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Residual genomic profile after cyclosporine may offer insights into atopic dermatitis reoccurrence

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To the Editor

Atopic dermatitis (AD) is characterized by activation of inflammatory pathways and epidermal barrier alterations. Cyclosporine A (CsA), a potent immunosuppressant, is considered the treatment of choice for severe AD. We recently showed that clinical resolution of AD with CsA is associated with strong suppression of immune and epidermal disease phenotypes.¹ Compared to narrowband UVB (NB-UVB) phototherapy, CsA caused greater reductions in Th2, Th22, Th9, and some Th1/IFN γ and Th17-related markers.¹

AD skin lesions tend to reoccur in the same areas after therapeutic clearance, suggesting that residual expression of a subset of disease-related genes that do not improve to non-lesional skin levels may predispose patients to reoccurrence. We have previously identified the residual disease genomic profile (RDGP) after NB-UVB,² and we now establish the unique RDGP after CsA, as well as the common RDGP across both therapeutics. Identifying a set of AD genes that do not show adequate improvement towards background skin expression

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levels across different therapeutics can serve as an important tool for understanding relevant AD-specific versus treatment-specific mechanisms associated with disease relapse. The CsA RDGP may also be a useful point of reference for future studies with topical calcineurin antagonists.

In order to identify the CsA RDGP, we analyzed the residual genomic and histologic profiles in clinically resolved lesions of 17 responders (>50% decrease in SCORAD) after 5 mg/kg/day CsA for 12 weeks¹ (see Methods in the Online Repository (OR)). The RDGP was defined as genes <75% improvement in the AD transcriptome (differentially expressed genes between lesional and nonlesional skin). The residual histologic disease phenotype was also evaluated, including cellular markers <75% improvement.

After 12 weeks of CsA treatment, the majority of genes had >75% improvement on gene-arrays. The RDGP after 12 weeks consisted of only a handful of genes compared to 2 weeks (Tables E1-E2 in OR). Leptin (LEP), an important protein involved in lipid metabolism and immune proliferation, did not improve at week 2 but improved by week 12. Similarly, claudin 8 (CLDN8), a tight junction protein, improved by only 72.7% at week 2 but greatly improved by week 12 (114%), demonstrating the importance of treatment duration (Tables E1-E2 in OR).

Compared to the NB-UVB RDGP, many barrier and immune genes already showed improvement at week 2 of CsA, and the RDGP at week 12 of CsA treatment was much smaller (Table 1, E2)². For example, chemotaxis-associated genes such as CXCL1 and CXCL2, which lacked sufficient improvement with NB-UVB (12% and 8%, respectively), showed significant improvement ($p<10^{-8}$, $p<10^{-6}$) with CsA at week 2 (146% and 136%, respectively), whereas CLDN8 and LEP significantly improved ($p<10^{-10}$, $p<10^{-3}$) at week 12 (114% and 80% respectively) (Table 1).

Claudin 23 (CLDN23), another tight junction protein, is part of the CsA and NB-UVB RDGP (Table 1). Decreased expression of CLDN23 is known to contribute to tight junction dysfunction, leading to higher allergen sensitization in AD patients.³ Immunohistochemistry confirmed the gene-array results, with faint pre-treatment lesional skin staining of CLDN8 and LEP versus increased staining at week 12 (Figure 1B-C). CLDN23 showed lack of histological improvement with weak and discontinuous epidermal staining in lesional skin before and after treatment, despite clinical resolution of AD lesions (Figure 1D, 1A).

Overall, RT-PCR and immunohistochemistry data demonstrated significant ($p<0.05$) genomic and cellular improvements with CsA (Table E3 in OR). Compared to NB-UVB, 12 weeks of CsA treatment showed greater reductions in epidermal hyperplasia (measured by epidermal thickness and K16 mRNA) (95% vs 88% and 117% vs 59%, respectively). Cytokine-induced genes such as MX1, S100A7, and S100A8, which did not improve after NB-UVB, showed significant improvement ($p<10^{-6}$, $p<10^{-5}$, $p<10^{-3}$) after only 2 weeks of CsA (102%, 114%, and 128%, respectively).

However, despite the smaller CsA RDGP, AD lesions tend to reoccur much faster after stopping CsA compared to NB-UVB (a two week relapse compared to eight weeks, respectively).^{4,5} A possible explanation for this phenomenon might be improved

differentiation of the stratum corneum (SC) after NB-UVB. The expression of terminal differentiation genes such as periplakin (PPL) and loricrin (LOR) was greatly increased after NB-UVB compared to CsA (Table E3 in OR). In addition, a significantly thicker SC was measured post-compared to pre- NB-UVB treatment, whereas no change in SC was detected after CsA (Fig E1 in OR).

