macrophages (Fig. 2b). Ziehl-Neelsen staining of the tissue was negative. Bacterial and fungal cultures of biopsy specimens obtained from the lesion were negative. Laboratory studies, including complete blood cell count, biochemical tests, and serum levels of angiotensin-converting enzyme were within normal limits. Enzyme-Linked ImmunoSpot assay, used for tuberculosis diagnosis, was negative. Serum levels of blood glucose and haemoglobin A1c were also normal. A diagnosis of AEGCG was made on the basis of clinical and histopathological findings.

Because the patient's AEGCG was refractory to a potent glucocorticoid ointment, we obtained informed consent and administered oral minocycline hydrochloride at 200 mg/day for 2 weeks, followed by 100 mg/day for 9 weeks. Eleven weeks later, the active erythematous infiltration had gradually decreased, and the lesion had faded with pigmentation (Fig. 1c). No adverse effects were reported.

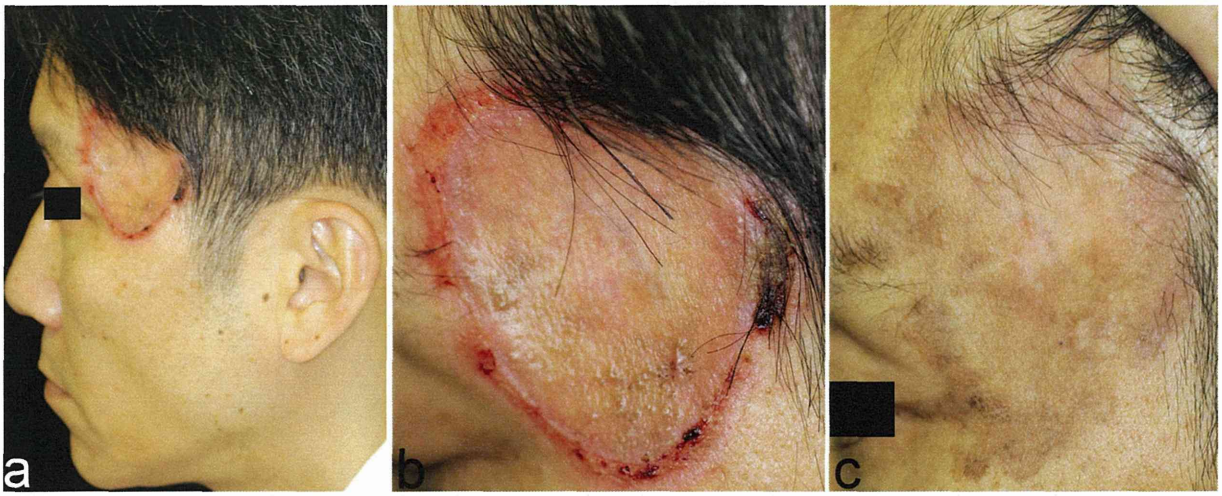

Fig. 1. Patient's clinical features. Pre-treatment, a well-demarcated, annular, erythematous plaque is seen on the left temple (a, b). Post-treatment with systemic minocycline, erythema is decreased and pigmentation is observed (c).

