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H　知的財産の出願・登録状況

なし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表（雑誌）

発表者氏名	論文タイトル名	発表雑誌	巻号	ページ	出版年
Saito N, Abe R, Yoshioka N, Murata J, Fujita Y, Shimizu H	Prolonged elevation of serum granulysin in drug-induced hypersensitivity syndrome.	Br J Dermatol			in press
Hirata Y, Abe R, Kikuchi K, Hamasaka A, Shinkuma S, Nomura T, Nishie W, Arita K, Shimizu H	Intraepidermal neutrophilic IgA pemphigus successfully treated with dapsone.	Eur J Dermatol			in press
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Ikeda Y, Nakamura M, Gonzalez G, Koyanagi KO, Watanabe H, Suzutani T.	sequence.				
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Ⅳ. 研究成果の刊行物・別刷

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Article type : Item of Correspondence

Prolonged elevation of serum granulysin in drug-induced hypersensitivity syndrome

N. Saito, R. Abe, N. Yoshioka, J. Murata, Y. Fujita, H. Shimizu

Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Key words

Drug-induced hypersensitivity syndrome (DIHS), Eosinophilia and systemic symptoms (DRESS), Granulysin

Correspondence to:

Riichiro Abe MD/PhD

Department of Dermatology

Hokkaido University Graduate School of Medicine

N15 W7, Kita-ku, Sapporo 060-8638, Japan.

Tel: +81-11-706-7387

Fax: +81-11-706-7820

e-mail: aberi@med.hokudai.ac.jp

MADAM,

Drug-induced hypersensitivity syndrome (DIHS)[1], also known as drug rash with eosinophilia and systemic symptoms (DRESS) [2], has been established as a clinical entity in severe cutaneous adverse drug reactions. DIHS is characterized by the limited number of causative drugs, late onset, clinical similarity to infectious mononucleosis-like syndrome and prolonged clinical course due to relapse[1].

Granulysin is a cytotoxic molecule produced against virus-infected cells, tumor cells, transplant cells, bacteria, fungi and parasites[3]. It plays an important role in the host defense against pathogens. A recent paper reported that granulysin is highly expressed in blisters of two other severe cutaneous adverse drug reactions: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)[4]. In addition, we found that serum granulysin is more elevated in patients with early-stage SJS/TEN than in those with ordinary drug-induced skin reactions[5].

This paper investigates the serum granulysin level of DIHS patients. We assembled serum samples of patients with DIHS and analyzed the correlation between granulysin concentrations with clinical manifestations and disease courses.

Sera of 15 patients with DIHS (10 men and 5 women; average age: 55.4 ±19.9 years) were obtained from multiple institutions. All the patients had actively progressing reactions meeting the criteria for DIHS, as previously defined[1]. The disease onset (day 1) was defined as when the skin eruption appeared. Sera of patients with ordinary drug skin reactions (ODSRs) (n=24) and healthy controls

(n=31) were also obtained. ODSRs included maculopapulartype and erythema multiforme-type reactions. The granulysin concentrations of the serum samples were measured with an ELISA as previously described[6]. In brief, serum samples were incubated on plates coated with RB1 antibody (MBL) and then reacted with biotinylated RC8 antibody (MBL). We performed assays in blind of the clinical features.

In serum samples taken from day 1 to day 10 (n=9), 8 samples showed elevated serum granulysin levels over 10 ng/ml (88.9%, 21.9 ±12 ng/ml). In serum samples taken from day 11 to day 20 (n=11), we detected prolonged high serum granulysin levels (63.6%, 16.1±14.8ng/ml). Serum granulysin levels decreased gradually after day 21 (n=20) (30%, 7.6 ± 3.4) (Figure 1). By day 20, the skin eruptions of all the DIHS patients had disappeared. As we reported previously, in 31 healthy control subjects, no increase of granulysin level was detected (0%, 1.6±0.6 ng/ml) and in 24 patients with ODSRs, elevated granulysin was detected in only one patient (4.16%,3.5±3.4 ng/ml) [5].

To distinguish DIHS from ODSRs, the following clinical information is helpful: limited causative drugs, late onset after medication, manifestations similar to infectious mononucleosis such as fever, lymphoadenopathy, hepatitis and hematological abnormalities. However, because of the diversity of ODSRs and similarity to viral exanthema, DIHS sometimes poses a diagnostic challenge. In addition, some cases suffer from multiple organ failure. Therefore, early diagnosis and appropriate treatment is essential.

Unique mechanisms have been implicated in DIHS development, including detoxification defects leading to reactive metabolite formation and subsequent

immunological reactions[7], and reactivation of HHV[8]. In addition, it is increasingly apparent that there is a genetic predisposition to adverse drug reactions. Human leucocyte antigen-related genes have been identified as predictors of DIHS[9].

