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学位論文題目	Studies on atopic dermatitis related genes and cytokines 1.Association between RANTES promoter polymorphism -401A and enhanced RANTES production in atopic dermatitis patients 2.Complex regulation of S100A8 by IL-17, Dexamethasone, IL-4 and IL-13 in HaCat cells (human keratinocyte cell line) （アトピー性皮膚炎の進展に関連する遺伝子とサイトカインについて研究 1.「アトピー性皮膚炎患者における RANTES の遺伝子および蛋白発現と遺伝子多型」 2.「HaCat 細胞（ヒトケラチノサイト細胞）での IL-17、デキサメタゾン、IL-4 および IL-13 による S100A8 の調節」）
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論　文　内　容　要　旨

※整理番号	556	氏　名（ふりがな）	柏　冰　雪（ばい　びん　しゅ）
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Atopic dermatitis (AD) is a common pruritic and chronically relapsing inflammatory skin disease with a steadily increasing prevalence. At least two distinct type of AD have been identified: an extrinsic type associated with IgE-mediated sensitization, which affects 70-80% of patients; and an intrinsic type without IgE-mediated sensitization, which affect 20-30% of patients. AD results from a complex interplay between strong genetic and immunological factors, the host's environment, and skin barrier defects.

Current evidence indicates that AD is strongly genetic, several chromosomal regions contain pathophysiologically relevant candidate genes, especially on chromosome 5q31-33 since it contains a clustered family of Th2 cytokine genes: ie interleukine (IL) 4 and 13, others including: chromosome 17q contains (regulated on activation, normal T-cell-expressed and secreted) RANTES and eotaxin 1, chromosome 1q21 contains the epidermal differentiaton complex including S100A8. In addition, recent studies provide insights into the underlying immunological mechanisms, suggesting the complex interdependent network of cytokines that lead to the development of AD. Although current researches enhanced knowledge of AD in many aspects, the association of the candidate gene with AD and the immunoregulatory mechanism of cytokines in AD are far from being elucidated completely. Our studies are to investigate 1) the association of a candidate gene-RANTES with AD 2) the regulation of AD related cytokines on S100A8 in human Keratinocytes, undoubtedly, they will result in a better understanding of the disease and might lead to the identification of novel therapeutic targets.

Project 1

Association between RANTES promoter polymorphism -401A and enhanced RANTES production in atopic dermatitis patients

Objective: Chemokine RANTES, located on 17q, is reported linked to AD. Enhanced RANTES production has been found in serum and skin lesions of AD patients. Our previous data has shown that RANTES –401A polymorphism is associated with Japanese AD patients with high IgE concentration. However, it remains unclear whether this polymorphism is correlated with serum RANTES levels in AD patients. This study was to investigate the association of the -401G/A polymorphism with RANTES mRNA as well as protein expression in AD patients.

Methods: Sixty-two Japanese AD patients and fourteen normal controls were studied. RANTES genotypes were determined using a polymerase chain reaction (PCR)-based assay, while the expression of RANTES mRNA from peripheral blood mononuclear cells (PBMC) was quantified with real-time reverse transcription (RT)-PCR. RANTES serum levels were measured by enzyme-linked

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等で印字すること。

２．※印の欄には記入しないこと。

immunosorbent assay (ELISA).

Results: There were no significant differences in mRNA expression between patients and normal controls with RANTES -401 genotypes. RANTES serum levels were significantly increased in AD -401A carriers as compared to the controls. AD -401A carriers with a high IgE concentration showed higher RANTES protein levels than normal controls or normal -401A carriers. Further, AD -401A carriers with severe AD showed significantly higher RANTES levels when compared to those with moderate or mild AD and normal controls.

Discussion: The RANTES –401A promoter polymorphism results in a binding element for GATA transcription factors which play roles in up-regulating RANTES promoter activity. It has been reported that levels of GATA-3 mRNA in PBMC were elevated in AD patients. Therefore, GATA transcription factor may at least partly, contribute to the high RANTES levels observed in AD -401A polymorphism carriers. Furthermore, AD -401A polymorphism carriers with a high IgE concentration also showed enhanced RANTES production as compared to normal controls, which might provide further support to the hypothesis that AD patients are genetically heterogeneous.

Conclusion: The RANTES promoter -401A polymorphism plays a role in the development of AD by up-regulating serum levels of RANTES in Japanese patients. These results provide an explanation for the association of this polymorphism with AD.