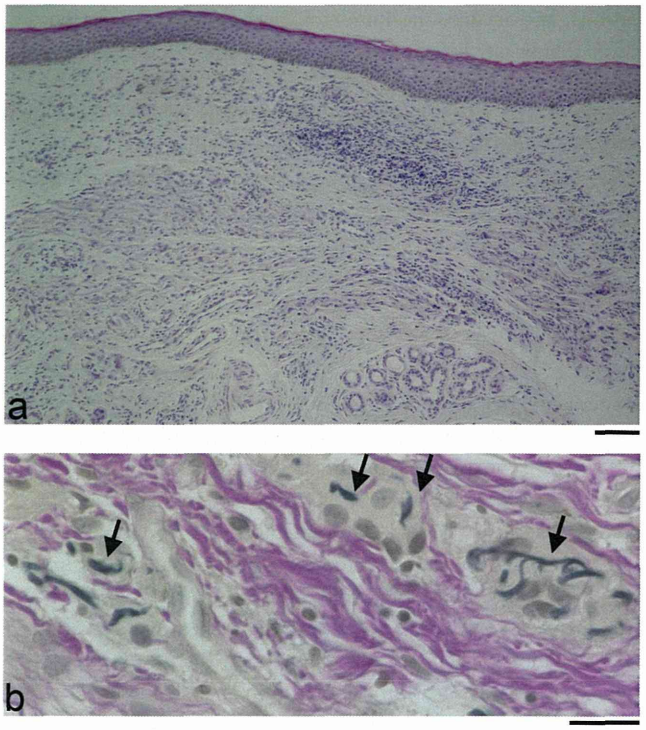

Fig. 2. Inflammatory cell infiltration and non-palisading granulomas in the upper to mid-dermis (haematoxylin and eosin) (a). Phagocytosis of elastic fibres by multinucleated giant cells and macrophages (Elastica van Gieson) (b). Arrows indicate elastic fibres in multinucleated cells and macrophages. Scale bars: 100 μm (a) and 20 μm (b).

DISCUSSION

AEGCG is a rare, reactive granulomatous dermatosis, usually associated with actinic damage (1). The pathogenesis of AEGCG is unidentified. Both normal and degenerated elastic fibres are phagocytosed by macrophages in AEGCG (3). Several treatments for AEGCG have previously been proposed, including topical or intralesional glucocorticoids, cyclosporine, topical calcineurin inhibitors, dapsone, hydroxychloroquine sulphate, clofazimine, cryotherapy, methotrexate, psoralen plus ultraviolet A therapy, narrowband ultraviolet B therapy, retinoids, fumaric acid esters, and tranilast. However, most treatments are unsatisfactory, and there is no definitive therapy for AEGCG (5–7). A triple antibiotic therapy regimen, which included minocycline, has previously been reported to provide some beneficial effects for granuloma annulare (8), although not curing the disease (9). Minocycline has anti-inflammatory effects that interfere with lymphocyte proliferation, especially that of T cells, as well as immunomodulating and anti-granulomatous effects (10, 11). We considered that these mechanisms of action of minocycline may have affected AEGCG in the present patient. However, minocycline should be used with care, as it may be associated with photosensitivity. This could potentially worsen, rather than improve AEGCG, as previously reported with doxycycline (12).

To our knowledge, this is the first time that AEGCG was successfully treated with minocycline. In conclusion, the present case suggests that minocycline may be a useful therapeutic option for AEGCG. We cannot, however, exclude a spontaneous resolution of the lesion, and further case reports and studies are needed to confirm our observation.