Another explanation for the slower relapse after NB-UVB might be the reduction in IL-31 with this therapy, compared to lack of improvement after CsA (Table E3 in OR). IL-31, a pruritus-associated cytokine, can induce release of inflammatory mediators and inhibit epidermal differentiation.³ Furthermore, IL-33 and IL-25 can be released from damaged epithelium due to scratching and augment the Th2 response.³ IL-25 can also further weaken the epidermal barrier by inhibiting FLG expression.³ Of note, the expression of both IL-33 and IL-25 increased after CsA treatment (Table E3 in OR).

Analyzing the RDGP may also uncover genes with possible dual inflammatory and anti-inflammatory functions. Thus, the increases in IL-25 and IL-33 after treatment may actually be beneficial. Recent airway and IBD studies suggest that IL-25 decreases IL-17A via an IL-10 increase.⁶ IL-33 may also be anti-inflammatory by reducing NF- κ B gene expression.⁷ Similarly, in addition to their inflammatory role, Langerhans cells are thought to also have immunoregulatory functions.⁸ Indeed, we see that langerin⁺ cells are largely reduced in lesional compared to non-lesional AD skin at baseline, and significantly increase upon clinical reversal after 12 weeks of CsA (Fig 1E and Table E3).

In addition to the RDGP, other possible mechanisms for disease recurrence in the same areas need to be considered, including: 1) regional differences (increased humidity/friction, TEWL, pH, and lipids) that allow increased antigen penetration; 2) epigenetic modifications; 3) microbiome differences.⁹

In summary, we have demonstrated that although the CsA RDGP is much smaller than the NB-UVB RDGP, important structural defects and residual inflammation remain and the overall size of the RDGP does not predict relapse kinetics. Given that NB-UVB and CsA have different courses of disease maintenance upon discontinuing therapy, some elements in the RDGP of each treatment might explain relevant treatment- and disease-specific mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Histologic changes corresponding with CsA RDGP

A) Representative images of AD lesions after CsA. Representative staining in nonlesional/NL and lesional/LS skin before and after 2 and 12 weeks/Wk of CsA, black arrows indicate increased staining of claudin 8/CLDN8 (**B**) and leptin/LEP (**C**). White arrows indicate faint/discontinuous staining of claudin23/CLDN23 (**D**). **E**) Increase in langerin+ cells after CsA. Scale bars = 100 μm

Table 1
Comparison of the NB-UVB RDGP with gene improvement after CsA treatment

Symbol	CsA				NB-UVB		
	Wk0 (LS/NL)	Wk2/Wk0 (LS)	Impvt (%) Wk2	Wk12/Wk0 (LS)	Impvt (%) Wk12	Wk0 (LS/NL)	Wk12/Wk0 (LS)
<75% after both							
GYG2	1.14	0.74	48	0.77	54	2.14	0.42
CLDN23	1.35	0.49	35	1.05	68	0.92	-0.03
>75% after CsA							
BTC	1.74	1.10	57	1.50	77	2.45	1.34
RBP4	1.94	1.79	69	1.90	77	2.79	0.48
LEP	2.13	1.70	55	1.99	80	2.69	-0.43
ALDH1A1	1.19	0.39	36	1.06	83	1.26	0.64
LPL	1.34	1.42	82	1.79	87	2.45	0.00
FABP4	-1.8	1.32	60	1.72	87	1.43	0.32
AOC3	1.12	0.72	57	1.25	104	0.95	0.21
PPARG	1.22	0.93	68	1.58	106	1.84	0.17
CLDN8	1.76	1.51	73	2.25	114	1.76	1.02
MMP1	3.28	-4.30	132	-3.9	128	3.35	-2.34
THRSP	2.31	1.93	77	3.77	148	4.09	1.83
CXCL2	1.42	-1.70	136	-2.1	163	0.82	-0.06
CXCL1	2.05	-2.9	146	-3.2	164	1.72	-0.2

Data is sorted in ascending order of improvement (%) at week 12 of CsA. Improvement (%) is defined in Methods in the Online Repository (OR). All data is in log₂FCH. LS/lesional; NL/nonlesional; Wk/week; Impvt/improvement NB-UVB data from (Suarez-Farinas et al., J Allergy Clin Immunol 2013 131(2):577-9).