アトピー性皮膚炎患者は、なぜヘルペスウィルスに感染しやすいのか？

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Pathological Role of Regulatory T cells in the Initiation and Maintenance of Eczema Herpeticum

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Introduction:

It remains unknown why the occurrence of eczema herpeticum (EH) caused by an extensive disseminated cutaneous infection with herpes simplex virus (HSV) 1 or 2 is associated with the exacerbation of atopic dermatitis (AD) lesions after withdrawal of treatment. Although regulatory T cells (Tregs) limit the magnitude of HSV-specific-T cell responses in mice, their role in the induction and resolution of EH is yet to be defined. In this study,we investigated the frequencies, phenotype and function of Tregs in the peripheral blood of ADEH patients at onset and after clinical resolution, AD patient without EH, and healthy controls; and the effect of Treg-depletion on cytokine production.

Methods:

Blood samples:
Obtained from these patients on or near the day of the initial presentation before starting treatment, and additional samples were subsequently obtained from these patients.

We investigated:
・Frequencies, phenotype and function of Tregs in the PBMC from the patients.
・Effects of depletion of Tregs on IFN-γ production by HSV-1 specific $CD8^+$ T cells.

Patients:

Clinical and biological Characteristic	ADEH	P value	$ADEH^-$	P value	Healthy Control
No. of Samples	16	———	10	———	12
Age (y) mean ± SEM	29. 2 ± 2.1	.001	37.9 ± 3.1	.152	43.4 ± 3.0
SCORAD mean ± SEM	31.6 ± 6.1	———	35.5 ± 3.4	———	ND
Serum IgE mean ± SEM (IU/mL)	10201.4 ± 4151.7	.008	4742.5 ± 1783.2	.154	175.6 ± 84.0
Serum HSV IgG mean ± SEM (EIA Unit)	49.14 ± 11.4	.467	143.4 ± 44.4	.005	5.4 ± 2.0

mean ± SEM, P value: compared with healthy control. ND: not done

Results:

1) $Foxp3^+CD25^{++}$Tregs were expanded at onset and contracted upon resolution in ADEH (**Fig 1~2**).
2) The expanded Tregs displayed a phenotype of $CLTA\text{-}4^+$ $CD39^+$ $CD127^{-/mid}$ $CD45RA^-$ induced Tregs (iTregs) (**Fig 3**).
3) The expanded iTregs expressed Ki-67 and Helios, a member of the Ikaros transcription factor family (**Fig 3**).
4) A sequential analysis of Treg frequency in ADEH patient suggested that the expansion of Tregs was not a consequence of HSV infection but may be a causal factor (**Fig 4**).
5) Treg suppressive capacity to inhibit proliferation of effector T cells was retained in these ADEH patients (**Fig 5**).
6) Impaired IFN-γ and TNF-α production by CD8+ T and NK cells was shown at onset of ADEH patients (**Fig 6**).
7) Treg depletion resulted in a 2-fold increase in the frequency of HSV-1-specific $CD8^+$ T cells producing IFN-γ at onset of ADEH patients (**Fig 7**).
8) Reduced proinflammatory cytokine production from CD14dim monocytes stimulated with TLR2-ligand in the acute stage of ADEH patients (**Fig 8**).

Fig.1 The percentages of $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, $CD56^+$ NK cells, and TCR-γ/δ^+ T cells, in PBMC from ADEH patients, $ADEH^-$ patients, and healthy controls. Results are expressed as the mean percentage of each subset ± SEM in PBMC (in lymphocyte gated).