REFERENCES

1. Hanke CW, Bailin PL, Roenigk HH, Jr. Annular elastolytic giant cell granuloma. A clinicopathologic study of five cases and a review of similar entities. J Am Acad Dermatol 1979; 1: 413–421.
2. Aso Y, Izaki S, Teraki Y. Annular elastolytic giant cell granuloma associated with diabetes mellitus: a case report and review of the Japanese literature. Clin Exp Dermatol 2011; 36: 917–919.
3. El-Khoury J, Kurban M, Abbas O. Elastophagocytosis: underlying mechanisms and associated cutaneous entities. J Am Acad Dermatol 2014; 70: 934–944.
4. Ozkaya-Bayazit E, Buyukbabani N, Baykal C, Ozturk A, Okcu M, Soyer HP. Annular elastolytic giant cell granuloma: sparing of a burn scar and successful treatment with chloroquine. Br J Dermatol 1999; 140: 525–530.
5. Babuna G, Buyukbabani N, Yazganoglu KD, Baykal C. Effective treatment with hydroxychloroquine in a case of annular elastolytic giant cell granuloma. Indian J Dermatol Venereol Leprol 2011; 77: 110–111.
6. Igawa K, Maruyama R, Katayama I, Nishioka K. Antioxidative therapy with oral dapsone improved HCV antibody positive annular elastolytic giant cell granuloma. J Dermatol 1997; 24: 328–331.
7. Errichetti E, Stinco G, Avellini C, Patrone P. Annular elastolytic giant cell granuloma treated with topical pimecrolimus. Indian J Dermatol Venereol Leprol 2014; 80: 475–476.
8. Marcus DV, Mahmoud BH, Hamzavi IH. Granuloma annulare treated with rifampin, ofloxacin, and minocycline combination therapy. Arch Dermatol 2009; 145: 787–789.
9. Simpson B, Foster S, Ku JH, Simpson EL, Ehst BD. Triple antibiotic combination therapy may improve but not resolve granuloma annulare. Dermatol Ther 2014; 27: 343–347.
10. Tilley BC, Alarcon GS, Heyse SP, Trentham DE, Neuner R, Kaplan DA, et al. Minocycline in rheumatoid arthritis. A 48-week, double-blind, placebo-controlled trial. MIRA Trial Group. Ann Intern Med 1995; 122: 81–89.
11. Webster GF, Toso SM, Hegemann L. Inhibition of a model of in vitro granuloma formation by tetracyclines and ciprofloxacin. Involvement of protein kinase C. Arch Dermatol 1994; 130: 748–752.
12. Lim DS, Triscott J. O'Brien's actinic granuloma in association with prolonged doxycycline phototoxicity. Austral J Dermatol 2003; 44: 67–70.

Pustular psoriasis-like lesions associated with hereditary lactate dehydrogenase M subunit deficiency without interleukin-36 receptor antagonist mutation: long-term follow-up of two cases

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Dear Editor, Lactate dehydrogenase (LDH) is a key enzyme involved in the catalysis of the interconversion of pyruvate and lactate in the final step of anaerobic glycolysis, and is present in almost all cells. It exists as five isozymes composed of tetramers with two different subunits: H (heart) and M (muscle).[1] The isozymes composed mainly of the M subunit (LDH4 and LDH5) are predominant in tissues that undergo anaerobic metabolism, such as skin, liver and muscle. LDH M subunit deficiency, first reported in two Japanese families,[2] is characterized by fatigability and myalgia with myoglobinuria and high creatine kinase after strenuous exercise. The diagnosis of LDH M subunit deficiency is usually based on the electrophoretic pattern of LDH, which shows a band for LDH1 only. Erythematosquamous skin lesions were documented first,[3] and several types of eruptions have since been reported, for example desquamating erythematosquamous lesions and annular erythematous plaques with desquamating borders.[4,5] Here, we report two patients with LDH M subunit deficiency with generalized pustular psoriasis (GPP)-like lesions, and include the immunological aspects of their long-term follow-up. Patient 2 is the same patient reported previously by Yoshikuni *et al.* in 1986.[3]

Patient 1, a 64-year-old man, was initially referred to us in October 2006 for evaluation of annular erythematous plaques (Fig. 1a), with pustules on the peripheries (Fig. 1b). He had suffered from asymptomatic scaly papules and erythematous patches on the elbows and knees since childhood, which were exacerbated in the summer. Laboratory tests revealed moderately elevated levels of aspartate transaminase (70 U L^{-1}; normal range 10–35 U L^{-1}) and alanine aminotransferase (79 U L^{-1}; normal range 5–40 U L^{-1}). The electrophoretic pattern of LDH showed 100% LDH1 and 0% LDH2–LDH5. A skin biopsy taken from abdominal skin showed a subcorneal infiltrate of neutrophils in the psoriasiform epidermis with spongiform pustules of a Kogoj-like pattern (Fig. 1c). The patient had been treated intermittently with oral ciclosporin 3·5 mg kg^{-1} daily, and with topical corticosteroid and calcipotriol for 3 years. In order to avoid the adverse effects of ciclosporin, the cessation period of ciclosporin treatment was taken into consideration during treatment. However, his pustular lesions worsened. Therefore, intravenous infliximab 5 mg kg^{-1} was started, and marked therapeutic effectiveness was seen.

