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Concise communication

Phosphorylated STAT3 in the epidermis in adult-onset Still's disease

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Abstract

Adult-onset Still’s disease (AOSD) is characterized by multiple systemic inflammation of unknown etiology. Although the typical eruption of AOSD is salmon-pink rheumatoid rash on the trunk and extremities, persistent pruritic papules and plaques have also been reported. Correlations between serum cytokines, including interleukin-6 and interleukin-18, and disease activity in AOSD have been reported. Activated signal transducer and activator of transcription 3 (STAT3) is transported into the nucleus, where it functions as a transcription factor that regulates genes involved in cell survival and inflammation.

To assess whether STAT3 was phosphorylated in skin samples from AOSD patients, we conducted immunohistochemical analysis of affected and unaffected lesions from four AOSD patients in comparison with ten normal controls. Quantitative analysis was conducted by measuring the ratio of epidermal keratinocytes with phosphorylated STAT3 (p-STAT3)-positive nuclei to total epidermal keratinocytes.

p-STAT3 was found to be more strongly expressed in the nuclei in the epidermis of AOSD than in normal controls. Quantification of the data revealed significant differences in staining for p-STAT3 between AOSD and normal skin.

Our findings suggest that phosphorylation of STAT3 might be a potential therapeutic target for AOSD.

KEY WORDS: adult-onset Still's disease (AOSD), signal transducer and activator of transcription 3 (STAT3)

(iii) text

Introduction

Adult-onset Still's disease (AOSD) is characterized by multi-system inflammation of unknown etiology. Although the typical eruption of AOSD is salmon-pink rheumatoid rash on the trunk and extremities, persistent pruritic papules and plaques have also been reported.[1] Additionally, various skin lesions are occasionally observed in association with AOSD.[2] Recently, atypical cutaneous manifestations associations with delayed malignancy were reported.[3] Correlations between serum cytokines, including interleukin-6 (IL-6) and interleukin-18 (IL-18), and disease activity in AOSD have been reported.[4] Indeed, the administration of biologics to block these cytokines is effective.[5] Moreover, the levels of mRNA expression of IL-6, IL-18 and interleukin-8 are significantly higher in tissue biopsied from Still's rash in AOSD patients than in controls.[6]

Activated signal transducer and activator of transcription 3 (STAT3) is transported into the nucleus, where it functions as a transcription factor that regulates genes involved in cell survival and inflammation.[7] The

activation of STAT3 in keratinocytes is well known in psoriasis.[8, 9] Here, we conducted immunohistochemical analyses to determine whether STAT3 is phosphorylated in skin samples from AOSD patients. Our findings suggest that activated STAT3 (phosphorylated STAT3, p-STAT3) may play a role in AOSD and that the STAT3-activating pathway may be a therapeutic target.

Materials and methods

Human skin tissues

Four patients with AOSD were enrolled. The diagnoses were based on the Yamaguchi criteria for AOSD.[10] Skin samples from ten healthy individuals were used as controls (mean age: 67.0 years; range: 53-87). All skin tissue samples were taken after written informed consent had been obtained. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine, and was conducted according to the Declaration of Helsinki Principles.

Immunohistochemistry

Immunohistochemical analysis of skin samples from the participants was performed as described previously, with slight modifications.[9] Thin sections (3μm) were cut from samples embedded in paraffin blocks. The sections were incubated for 30 minutes in PBS with 4% BSA followed by an incubation with the polyclonal rabbit anti-phosphorylated STAT3 (Phospho-Tyr705) antibody (Signalway Antibody, College Park, MD) (10 ng/ml) in PBS containing 1% BSA. Thin sections were stained with avidin-conjugated goat anti-rabbit immunoglobulin secondary antibodies for 1 hour. Vectastain Elite ABC-PO kit (Vector Laboratories, Burlingame, CA) was used for staining. We counted the keratinocytes with positively stained nuclei and we counted all the keratinocytes in two high-magnification views for each skin biopsy specimen. To identify the keratinocytes, we made serial sections of the biopsy specimens and stained the section next to the immunohistochemically stained section with haematoxylin-eosin stain. Then, we calculated the average ratios of keratinocytes with positively stained nuclei to all keratinocytes in each skin specimen. Quantitative analysis was conducted by measuring the ratio of cells with p-STAT3-

positive nuclei to total cells. An unpaired t-test was used to analyze the results. $P < 0.01$ was considered statistically significant.

Results

Clinical features of the participants

Case 1: A 41-year-old Japanese woman was admitted to the hospital with a spiking fever, polyarthralgia and chronic urticaria. Clinical examination showed diffuse edematous erythema on the upper eyelids, scaly erythematous plaques and small pustules on the chest (Fig. 1a, b), and hyperkeratotic pigmented macules on her metacarpophalangeal joints. Diffuse scratched erythema with pigmentation was found on her abdomen and back. In addition, she had distinctive salmon-pink rheumatoid eruptions on the right thigh (Fig. 1c).

Case 2: A 37-year-old man showed diffuse widespread erythema on the trunk (Fig. 1d) and extremities accompanied by persistent high fever and sore throat. He had recurrent urticarial eruption, especially on the back. Bone marrow aspiration led to the diagnosis of hemophagocytic

lymphohistiocytosis.

Case 3: Case 3 is a 74-year-old woman. She was hospitalized for anorexia, fever of unknown origin and polyarthritis. Clinical examination revealed salmon-pink rashes on the forehead, back and extremities. On one occasion, diffuse erythematous plaques with small pustules were seen on the chest (Fig. 1e). Haematoxylin-eosin staining of a skin specimen from this eruption showed some dyskeratotic cells in the upper layers of the epidermis, including in the horny layers.