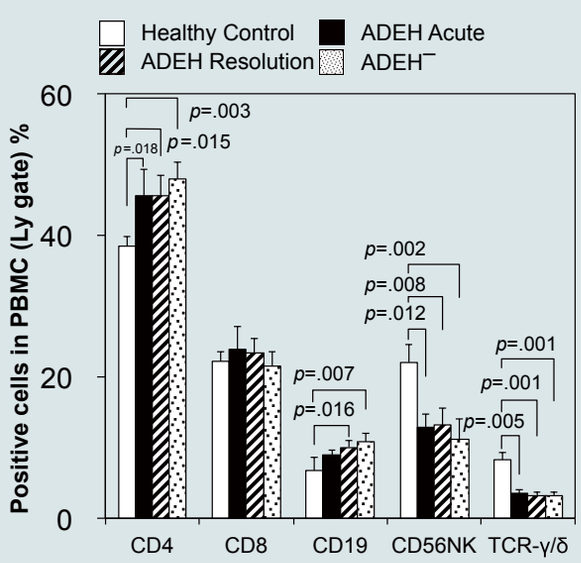


Fig.2 Expansion of Foxp3+CD25+Tregs at the acute stage of ADEH, but not the resolution stage, in ADEH patients and expression of skin-homing addressins on Tregs expanded at the acute stage of ADEH. **A**, Representative flow cytometry dot plots. **B**, The mean frequency of Foxp3+CD25+, Foxp3+CLA+, and Foxp3+CCR4+ Tregs in CD4+ T cells. Results are expressed as the mean ± SEM.

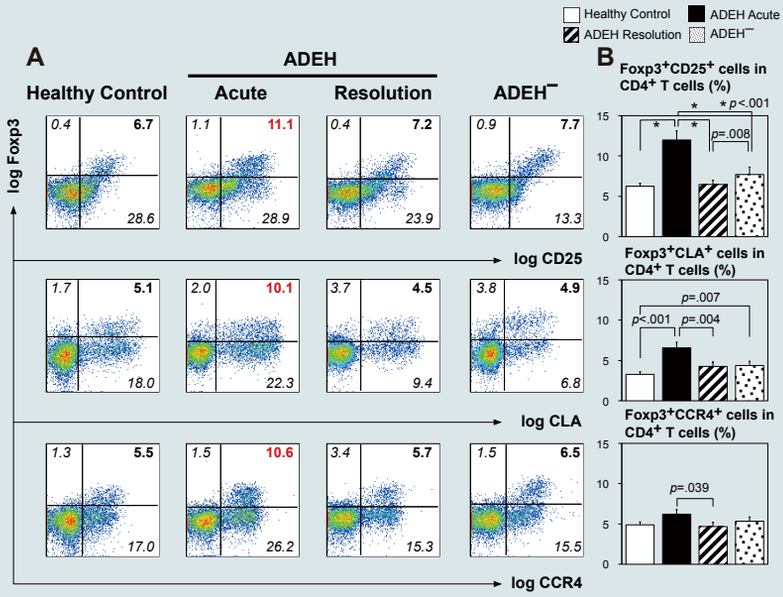


Fig.3 Representative flow cytometry dot plots showing the expression of Foxp3 vs. CD152 (CTLA-4), Foxp3 vs. CD39, Foxp3 vs CD127, Foxp3 vs HELIOS, Foxp3 vs Ki67, and CD45RA vs Foxp3 in CD4+ T cells and HELIOS vs. Ki67 in CD4+Foxp3+ T cells.

