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Letter to the Editor

Autoantibodies detected in patients with vitiligo vulgaris but not in those with rhododendrol-induced leukoderma

Cosmetics containing a skin-whitening agent, namely, rhododendrol, caused leukoderma in 16,000 users. Although the cytotoxic effects of rhododendrol metabolites on the melanocytes and degeneration of melanosomes have been proposed, the reason why only 2% of users develop leukoderma remains unclear. The clinical manifestations of leukoderma are similar to those of vitiligo vulgaris, except that leukoderma is apparent at the topical application site [1–7]. Vitiligo vulgaris is frequently associated with autoantibody- (autoAb-) dependent autoimmune disorders, such as autoimmune thyroiditis or myasthenia gravis. Reportedly, both anti-thyroid antibodies (Abs) and several melanocyte-related autoAbs are elevated in the sera of patients with vitiligo vulgaris [8,9]. In order to explain the role of autoAbs in rhododendrol-induced leukoderma, the autoAbs were analyzed in the patients with rododendrol-induced leukoderma.

As shown in Fig. 1A, using a chemiluminescence enzyme immunoassay (CLEIA) method, the serum anti-thyroid peroxidase (anti-TPO) Ab titers were found to be significantly higher in patients with vitiligo vulgaris than in healthy participants, as previously reported [8]. However, the anti-TPO Ab titers were not significantly elevated in patients with rhododendrol-induced leukoderma compared to those in healthy participants. The anti-thyroglobulin (anti-TG) Ab titers were not elevated in the sera of patients with rhododendrol-induced leukoderma (Fig. 1B).

The positive rate of anti-TG or anti-TPO autoAbs was also analyzed for the patients with rhododendrol-induced leukoderma in a multicenter study. The titers of the Abs for the patients were analyzed at each hospital. The total percentage of anti-TPO Ab positive was 15.8% and that of anti-TG Ab positive was 20.7% in patients with rhododendrol-induced leukoderma. In total, 24.3% of the patients were positive for either Ab, whereas 21% of 100 healthy females showed elevated levels of either Ab titer. Using Fisher's exact test, no statistically significant differences were noted in a multicenter study (Fig. 1C).

Next, the serum TPO autoAb titer was analyzed by Ab binding to the TPO-expressing cell line (Fig. 1D) [10]. The Ab titer with serial dilutions of TPO Ab positive serum showed a linear correlation with the fluorescence intensity determined by Ab binding (Fig. 1E). Using this method, autoAbs against TPO transfectants were significantly elevated in patients with vitiligo vulgaris, but not in those with rhododendrol-induced leukoderma (Fig. 1F), consistent with the results determined using CLEIA (Fig. 1A).

Previously, melanosomal autoAb titers were reported to be elevated in patients with vitiligo vulgaris [9]. Thereafter, autoAbs binding against melanosomal antigens, tyrosinase-related protein 2 (TYRP2), tyrosinase-related protein 1 (TYRP1), and tyrosinase (TYR), were analyzed by expressing them on the cells (Fig. 2 lower histgrams). The anti-TYRP2 Ab titer was significantly increased in patients with vitiligo vulgaris, but not in those with rhododendrol-induced leukoderma (Fig. 2A). Anti-TYRP1 and anti-TYR Abs were not significantly detected in the sera of patients with rhododendrol-induced leukoderma either (Fig. 2B and C).

Because the surface expressions of these melanosomal antigens were relatively lower than the conventional membrane proteins (Fig. 2, lower histograms), low autoAb binding may affect Ab positivity. Therefore, modifications of the cytoplasmic sorting signals within TYRP1, TYRP2, and TYR were introduced into these constructs to upregulate the surface expression of these antigens (Fig. 2, lower histograms, mut). The Ab titers were elevated in TYRP2 (mut) transfectants in patients with vitiligo vulgaris, but not in those with rhododendrol-induced leukoderma (Fig. 2D). A slight increase in the serum anti-TYRP1 (mut) Ab and anti-TYR (mut) Ab titers was noted in patients with vitiligo vulgaris patients compared to the healthy control group (Fig. 2E and F); however, it was not significant. Anti-TYRP1 (mut) and anti-TYR (mut) Abs were not significantly elevated in the sera of patients with rhododendrol-induced leukoderma either (Fig. 2E and F). These results were consistent with the analysis using transfectants without the modifications of sorting signals (Fig. 2A–C).