Case 4: A 37-year-old woman presented with recurrent episodes of continuous high fever, polyarthritis and urticarial edematous erythema on the extremities. In addition, she showed punctate purpura (Fig. 1f). She had been treated with tocilizumab, a humanized monoclonal antibody against the IL-6 receptor (IL-6R), based on her elevated serum IL-6 of 52.3 pg/mL (normal range: 0-4.0).

Strong nuclear staining of p-STAT3 in the epidermis of the active

phase of AOSD patients

p-STAT3 was found to be strongly expressed in the nuclei of keratinocytes in the patients (Fig. 2b) compared with almost cytoplasmic staining in normal control skin samples (Fig. 2a). Similar staining patterns were found for all four AOSD patients. We counted epidermal keratinocytes with p-STAT3-positive nuclei and total epidermal keratinocytes at higher magnification (x400) for each skin sample by conventional light microscopy. Table 1 shows the ratios of epidermal keratinocytes with p-STAT3-positive nuclei to total epidermal keratinocytes in the examined skin samples. Statistical analyses of the data revealed significant differences in the rates of epidermal keratinocytes with p-STAT3-positive nuclei ($P < 0.01$) between AOSD patient lesional skin samples and normal control skin samples (Fig. 2e). Additionally, skin biopsy specimens from unaffected skin of the patients showed stronger nuclear expression of p-STAT3 than in the normal control skin, although the difference is not significant (Fig. 2c). In contrast, the ratios of epidermal keratinocytes with p-STAT3-positive nuclei to total epidermal keratinocytes were lower than those in the affected lesions (Table 1). The

affected lesion of Case 2 was stained only by the secondary antibody as a negative control (Fig. 2d). The laboratory test results for skin biopsies taken simultaneously from affected and unaffected lesions are given in Supplementary Table 1.

Discussion

We described four cases of AOSD with various skin eruptions, including salmon-pink rheumatoid rash and persistent pruritic papules and plaques (Fig. 1). Notably, Case 1 showed sharply demarcated erythema with sliver-white scales on the chest, mimicking psoriasis (Fig. 1b). We needed to rule out generalized pustular psoriasis. Histologically, the dyskeratotic cells were found in the upper layers of the epidermis, including in the horny layers, in Case 3. This finding is seen in one of the four present patients and is consistent with the histological features of persistent pruritic eruption in the previously reported AOSD cases.[2] Case 4 had punctate purpura (Fig. 1f). Though thrombotic thrombocytopenic purpura has rarely been reported in association with AOSD, awareness of the possible coexistence of these two diseases is important for diagnosis

and treatment when the patient shows such purpura.[11] So far, no malignancies have been found in any of the four patients. However, atypical cutaneous manifestations associated with delayed malignancy were reported previously[3] and we think we should pay attention to the occurrence of late-onset malignancies in AOSD.

We report that p-STAT3 was found to localize to the nucleus in keratinocytes of the skin in four active AOSD patients (Fig. 2). These findings are similar to that seen in the psoriatic condition.[8] As in the active phase of AOSD, the expression of IL-6 is significantly increased in psoriatic lesions.[9] IL-6 is a multifunctional cytokine, and it signals through a heterodimeric IL-6R/gp130 complex that triggers the activation of the Janus (JAK) kinases and downstream effectors, including STAT3 in keratinocytes.[9, 12] Thus, it is possible that STAT3 might be activated by the IL-6 signaling pathway in the epidermis in AOSD. A similar molecular mechanism might lead to epidermal inflammation in both psoriasis and AOSD. There are several reports of tocilizumab being effective for patients with psoriatic arthritis as well as AOSD.[5, 13, 14] Moreover, several JAK inhibitors are known to be effective treatments for psoriasis vulgaris.

Therefore, the JAK inhibitor may also be effective against AOSD by blocking the IL-6/STAT3 pathway.

In conclusion, we demonstrated that there is strong nuclear staining of p-STAT3 in the epidermis of AOSD patients in the active phase. This finding suggests that phosphorylation of STAT3 might be a potential therapeutic target for AOSD.

(iv) acknowledgments

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(v) conflict of interest

The authors have no conflict of interest to declare.

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(vii) supporting information

Supplementary Table 1

(viii) figure legends

Figure 1. Clinical characterizations of the AOSD patients

(a-c) Case 1. (a) An edematous erythema on the upper eyelids resembles heliotrope rash. (b) Scaly erythematous plaques and small pustules are seen in the chest. (c) Typical salmon-pink rheumatoid eruptions are seen on the right thigh. (d) Case 2: Diffuse faint erythema and chronic pigmentation on the back. (e) Case 3: Diffuse scratched erythema with pustulosis on the chest. (f) Case 4: Atypical non-palpable purpuras of various sizes are seen in both legs.

Figure 2. p-STAT3 immunostaining in AOSD and normal epidermis

(a-c) The epidermis in an affected lesion (b) and in an unaffected lesion (c) from Case 2, and normal skin from a healthy donor (a) were stained with anti-p-STAT3 antibody. Scale bars: 50μm. (d) A negative control (by using only the secondary antibody) for an affected lesion of Case 2. Scale bar: 50μm. (e) The results were quantified by calculating the rates of epidermal keratinocytes with p-STAT3-positive nuclei to total epidermal keratinocytes in the examined skin samples. Results are presented as the interquartile

range ± SEM. The statistical significance was assessed using unpaired Student’s t-test. *$P < 0.01$. Abbreviation: KCs, keratinocytes