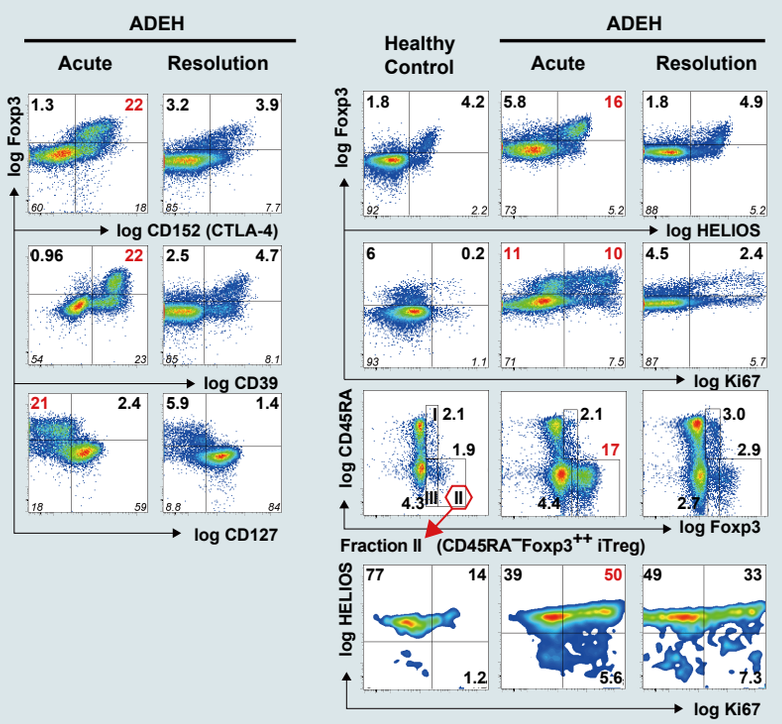


Fig.4 A sequential analysis of Treg cell frequency, serum HSV IgG level, and clinical course in a single AD patient with EH induced by a primary infection with HSV. The frequency of Tregs observed at onset was in the same range as what we observed in other AD patients with recurrent EH induced by reactivation of HSV. In contrast to what was observed in a recurrent form, however, the frequency of Tregs after clinical resolution did not correlate with the improvement of clinical symptoms but was shown to be even increased after resolution on day 13 as compared with that at onset in this patient. Symptomatic flares (on day 36), although subsequently followed by the clinical improvement of EH, were observed during this period in this patient, despite a persistent elevation in circulating Tregs.

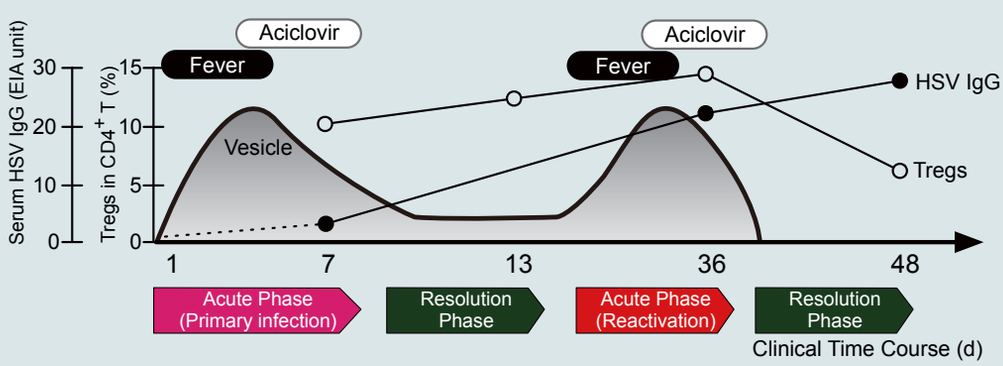


Fig.5 Functional analysis of Tregs at the different stages of ADEH, ADEH⁻, and healthy controls. Purified CD4⁺CD25⁺⁺ Treg cells from patients were co-cultured with purified CD4⁺CD25⁻ effector T cells in the presence of MitomycinC-treated allogeneic APCs and anti-CD3 and anti-CD28 mAbs. Proliferation was assessed by a [3H]thymidine incorporation assay. The results are expressed as the percent proliferation of CD4⁺CD25⁻ effector T cells in the absence of CD4⁺CD25⁺⁺Tregs.

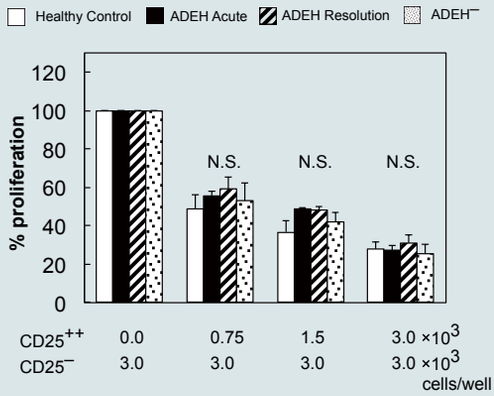


Fig.6 Intracellular expression of IFN-γ, TNF-α, IL-17, and IL-4 by CD8⁺ T, CD4⁺ T, CD56⁺ NK, and TCR-γ/δ⁺ from ADEH patients, ADEH⁻ patients, and healthy control. IFN-γ and TNF-α production by CD8⁺ T cells and CD56⁺ NK cells were impaired in AD patients with EH at onset as compared with that in those after resolution. Upon a contraction of Tregs after resolution, however, the impaired ability of these cells to produce IFN-γ and TNF-α was restored to levels comparable to that in healthy controls.