Regarding patients with rhododendrol-induced leukoderma, they were categorized into those who did not show any improvement ($n = 8$) and those who showed an improvement ($n = 32$) after discontinuation of the cosmetics. Among these groups, the positivity of autoAbs—anti-TPO Ab or anti-TYRP2 (mut) Ab—was analyzed using Fisher's exact tests. No statistical correlation was found between the no improvement of skin symptoms and these autoAbs. However, these outcomes might be a result of the limited number of patients analyzed. Detailed information of patients with vitiligo vulgaris was described in Supplemental Table 1.

Vitiligo vulgaris is frequently associated with autoimmune comorbidities and diseases, such as collagen disease, diabetes, myasthenia gravis, or autoimmune thyroiditis. In a systematic review, autoimmune thyroid disease was found to be accompanied by vitiligo vulgaris, with a prevalence of 14.3%. Thyroid-specific autoAbs showed a mean prevalence of 20.8% in patients with

Abbreviations: autoAb, autoantibody; Ab, antibody; CLEIA, Chemiluminescence enzyme immunoassay; TPO, thyroid peroxidase; TG, thyroglobulin; CTL, cytotoxic T lymphocyte; TYRP, Tyrosinase Related Protein; TYR, tyrosinase; MFI, mean fluorescent intensity; Ag, antigen; mAb, monoclonal Ab.

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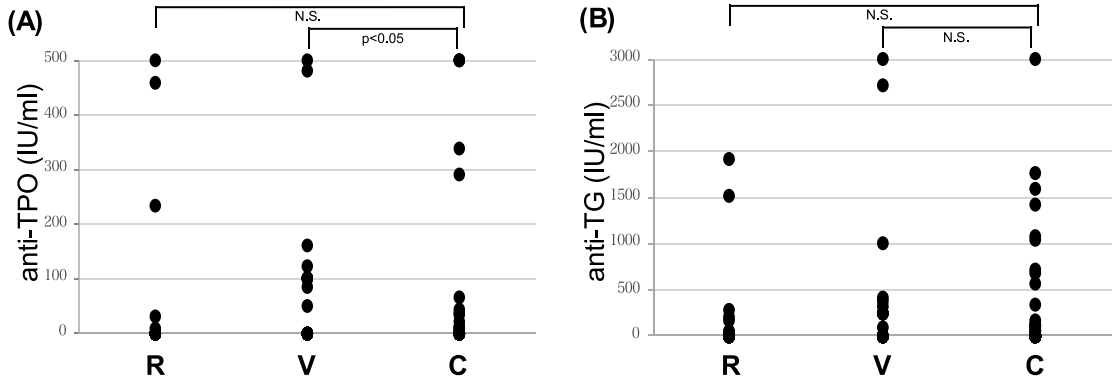
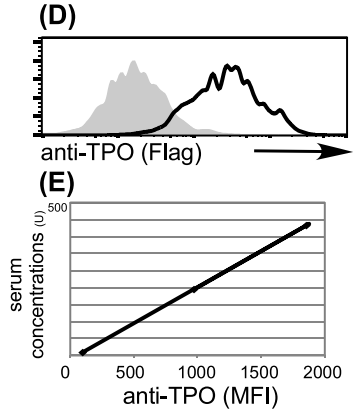


(C)

Centers	TPO Ab examined	TPO Ab (+)	TG Ab examined	TG Ab (+)	Total patients examined	TPO or TG Ab (+)
A	56	8	57	11	57	13 (n)
B	4	0	4	0	4	0
C	38	6	38	8	39	10
D	*n.d.*	*n.d.*	26	7	26	7
E	61	8	61	14	61	15
F	*n.d.*	*n.d.*	9	5	9	5
G	39	4	69	8	70	11
H	51	9	52	10	52	13
I	16	6	14	7	16	8
J	33	6	33	5	33	7
Total	298	47	363	75	367	89 (n)
		15.8 (%)		20.7 (%)		24.3 (%)
Control	100	16	100	18	21	100 (n)
		16.0 (%)		18.0 (%)		21.0 (%)