Patient 2, at the age of 50 years, had suffered from small follicular erythematous papules with scales and large desquamating erythematous plaques on the elbows and legs since early childhood, with myalgia after exercise (Fig. 1d).[3] At the age of 56 years, pustules emerged on large desquamating ery-

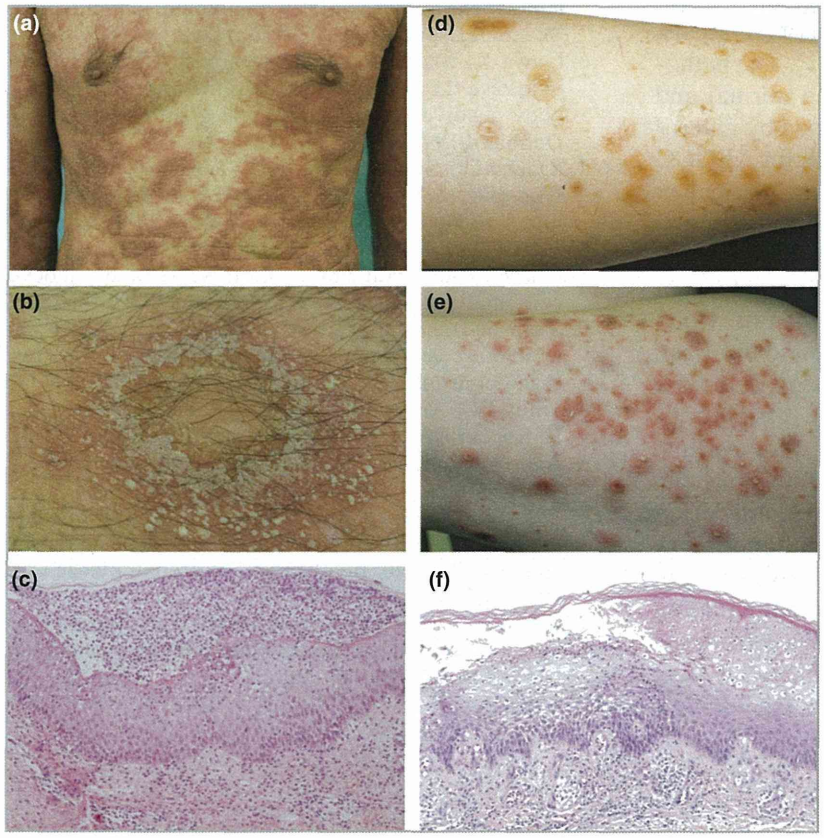

Fig 1. (a, b) Erythematous plaques with pustules on the trunk and extremities of patient 1. (c) Biopsy specimen from patient 1 showing subcorneal infiltration of neutrophils (spongiform pustules of Kogoj) in a psoriasiform epidermis with perivascular infiltration of lymphocytes and a few neutrophils in the dermis. (d) Initial skin lesions in patient 2, showing small follicular erythematous papules with scales and large desquamating erythematous plaques on her elbows and legs. (e) Skin lesions in patient 2 at the age of 56 years, which spread to the lower extremities with pustules. (f) Biopsy specimen from patient 2, showing subcorneal infiltration of neutrophils (spongiform pustules of Kogoj) and lymphocyte infiltration in the upper dermis.

thematous plaques (Fig. 1e). A skin biopsy revealed spongiform pustules of Kogoj and a lymphocytic infiltrate in the upper dermis (Fig. 1f).

