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Identification of thymus and activation-regulated chemokine (TARC/CCL17) as a potential marker for early indication of disease and prediction of disease activity in drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS)

Kohei Ogawa [a], Hironori Morito [a], Ayako Hasegawa [a], Natsuko Daikoku [a], Fumi Miyagawa [a], Aiko Okazaki [a], Takaya Fukumoto [a], Nobuhiko Kobayashi [a], Takahiko Kasai [b], Hideaki Watanabe [c], Hirohiko Sueki [c], Masafumi Iijima [c], Mikiko Tohyama [d], Koji Hashimoto [d], Hideo Asada [a,*]

[a] Department of Dermatology, Nara Medical University School of Medicine, Nara, Japan
[b] Department of Diagnostic Pathology, Nara Medical University School of Medicine, Nara, Japan
[c] Department of Dermatology, Showa University School of Medicine, Tokyo, Japan
[d] Department of Dermatology, Ehime University Graduate School of Medicine Ehime, Matsuyama, Japan

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ABSTRACT

Background: Drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS) is a serious acute drug reaction with fever, cutaneous eruption, lymphadenopathy, and several visceral dysfunctions. Eosinophilia is a common hematological abnormality in DIHS/DRESS suggesting that the Th2-type immune response is involved. Thymus and activation-regulated chemokine (TARC/CCL17) is a family of CC chemokines known to play an important role in Th2-mediated immune-inflammatory processes.
Objective: We investigated the pathogenic role of TARC in patients with DIHS.
Methods: Sera were obtained from 8 patients with DIHS, 7 patients with Stevens–Johnson syndrome/Toxic epidermal necrolysis (SJS/TEN), and 14 patients with drug-induced maculopapular exanthema (MPE). Serum TARC levels were measured by ELISA. TARC levels were then compared with clinical symptoms and various hematological parameters. In addition, a biopsy was taken from the lesional skin of patients with DIHS and stained with anti-TARC Ab and anti-CD11c Ab.
Results: Serum TARC levels in patients with DIHS were significantly higher than those in patients with SJS/TEN and MPE during the acute phase. Serum TARC levels in DIHS patients correlated with skin eruptions, serum sIL-2R levels, eosinophil counts, and serum IL-5 levels. Immunohistochemical staining revealed that TARC was mainly expressed on CD11c+ dermal dendritic cells in patients with DIHS.
Conclusion: Serum TARC levels may be associated with the initial presentation of DIHS as well as disease activity during the course. Thus, they could be useful as an indicator for early diagnosis and assessment of disease activity in DIHS. CD11c+ dendritic cells may be the main source of TARC in patients with DIHS.

1. Introduction

Drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms (DRESS) is a severe acute adverse drug-induced reaction characterized by cutaneous eruption often develop to erythroderma, fever, leukocytosis with eosinophilia, and/or atypical lymphocytosis, lymph node enlargement, and several visceral dysfunctions. DIHS has a delayed onset and it usually occurs 3 weeks to 3 months after the initiation of medication with a limited number of drugs including carbamazepine, phenytoin, phenobarbital, dapsone, mexiletine, salazosulfapyridine, allopurinol, and minocycline [1]. Recently, it has been suggested that the severe systemic symptoms of DIHS are associated with reactivation of human herpesvirus-6 (HHV-6). HHV-6 reactivation, evidenced by the rise in HHV-6 IgG titers and HHV-6 DNA levels, usually occurs 2 to 3 weeks after the onset of a rash. It has been observed despite

* Corresponding author at: Department of Dermatology, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan. Tel.: +81 744 29 8891; fax: +81 744 25 8511.
E-mail address: asadah@naramed-u.ac.jp (H. Asada).

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the high variability of clinical manifestations in the vast majority of patients with DIHS [2]. Additionally, it is also known that other herpes viruses such as Epstein–Barr virus (EBV), HHV-7, or cytomegalovirus (CMV) are reactivated sequentially in the course of the disease [1]. Thus, this syndrome is regarded as a reaction induced by a complicated interplay between drug-specific immune responses and several herpes viruses. Recently, dramatic expansions of functional regulatory T (Treg) cells in the acute stage and functional deficiency in the resolution stage of DIHS have been reported. Treg cells were suggested to be involved in the pathological condition of DIHS, such as delayed onset and the risk of subsequently developing autoimmune disease [3].

Thymus and activation-regulated chemokine (TARC/CCL17) is a member of the CC chemokines [4]. It is a ligand for CC chemokine receptor (CCR) 4 that is expressed on type 2 helper T (Th2) lymphocytes [5–7]. TARC plays important roles in Th2-type immune responses by selectively recruiting CCR4+ Th2-polarized memory/effector T cells into inflamed tissues. It has been reported that atopic dermatitis (AD) is characterized by an expansion of the population of Th2 cells in the acute phase [8] and serum TARC levels are associated with disease activity [9]. It has been also reported that TARC levels were significantly increased in inflammatory erythroderma and Sézary syndrome [10]. Furthermore, Treg cells are known as another subset of CD4(+) T cells expressing CCR4 and responding to TARC [11].