In particular, the observation that HHV reactivation occurs during the acute phase of DIHS has led to suggestions of a pathogenic link. Shiohara *et al.* identified early reactivation of HHV6 and EB virus, with later involvement of HHV7 and CMV[8]. The resulting expansion of virus-specific T cells might mediate the clinical disease. Recent paper showed that cutaneous and visceral symptoms of DIHS/DRESS are mediated by activated $CD8^+$ T lymphocytes, which are directed against herpes viruses such as EBV[10].

Granulysin exhibits potent cytotoxicity against a broad panel of microbial targets, including tumor cells, transplant cells, bacteria, fungi and parasites, damaging negatively charged cell membranes because of its positive charge[3]. Granulysin plays important roles in the host defense against pathogens and induces apoptosis of the target cells in a mechanism involving caspases and other pathways [3]. In the present study, we showed that granulysin levels of sera were significantly elevated in DIHS patients compared to those of ODSRs. It is suggested that, in DIHS, activation of virus-specific cytotoxic T cells resulted in granulysin release in circulated blood. In contrast, granulysin was identified as the most highly expressed cytotoxic molecule in blisters of SJS/TEN resulting massive keratinocyte apoptosis[4], and we revealed that serum granulysin increased in early stage of SJS/TEN[5]. We speculated that granulysin is involved in SJS/TEN pathogenesis, inducing keratinocyte death in the early stage of

these diseases, whereas serum granulysin in DIHS might be released against virus-infected cells. This speculation is consistent with the present data that show the duration of DIHS manifestation to coincide with the timing of elevated serum granulysin levels. Recently we developed a rapid immunochromatographic test to detect high serum granulysin level in 15 minutes[6]. We expect that monitoring of serum granulysin by the rapid test might contribute to the early diagnosis of DIHS as well as of SJS/TEN. In conclusion, serum granulysin might help early diagnosis and predict disease prognosis.

Figure Legend

Fig 1. Granulysin levels of healthy controls, and of ODSRs patients and DIHS patients at different stages of the diseases.

In DIHS patients, we examined the concentration of granulysin for three terms: day 1 to 10, day 11 to 20, and after day 21. The granulysin level was elevated from day 1 to 20, compared to those for ODSRs and normal controls (*:$p<0.01$).

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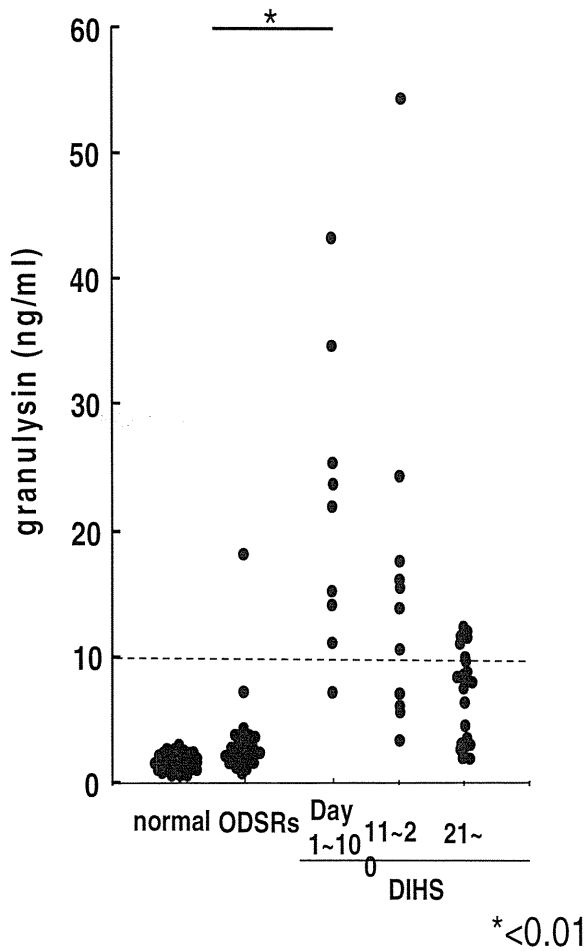
Fig 1


*
60
50
40
30
20
10
0
granulysin (ng/ml)
normal
ODSRs
Day
1~10
11~2
21~
0
DIHS
*<0.01