After obtaining written consent, genomic DNA was prepared from peripheral blood of both patients. Seven fragments containing seven exons and exon–intron junctions were amplified and subjected to direct DNA sequencing for *LDHA*. Exon 6 of *LDHA* showed a 20-base pair deletion in both cases, which is the most common mutation in LDH M subunit deficiency (Fig. 2a), resulting in frame shift and premature termination. Six of eight Japanese patients who suffered from LDH M subunit deficiency had the same mutation (Table S1; see Supporting Information).

Intracytoplasmic cytokine expression was analysed for patient 1. Peripheral blood mononuclear cells, collected upon the appearance of pustules, were stained with mouse monoclonal antibodies to human interleukin (IL)-17A, IL-22 and interferon (IFN)-γ (BD Bioscience, Franklin Lakes, NJ, U.S.A.), as reported previously.[6] As CD4 expression on T cells is downregulated in the presence of stimulants, T helper cell (Th)17 cells were expressed as IL-17A$^+$CD3$^+$ and IL-17A$^+$CD8$^-$ cells. The percentages of IL-17A$^+$CD8$^-$ T cells and IL-22$^+$CD8$^-$ T cells were 23·2% (Fig. 2b; normal 0·4%) and 15·4% (Fig. 2c; normal < 1%), respectively. Even compared with drug eruptions,[6] which show high numbers of Th17 cells, the percentage of Th17 cells in this case was extremely high.

IL-8 is a well-known chemokine in neutrophil biology, and chemerin attracts plasmacytoid dendritic cells in relation to the pathogenesis of psoriasis.[7] In patient 2, the serum levels of IL-8 ($P = 0·02$; Fig. 2d) and chemerin ($P = 0·01$; Fig. 2e) were significantly higher than those in healthy participants ($n = 3$), as assessed by enzyme-linked immunosorbent assay.

A recent study revealed that the majority of GPP is caused by a deficiency in the IL-36 receptor antagonist due to mutations of *IL36RN*.[8] Neither of our patients had mutations of *IL-36RN*.

LDH catalyses the interconversion of pyruvate and lactate in the final step of anaerobic glycolysis.[9] Therefore, the lack of LDH activity might affect keratinocyte metabolism via impaired adenosine triphosphate (ATP) production in the anaerobic stage. Recent studies have revealed that physical and chemical damage induces the extracellular release of ATP,