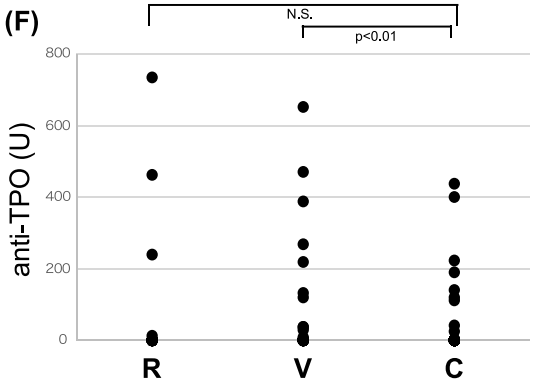


Fig. 1. Thyroid-specific autoantibodies (autoAbs) are detected in patients with vitiligo vulgaris, but not in those with rhododendrol-induced leukoderma.
Titers of serum autoAbs against thyroid peroxidase (TPO) **(A)** and thyroglobulin (TG) **(B)** were compared among patients with rhododendrol-induced leukoderma (**R**, number of the samples, mean ± S.D. of Ab titers,$n = 35$, TPO Ab: 35 ± 118 IU/mL; TG Ab: 88 ± 408 IU/mL), female patients with vitiligo vulgaris (**V**, $n = 45$, TPO Ab: 94 ± 173 IU/mL; TG Ab: 293 ± 724 IU/mL), and healthy women (**C**, $n = 100$, TPO Ab: 29 ± 107 IU/mL, TG Ab: 135 ± 438 IU/mL). Ab titers of >500 (anti-TPO) and >3000 (anti-TG) were plotted as 500 and 3000, respectively. Proportions of anti-TPO Ab positive were 14.3% in patients with Rhododendrol-induced leukoderma, 28.8% in patients with vitiligo vulgaris, and 15.0% in healthy females, respectively (cut-off value: 5.3 IU/mL). Proportions of anti-TG Ab positive were 22.9% in patients with Rhododendrol-induced leukoderma, 22.2% in those with vitiligo vulgaris, and 18.0% in healthy females (cut-off value: 40 IU/mL). **(C)** The proportions of patients with rhododendrol-induced leukoderma who were positive for anti-TPO or anti-TG Abs were analyzed in a multicenter study. The Ab titers were examined at each hospital, and the number of the patients who were positive for the Abs are described. The average was calculated and compared with those of 100 healthy female adult individuals. **(D)** Surface expression of TPO on the TPO transfectants was analyzed using flow cytometry (upper histogram). The shaded histogram represents secondary Ab only. **(E)** The linear proportional increase of serum concentrations (*y*-axis) with mean fluorescence intensities (MFIs) of serum binding to the TPO transfectant (*x* -axis) was analyzed using flow cytometry. **(F)** Anti-TPO Ab titers were determined by serum binding (MFI) to the TPO transfectant among patients with rhododendrol-induced leukoderma (**R**, $n = 32$, 45 ± 154), female patients with vitiligo vulgaris (**V**, $n = 30$, 67 ± 160), and healthy women (**C**, $n = 96$, 18 ± 69). Ab titers were converted to the unit according to Fig. 1 (E).