In this study, to examine the role of TARC in the pathophysiology of DIHS, that is erythroderma, the Th2-shift, and increased Treg cells, we measured serum TARC levels of DIHS, Stevens–Johnson syndrome/Toxic epidermal necrolysis (SJS/TEN), and drug-induced maculopapular exanthema (MPE). In addition, we examined the association between serum TARC levels and the severity of skin eruption and other laboratory data such as soluble interleukin-2 receptor (sIL-2R), IL-5, eosinophil numbers in peripheral blood, and ALT. To the best of our knowledge, the association between TARC and DIHS has not yet been described.

2. Materials and methods

2.1. Patients

In the period between December, 2009 and December, 2011, 8 patients with DIHS (6 men and 2 women with a median age of 46.0 years old, ranging from 16 to 79 years old), 7 patients with SJS/TEN (3 men and 4 women with a median age of 49.1 years old, ranging from 33 to 72 years old), and 14 patients with MPE (8 men and 6 women with a median age of 59.5 years old, ranging from 32 to 75 years old) were enrolled in this study. Criteria for diagnosis of DIHS were maculopapular rash and/or erythroderma, high fever, leucocytosis with hypereosinophilia, and/or atypical lymphocytosis, lymphadenopathy, and liver dysfunction or other organ involvement [12]. Characteristics of these patients are summarized in Table 1. Causative drugs were identified by the lymphocyte transformation test (LTT) and/or history of drug administration and clinical course; allopurinol and salazosulfapyridine were involved in one patient each and carbamazepine and lamotrigine in three patients each. HHV reactivation was detected in all 8 cases. Of these, HHV-6 was detected in 6 cases, and HHV-7 and CMV in one each. We defined "day 0" as the onset day of characteristic clinical symptoms of DIHS, that is development of skin eruption. In case 4, the serum anti HHV-6 IgG titer was elevated from ×10 on day 4 to ×80 on day 25. In case 8, the serum anti HHV-6 IgG titer was elevated from ×40 on day 21 to ×640 on day 44. In case 6, unfortunately, detailed clinical symptoms and laboratory data were unspecified before consultation on day 15. Case 6 did not fulfill the diagnostic criteria of DIHS exactly, but fulfilled the criteria of DRESS (probable case) based on a skin rash, prolonged clinical course, liver dysfunction, and lymphadenopathy. Four cases (cases 1, 2, 5, and 7) developed erythroderma and the other 4 cases remained as maculopapular erythema. Liver dysfunction developed in 7 cases (cases 1–3, and 5–8) and renal disorder occurred in 3 cases (cases 2, 3, and 4) during the course (Table 1).

2.2. Detection of human herpes virus DNA

Peripheral blood was obtained twice or three times a week until the remission period. DNA was extracted from whole blood using a QIAamp DNA Blood mini-kit (Qiagen, Tokyo, Japan), according to the manufacturer's instructions, and subjected to a real-time polymerase chain reaction (PCR) for the detection of HHV-6, HHV-7 [13], and CMV [14].

2.3. Assay for serum TARC and other cytokines

Serum samples from patients were obtained several times from the acute stage to the remission stage and were stored at −80 °C until use. Serum levels of TARC, sIL-2R, and IL-5 were retrospectively measured by enzyme-linked immunosorbent assays (ELISA) (R&D systems, Minneapolis, USA).

2.4. Immunohistochemical staining

Lesional skin biopsies were performed in 7 patients with DIHS in acute stage (day 4–21) and 4 patients with MPE (day 2–13). Biopsy samples were placed in 10% buffered formalin for processing to paraffin blocks and sections were stained with goat polyclonal anti-human TARC antibodies (R&D systems,

Table 1
Summary of DIHS patients' number, age, sex, causative drugs, types of human herpes virus, day of viral reactivation, skin rash, body temperature, existence or non-existence of lymphadenopathy, and laboratory data.

Case	Age/Sex	Causative drug	Viral reactivation	Day of viral detection after onset	Skin rash	BT (°C)	Leukocytosis (μl)	Eosinophil (μl)	Aty-lym (%)	ALT (U/l)	Lymph adenopathy
1	32/M	Allopurinol	HHV-6	day 16	E	39.3	18,000	3400	12	449	(+)
2	36/F	Lamotrigine	HHV-6	day 19	E	39.7	32,700	1200	5	107	(+)
3	16/M	Lamotrigine	CMV	day 18	MP	37.4	17,400	3100	0	60	(+)
4	79/M	Carbamazepine	HHV-6		MP	38.5	18,100	3500	1	32	(−)
5	44/M	Carbamazepine	HHV-6	day 16	E	39.8	22,700	2400	17	108	(+)
6	60/M	Lamotrigine	HHV-7	day 22	MP	36.5	10,700	700	0	104	(+)
7	57/F	Salazosulufa-pyridine	HHV-6	day 19	E	38.6	22,300	5800	33	383	(+)
8	44/M	Carbamazepine	HHV-6		MP	40.0	16,200	3200	15	80	(+)

BT: body temperature; Aty-lym: atypical lymphocyte; ALT: alanine aminotransferase; HHV: human herpes virus; CMV: cytomegalovirus; MP: maculopapular rash; E: erythroderma.