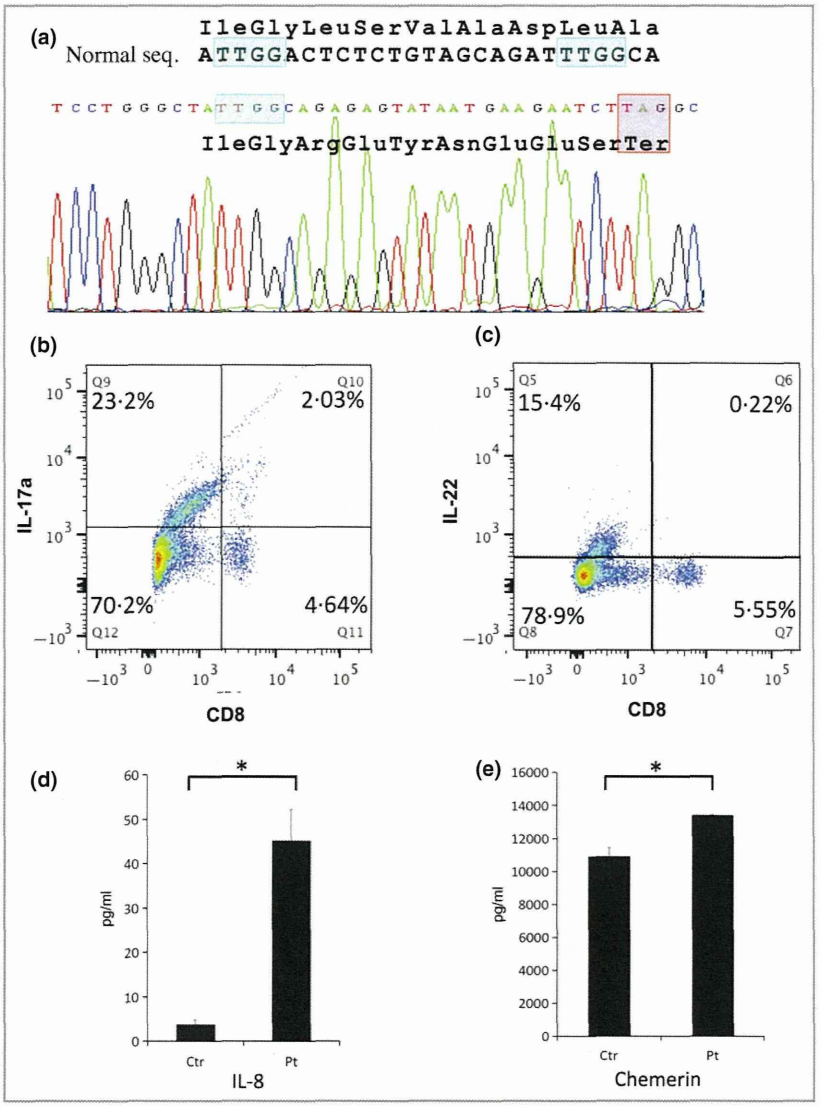

Fig 2. (a) Direct DNA sequencing of exon 6 of *LDHA* showing a 20-base pair deletion in exon 6. (b, c) Intracytoplasmic cytokine analysis showing that the frequencies of interleukin (IL)-17A$^+$CD8$^-$ cells (representing IL-17A$^+$CD4$^+$ T cells) and IL-22$^+$CD8$^-$ cells (representing IL-22$^+$CD4$^+$ T cells) were 23·3% and 15·4%, respectively, in patient 1. (d, e) Enzyme-linked immunosorbent assay data of the serum levels of chemerin and IL-8 in patient 2, which are significantly higher than those in a healthy participant (* $P = 0·02$ and $P = 0·01$, respectively). Ctr, control; Pt, patient.

followed by the production of cytokines and chemokines.[10] In association with this change, keratinocytes might release various psoriatic pathogenic factors, such as IL-8, cathelicidin LL-37 and vascular endothelial growth factor. Moreover, inflammatory and/or plasmacytoid dendritic cells can be stimulated to produce IL-23 or tumour necrosis factor-α and IFN-α, respectively.[7] It is possible that these alterations induce pustular lesions in patients with LDH M subunit deficiency. Our study suggests that abnormal LDH activity is involved in the pathogenesis of GPP.

Departments of [1]Dermatology and [3]Laboratory Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan
[2]Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan
E-mail: itoutai@hama-med.ac.jp

T. Ito[1]
M. Aoshima[1]
K. Sugiura[2]
T. Fujiyama[1]
N. Ito[1]
J.I. Sakabe[1]
M. Akiyama[2]
M. Maekawa[3]
Y. Tokura[1]

T.I. and M.Ao. contributed equally to this work.