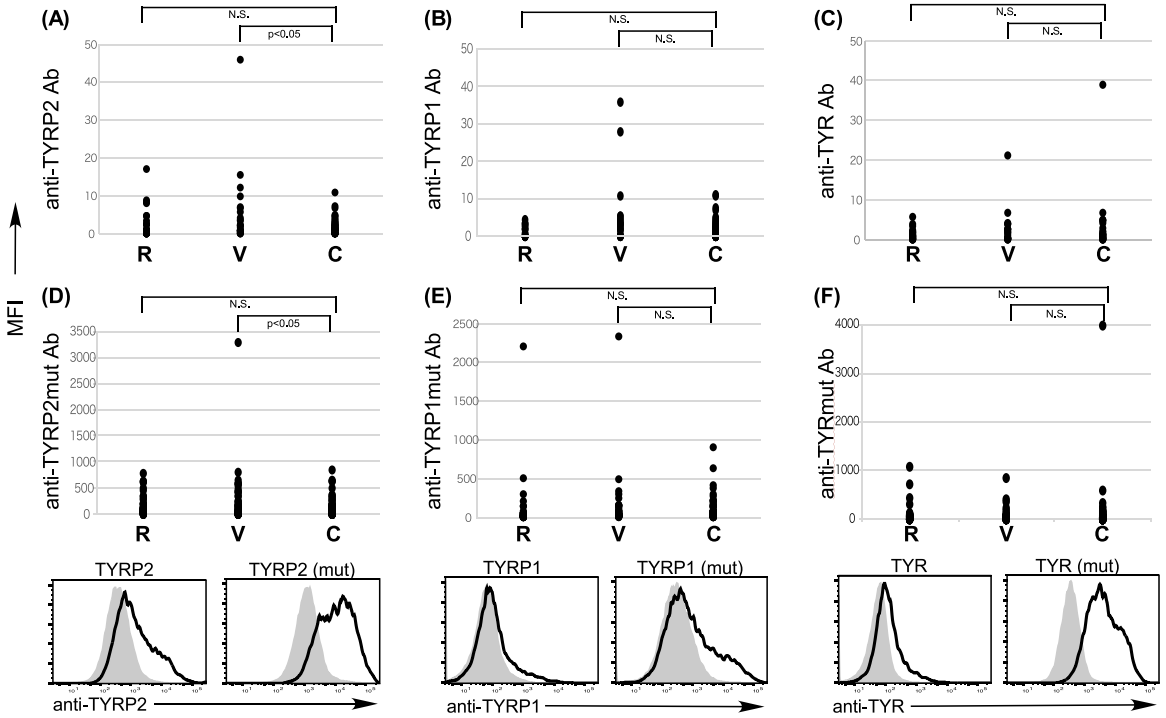


Fig. 2. Vitiligo-related autoantibodies (autoAbs) are not observed in patients with rhododendrol-induced leukoderma.
(A–C) Serum autoAb titers against TYRP2, TYRP1, and tyrosinase (TYR) were compared among patients with rhododendrol-induced leukoderma (**R**), patients with vitiligo vulgaris patients (**V**), and healthy women (**C**) using each transfectant. The number of analyzed samples and mean ± SD of Ab titers (mean fluorescence intensity, MFI) were as follows. **(A) R**, $n = 24$, 2.4 ± 4.4; **V**, $n = 24$, 5.3 ± 9.6; **C**, $n = 45$, 1.6 ± 2.7. **(B) R**, $n = 24$, 0.8 ± 10.9; **V**, $n = 24$, 4.5 ± 9.0; **C**, $n = 44$, 1.8 ± 3.0. **(C) R**, $n = 24$, 1.2 ± 1.5; **V**, $n = 20$, 2.8 ± 4.7; **C**, $n = 24$, 3.3 ± 7.8. **(D–F)** AutoAbs binding against the transfectants mutated in the cytoplasmic sorting signals in order to augment surface expression. The numbers of analyzed samples and mean ± SD of Ab titers (MFI) were as follows. **(D) R**, $n = 42$, 152.3 ± 188.8; **V**, $n = 44$, 275.0 ± 511.2; **C**, $n = 83$, 121.9 ± 164.0. **(E) R**, $n = 42$, 97.4 ± 346.4; **V**, $n = 44$, 126.5 ± 354.7; **C**, $n = 99$, 94.0 ± 295.0. **(F) R**, $n = 42$, 90.2 ± 203.5; **V**, $n = 44$, 72.6 ± 148.1; **C**, $n = 98$, 82.7 ± 405.8. The surface expression of TYRP2, TYRP1, and TYR was analyzed by staining each transfectant with monoclonal Abs. The shaded histograms represent staining with secondary Abs only (lower histograms).

vitiligo vulgaris [8]. In the present study, anti-TPO autoAbs were significantly detected in the sera of patients with vitiligo vulgaris, but not in those with rhododendrol-induced leukoderma. These results indicate that the predisposing factors involved may differ between vitiligo vulgaris and rhododendrol-induced leukoderma.