Eur J Dermatol 2012; 22(2): 1-2

CORRESPONDENCE

Intraepidermal neutrophilic IgA pemphigus successfully treated with dapsone

A 25-year-old woman presented with a 2-month history of erythematous, intensely itchy macules and vesicles on the extremities and trunk. Before onset, she was in good health and took no medication. Physical examination revealed pinkish or reddish, edematous, well-demarcated erythema *(figure 1A)*. The lesions tended to coalesce, forming annular patterns, some of which had vesicles around the margins, forming a sunflower-like configuration. The oral cavity and genital area were unaffected. Histopathological findings of a pustule revealed intraepidermal blisters with neutrophil infiltrates without prominent acantholysis *(figure 1B)*. Laboratory examinations, including serum immunoglobulins, and ELISA for anti-desmoglein 1 and 3 were within normal ranges. Chest X-ray, electrocardiogram, and blood tests revealed no other related diseases and monoclonal gammopathy. DIF of the erythematous lesion revealed IgA deposition in the intercellular space throughout the epidermis *(figure 1C)*. IIF revealed circulating IgA autoantibodies binding to the cell surfaces of the entire epidermis of normal human skin (titer: 64×). Immunoblot analysis using epidermal extracts from normal human skin and recombinant desmocollin 3 showed no specific bands for either IgA or IgG antibodies. These findings led to the diagnosis of IEN-type IgA pemphigus. Treatment was initiated with topical corticosteroids, achieving only a slight effect; dapsone (50 mg per day) was therefore started. The pruritus and lesions improved but the symptoms recurred after four weeks. For that reason the dose was raised to 75 mg dapsone and the itchness subsided within a few days. Two weeks later, only pigmented macules with no active lesions were observed. The titer of IIF also decreased from 64× to 16×.

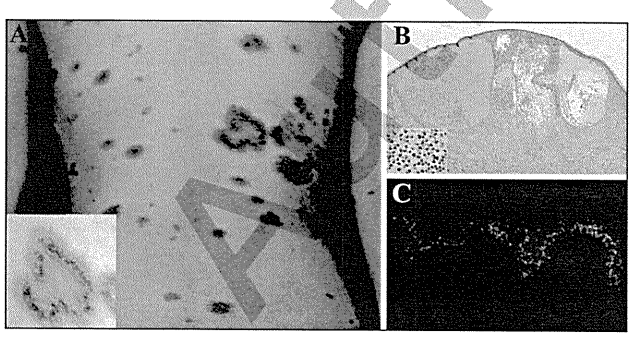


Figure 1. A) Pinkish and reddish edematous erythema with vesicles around the margins are scattered on the trunk. **B)** Histopathological findings of a pustule reveal intraepidermal blisters with neutrophil infiltrates. **C)** Direct immunofluorescence of the perilesional skin biopsy specimen reveals IgA deposits on the keratinocyte cell surfaces.

IgA pemphigus is a distinct group of auto-immune intraepidermal blistering diseases that present with vesiculopustular eruption, neutrophil infiltration with or without acantholysis. IgA autoantibodies that target keratinocyte cell surfaces and desmosomal components in the epidermis have been detected in DIF and IIF [1]. IgA pemphigus is divided into two major subtypes: the IEN type, and the SPD type. While SPD-type IgA pemphigus shows subcorneal pustules, the IEN type is characterized by pustule formation, mainly in the middle or lower epidermis.

In DIF, SPD-type IgA pemphigus involves cell surface IgA binding only in the upper epidermis, whereas IEN-type IgA pemphigus shows binding throughout the epidermis [2]. Desmocollin 1 has been identified as an autoantigen in SPD-type IgA pemphigus, suggesting that it plays an important role in the pathogenesis of this disease subtype [3]. Although autoantibodies against desmogleins [4] and desmocollins [5] have been reported in some cases of IEN-type IgA pemphigus, the specific autoantigen remains unidentified. In our case, we were also unable to detect specific autoantibodies using immunoblot analysis. Interestingly, a case with clinical and histological features compatible with SPD-type IgA pemphigus, but for which anti-desmocollins antibodies were not detected, was diagnosed as IEN-type IgA pemphigus [6]. That report suggested that the subtypes of IgA pemphigus might be considered to be divided by autoantigens.

In contrast to the common types of pemphigus, like pemphigus vulgaris, treatment for some cases of IgA pemphigus does not require corticosteroid or other immunosuppressive therapy. These cases of IgA pemphigus are well controlled using only anti-inflammatory treatments, such as dapsone, colchicine or isotretinoin [1]. Dapsone may be useful in treating IgA pemphigus due to its effect in suppressing neutrophilic infiltration. However refractory cases require plasmapheresis or cyclophosphamide. In the present case, oral administration of dapsone quickly caused the symptoms to subside. In IgA pemphigus, it is important to make the correct diagnosis and to choose a suitable therapy to avoid the side effects by the prolonged use of systemic corticosteroids. ■

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Department of Dermatology,
Hokkaido University Graduate
School of Medicine
N15 W7,
Sapporo 060-8638, Japan
<aberi@med.hokudai.ac.jp>

Yu HIRATA
Riichiro ABE
Kazuhiro KIKUCHI
Asuka HAMASAKA
Satoru SHINKUMA
Toshifumi NOMURA
Wataru NISHIE
Ken ARITA
Hiroshi SHIMIZU

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