Project 2

Complex regulation of S100A8 by IL-17, Dexamethasone, IL-4 and IL-13 in HaCat cells (human keratinocyte cell line)

Objective: S100A8, a calcium binding protein, is associated with keratinocyte differentiation, inflammation and wound healing. We previously reported that S100A8 is the most greatly changed gene in atopic dermatitis (AD). S100A8 protein is thought to have an inflammatory function and also possess anti-inflammatory properties. Although S100A8 has attracted much more interest recently, little is known regarding its regulation in human keratinocytes. In this study, we investigated the regulation of S100A8 by IL-17, dexomethasone (DEX), IL-4 and IL-13 in HaCat cells (human keratinocyte cell line).

Methods: The mRNA expression and protein levels of S100A8 were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. S100A8 promoter activity was analyzed using the luciferase reporter assay.

Results: IL-17 and DEX were found to enhance S100A8 mRNA and protein expression.IL-4 and IL-13 inhibited S100A8 expression, and also suppressed IL-17-stimulated S100A8 expression. Furthermore, Reporter assay confirmed that these regulations are mediated at the promoter level.

Discussion: T cells have been implicated in inflammatory skin disorders such as AD and psoriasis. T cell derived cytokine such as IL-17, IL-4 and IL-13 play very important role in AD, their mRNA were detected in AD, suggesting involvement of a complex cytokine network in skin lesions of AD. Our results showed that IL-17 increased S100A8 production, thus IL-17 may be associated with development of AD through up-regulation of S100A8 production. Furthermore, This study showed that IL-4 or IL-13 suppressed S100A8 expression. However, other important cytokines in AD such as TNF alpha, were reported to greatly enhance S100A8 expression. Thus high expression of S100A8 observed in our previous study, may result from complex regulation by many stimulatory factors for S100A8 in AD patients.

DEX, a strong anti-inflammatory drug, unexpectedly enhanced S100A8 production in this study, suggesting a protective role of S100A8 in inflammatory diseases. NF1 is thought to regulate murine S100A8 promoter activity in treatment with glucocorticoids. NF1 is also located in human S100A8

promoter (-54 to -37; -586 to -569) used in the present study, thus DEX appears to enhance S100A8 expression through NF1 in HaCat cells. S100A8 production was enhanced by both pro-inflammatory and anti-inflammatory factors. It is difficult to explain these competing results. However, we speculate as follows: modification of S100A8 protein may be involved in this discrepancy. For example, S100A8 was shown to bephosphorylated by PMA stimulation. Thus, the nature of S100A8 treated with IL-17 and DEX may be different. This interesting possibility warrants further investigation.

Conclusion: The complex regulation of S100A8 by IL-17, IL-4 and IL-13 seems to contribute to pathogenesis of inflammatory skin diseases with high S100A8 expression such as AD. Promoting effect of DEX (glucocorticoid) on S100A8 supports the idea that S100A8 may have an anti-inflammatory as well as inflammatory function. The nature of this protein may differ depending on the regulators.

Summary: Taken together, our study on RANTES promoter -401A polymorphism provided an understanding of the genesis basis of AD, while study on complex regulation of AD related cytokines on S100A8 represented susceptibility factors for the development of AD. Thus these studies are likely to assist with disease recognition and may provide new thought for novel efficacious treatment options.

学位論文審査の結果の要旨

整理番号	５５６	氏名	柏　冰雪

（学位論文審査の結果の要旨）

アトピー性皮膚炎(AD)の進展に関連する遺伝子としてRANTES、S100A8をとりあげ、血清中RANTESの上昇と遺伝子多型との関係、さらに角化細胞における各種サイトカイン(IL-17、デキサメタゾン(DEX)、IL-4、IL-13）によるS100A8発現の制御について研究した。

その結果、以下のことを明らかにした。

１）IgE高値のADで401A遺伝子多型の患者では、統計学的に有意に血清中RANTESレベルが高い。

２）IL-17とDEXは、S100A8の発現を促進したが、IL-4とIL-13はS100A8の発現に対して抑制的に作用した。

以上より、本研究は、血清RANTESの上昇とRANTESプロモータの遺伝子多型との関係を明らかにするとともに、S100A8の発現が多彩なサイトカインの複雑な制御下にあることを示した論文であり、博士（医学）の学位を授与するに値すると認める。

なお、本学位授与申請者は平成１９年８月２９日実施の論文内容とそれに関連した試問を受け、合格と認められた。

（平成１９年　８月３０日）