Anti-TYRP2 Abs were also significantly detected in patients with vitiligo vulgaris, but not in those with rhododendrol-induced leukoderma. Because melanosomal antigens were induced on the surface of the melanocytes under certain conditions (N.A., unpublished observations), autoAbs against melanosomes might play a role in damaging the melanocytes by Ab-dependent cellular cytotoxicity in patients with vitiligo vulgaris. Alternatively, autoAbs may just be a consequence of damaged melanocytes. In patients with vitiligo vulgaris, melanocyte damage may be more severe than in patients with rhododendrol-induced leukoderma. Because the pathogenicity of autoAbs in vitiligo has yet to be established, future studies will be required. Due to the fact that no significant increase was noted in these Abs in patients with rhododendrol-induced leukoderma, the underlying mechanisms involved in the development of vitiligo vulgaris and rhododendrol-induced leukoderma may be different.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.06.009.

References

[1] C. Nishigori, Y. Aoyama, A. Ito, K. Suzuki, T. Suzuki, A. Tanemura, M. Ito, I. Katayama, N. Oiso, Y. Kagohashi, S. Sugiura, K. Fukai, Y. Funasaka, T. Yamashita, K. Matsunaga, Guide for medical professionals (i.e., dermatologists) for the management of Rhododenol-induced leukoderma, J. Dermatol. 42 (2) (2015) 113–128.
[2] M. Sasaki, M. Kondo, K. Sato, M. Umeda, K. Kawabata, Y. Takahashi, T. Suzuki, K. Matsunaga, S. Inoue, Rhododendrol, a depigmentation-inducing phenolic compound, exerts melanocyte cytotoxicity via a tyrosinase-dependent mechanism, Pigment Cell Melanoma Res. 27 (5) (2014) 754–763.
[3] S. Ito, M. Ojika, T. Yamashita, K. Wakamatsu, Tyrosinase-catalyzed oxidation of rhododendrol produces 2-methylchromane-6,7-dione, the putative ultimate toxic metabolite: implications for melanocyte toxicity, Pigment Cell Melanoma Res. 27 (5) (2014) 744–753.
[4] Y. Abe, K. Okamura, M. Kawaguchi, Y. Hozumi, H. Aoki, T. Kunisada, S. Ito, K. Wakamatsu, K. Matsunaga, T. Suzuki, Rhododenol-induced leukoderma in a mouse model mimicking Japanese skin, J. Dermatol. Sci. 81 (1) (2016) 35–43.
[5] N. Arase, L. Yang, A. Tanemura, F. Yang, T. Suenaga, H. Arase, I. Katayama, The effect of rhododendrol inhibition of NF-kappaB on melanocytes in the presence of tyrosinase, J. Dermatol. Sci. (2016).
[6] T. Fujiyama, S. Ikeya, T. Ito, K. Tatsuno, M. Aoshima, A. Kasuya, J. Sakabe, T. Suzuki, Y. Tokura, Melanocyte-specific cytotoxic T lymphocytes in patients with rhododendrol-induced leukoderma, J. Dermatol. Sci. 77 (3) (2015) 190–192.
[7] R. Tsutsumi, K. Sugita, Y. Abe, Y. Hozumi, T. Suzuki, N. Yamada, Y. Yoshida, O. Yamamoto, Leukoderma induced by rhododendrol is different from leukoderma of vitiligo in pathogenesis: a novel comparative morphological study, J. Cutan. Pathol. 46 (2) (2019) 123–129.
[8] C. Vrijman, M.W. Kroon, J. Limpens, M.M. Leeflang, R.M. Luiten, J.P. van der Veen, A. Wolkerstorfer, P.I. Spuls, The prevalence of thyroid disease in patients with vitiligo: a systematic review, Br. J. Dermatol. 167 (6) (2012) 1224–1235.
[9] E.H. Kemp, D.J. Gawkrodger, P.F. Watson, A.P. Weetman, Immunoprecipitation of melanogenic enzyme autoantigens with vitiligo sera: evidence for cross-reactive

autoantibodies to tyrosinase and tyrosinase-related protein-2 (TRP-2), Clin. Exp. Immunol. 109 (3) (1997) 495–500.
[10] N. Arase, K. Tanimura, H. Jin, T. Yamaoka, M. Kishibe, M. Nishioka, E. Kiyohara, M. Tani, S. Matsuoka, K. Ohmura, K. Takasugi, T. Yamamoto, H. Murota, H. Arase, I. Katayama, Novel autoantibody against the beta2-glycoprotein I/human leucocyte antigen-DR complex in patients with refractory cutaneous ulcers, Br. J. Dermatol. 178 (1) (2018) 272–275.

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