References

1 Uitto J, Turto H, Lindy S. Assay of lactate dehydrogenase in human skin: total activity and isoenzyme composition. *Clin Chim Acta* 1972; **36**:43–50.
2 Kanno T, Sudo K, Takeuchi I *et al.* Hereditary deficiency of lactate dehydrogenase M-subunit. *Clin Chim Acta* 1980; **180**:267–76.
3 Yoshikuni K, Tagami H, Yamada M *et al.* Erythematosquamous skin lesions in hereditary lactate dehydrogenase M-subunit deficiency. *Arch Dermatol* 1986; **122**:1420–4.
4 Takayasu S, Fujiwara S, Waki T. Hereditary lactate dehydrogenase M-subunit deficiency: lactate dehydrogenase activity in skin lesions and in hair follicles. *J Am Acad Dermatol* 1991; **24**:339–42.
5 Nazzari G, Crovato F. Annually recurring acroerythema and hereditary lactate dehydrogenase M-subunit deficiency. *J Am Acad Dermatol* 1992; **27**:262–3.
6 Fujiyama T, Kawakami C, Sugita K *et al.* Increased frequencies of Th17 cells in drug eruptions. *J Dermatol Sci* 2014; **73**:85–8.
7 Parolini S, Santoro A, Marcenaro E *et al.* The role of chemerin in the colocalization of NK and dendritic cell subsets into inflamed tissues. *Blood* 2007; **109**:3625–32.
8 Sugiura K, Takemoto A, Yamaguchi M *et al.* The majority of generalized pustular psoriasis without psoriasis vulgaris is caused by deficiency of interleukin-36 receptor antagonist. *J Invest Dermatol* 2013; **133**:2514–21.
9 Kanno T, Sudo K, Kitamura M *et al.* Lactate dehydrogenase A-subunit and B-subunit deficiencies: comparison of the physiological roles of LDH isozymes. *Isozymes Curr Top Biol Med Res* 1983; **7**:131–50.
10 Dixon CJ, Bowler WB, Littlewood-Evans A *et al.* Regulation of epidermal homeostasis through P2Y2 receptors. *Br J Pharm* 1999; **127**:1680–6.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Reported cases with hereditary lactate dehydrogenase M subunit deficiency.

Combining biologics with methotrexate in psoriasis: a systematic review

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Dear Editor, Biological drugs ('biologics') have revolutionized the treatment of patients with extensive and therapy-resistant psoriasis.[1] Unfortunately, biologics [the antitumour necrosis factor (TNF)-α agents adalimumab, infliximab and etanercept, and the anti-p40 (interleukin12/23) ustekinumab] appear to lose efficacy over time and many patients are eventually switched to another biologic.[2]

For the monoclonal antibody-based anti-TNF-α drugs, this loss of efficacy has been partly attributed to immunogenicity. Neutralizing antidrug antibody (ADA) formation against the biologic drug leads to inhibition of function and formation of drug–antibody complexes, resulting in accelerated clearance from the circulation.[3]

To overcome both these efficacy problems, the feasibility of off-label therapies that combine biologics with traditional systemic agents are being explored. However, there is insufficient evidence to suggest that these agents can significantly prevent immunogenicity in psoriasis.[4] In contrast, combined treatment with methotrexate (MTX) may improve short-term clinical efficacy and drug survival in particular. The latter effect may be due to MTX's ability to reduce neutralizing ADA formation and thus maintain adequate biologic drug levels. However, European S3 guidelines on the systemic treatment of psoriasis do not recommend this combination therapy.[5] Herein, we review the combined therapy of biologics and MTX in psoriasis.

Pivotal electronic databases were searched up to 27 October 2014 to identify studies on the combination therapy of MTX and biologics for the treatment of psoriasis (Fig. 1). The searches were limited to English-language articles. Two independent investigators performed a preliminary selection of eligible trials based on the title and abstract, followed by a second selection based on the full text (see Fig. 1). Any discrepancies were resolved by discussion or by referral to a third investigator.

Eight studies were selected and reviewed (see Table 1). These studies generally showed that combination therapy of a biologic and MTX had higher efficacy than biologic monotherapy. Combination therapy was well tolerated and not associated with higher rates of clinically relevant adverse events.

However, the following limitations of the studies reviewed should be taken into account. Firstly, most studies were performed with relatively small numbers of patients (range 11–32). The only exception was the randomized controlled trial (RCT) by Gottlieb *et al.*,[6] which prospectively investigated the efficacy and safety of etanercept monotherapy vs. MTX combination treatment in a relatively large number of patients (n = 239). Secondly, most treatment durations were short (24 weeks) and had a retrospective design, with the inherent