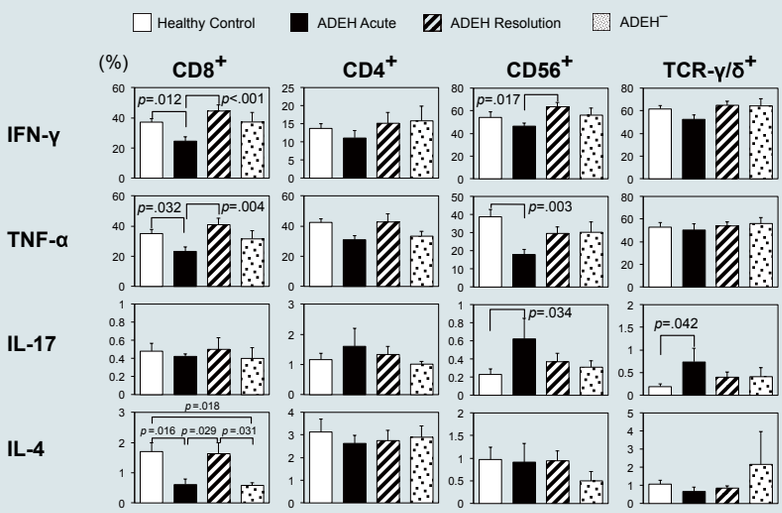
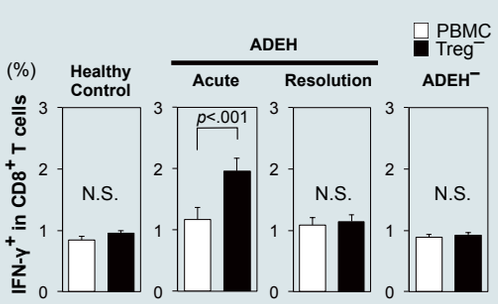
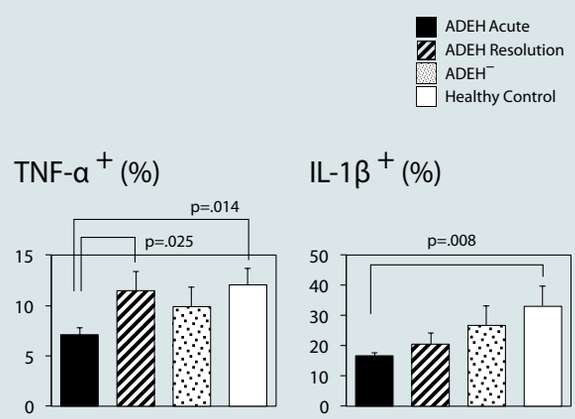


Fig.7 To demonstrate that IFN-γ production by HSV-1 specific CD8⁺ T cells could be abrogated by expanded Tregs at onset, we analyzed the effects of depletion of Tregs on IFN-γ production by HSV-1 specific CD8⁺ T cells. PBMCs and CD4⁺CD25⁺CD127⁻/mid Treg-cell depleted PBMCs (Treg⁻), respectively, were stimulated with 25μg/mL HSV-1gD peptide or medium for 24h with CD28/CD49d Abs. After 24h of culture, Brefeldin A was added and then additional culture was done for 4h. Intracellular cytokine was detected by FACS.

Fig.8 Proinflammatory cytokine (TNF-α & IL-1β) production from CD14dim in response to TLR2 (recognized HSV) was significantly reduced in the acute stage of ADEH. PBMCs were stimulated with Pam3Cys (TLR2-ligand) and then intracellular cytokines were analyzed by FACS.





Conclusions:

Expansions of functional Tregs initially required for ameliorating excessive inflammation occurring after withdrawal of topical corticosteroids could in turn contribute to the initiation and progression of HSV reactivation, resulting in the onset of EH. This study provides evidence suggesting that Treg cell-dependent suppression of IFN-γ production by CD8⁺ T cells plays a key role in triggering HSV reactivation before the onset of symptoms in EH. Our results suggest that therapeutic strategies that inhibit expansion of Tregs may improve control of HSV reactivation in AD patients with a history of EH.