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Chemokine expression in diverse nonimmediate drug hypersensitivity reactions: focus on thymus activation-regulated chemokine, cutaneous T-cell–attracting chemokine, and interleukin-10

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ABSTRACT

Background: Skin infiltration of different types of T lymphocytes is responsible for inflammatory profiles of nonimmediate drug hypersensitivity reactions (niDHRs). Important chemokines attracting skin-specific homing T cells include thymus activation-regulated chemokine (TARC) and cutaneous T-cell–attracting chemokine (CTACK). Interleukin-10 (IL-10) is a potent chemokine attracting CD8⁺ T cells.

Objective: To investigate serum levels of TARC, CTACK, and IL-10 in patients with niDHRs and evaluate the correlation among these 3 chemokines.

Methods: Forty patients, including 19 patients with Stevens-Johnson syndrome and toxic epidermal necrolysis and 21 patients with maculopapular exanthema, and 21 healthy donors were recruited into the study. Clinical data of patients were obtained. Serum TARC, CTACK, and IL-10 levels were determined by enzyme-linked immunosorbent assay.

Results: Serum levels of TARC, CTACK, and IL-10 were significantly elevated in patients with niDHRs compared with those in normal controls ($P < .05$, $P < .001$, $P < .001$, respectively). The CTACK and IL-10 levels were significantly higher ($P < .05$, $P < .001$) in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis than in normal controls. Patients with maculopapular exanthema exhibited higher levels of TARC, CTACK, and IL-10 compared with normal controls ($P < .001$, $P < .001$, $P < .05$). Serum CTACK levels were positively correlated with TARC levels in all 40 patients ($r_s = 0.3422$, $P < .05$). Serum CTACK levels positively correlated with detachment of body surface area in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis ($r_s = 0.510$, $P < .05$).

Conclusion: These results support a role for TARC, CTACK, and IL-10 in the pathogenesis of niDHRs for their chemotactic ability to attract different T-cell subtypes and different functional severities in niDHRs.

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Introduction

The skin is the most common target of drug hypersensitivity reactions (DHRs), with different clinical manifestations ranging from immediate DHRs, such as urticaria, to nonimmediate DHRs (niDHRs), such as maculopapular and bullous exanthema.¹ Although the exact mechanisms responsible for niDHRs have remained poorly understood, increasing evidence has indicated that drug-specific T cells expressing a skin-homing cutaneous lymphocyte antigen (CLA) play a central part in the pathogenesis.^{2–4} Maculopapular exanthema (MPE) is the most frequent form of niDHR. Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are the same entity with similar

clinicopathologic and immunologic features but of different severities.⁵ SJS-TEN is the most severe, life-threatening niDHR. The immunohistology of MPE and SJS-TEN shares some common features, such as lesions involving migrating T cells and MHC upregulation on keratinocytes, but the decisive difference is the diversity of infiltrating T cells.⁶ MPE is characterized by a predominance of CD4⁺ T lymphocytes, whereas SJS-TEN is related to CD8⁺ T-lymphocyte–mediated cytotoxicity.⁷

In inflammatory immune processes, chemokines control effector T-cell recruitment to the site where inflammatory chemokines are produced. Among inflammatory chemokines, 2 potential candidates, thymus activation-regulated chemokine (TARC) and cutaneous T-cell–attracting chemokine (CTACK), for regulating the trafficking of T cells to skin, are associated with cutaneous inflammatory diseases.⁸ TARC is a member of the CC chemokine family, with strong chemotaxis for T-helper cell type (T_H) 2 lymphocytes. TARC is expressed by various cells including epidermal keratinocytes, mononuclear cells, and even fibroblasts.⁹

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Several studies have reported increased TARC levels in patients with allergic diseases, such as asthma, atopic dermatitis, and allergic rhinitis.¹⁰ CTACK, only expressed by keratinocytes, also belongs to the CC chemokine family and selectively attracts a subset of memory and effector T cells expressing CLA from peripheral blood.¹¹ CTACK has been found to regulate T-cell recruitment to the epidermis in mouse models of acute skin inflammation.^{12,13} Interleukin (IL)-10 has been found to be an endogenous antipyretic agent,¹⁴ and keratinocytes are the major source of IL-10 production in the skin. In addition its downregulatory role, IL-10 is a chemokine for peripheral blood CD8⁺ T cells.¹⁵

The study of the role of chemokines in the onset of niDHRs is still in its infancy, and questions about why T cells infiltrate disparately in various subtypes of drug eruptions have not been resolved. In this study, the authors investigated serum TARC, CTACK, and IL-10 levels in patients with SJS-TEN and MPE to explore the behavior and effect of chemokines in the mechanisms of niDHRs.

Methods

Patients and Controls

Nineteen adult patients with SJS-TEN were consecutively enrolled from January 2012 to December 2013, and 21 age-matched patients with drug-induced MPE were enrolled in the study. All patients were visiting the Department of Dermatology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, for consultation of their skin problems. The most probable culprit drug was identified according to the definition of adverse drug reactions provided by the World Health Organization.¹⁶ All symptoms appeared at least 24 hours after drug intake and were alleviated after withdrawal from the culprit drugs. MPE consisted of erythematous macules or papules that often were symmetric. The diagnosis of SJS-TEN was based on skin lesions (typical targets, raised or flat atypical targets, and purpuric macules), involvement of mucous membranes, and extent of the epidermal detachment, as follows: SJS, detachment less than 10% of body surface area (BSA), involvement of at least 2 mucosal sites plus widespread erythematous or purpuric macules or flat atypical target appearance; TEN, detachment greater than 30% of BSA, prominent mucous membrane erosions and ulcerations plus widespread purpuric macules, flat atypical targets or large epidermal sheets; cases with detachment of 10% to 30% of BSA were classified as SJS-TEN overlap. Patients enrolled into the study were at first diagnosis and had not used any immunosuppressive or immunoregulatory agent to treat the underlying diseases and drug-induced reactions. Patients who had positive serologic results for human immunodeficiency virus or asthma or rheumatoid diseases were excluded.

Twenty-one healthy age-matched adults with no history of DHRs and no cutaneous or immunologic diseases or on any medicine at the time of selection were selected as controls.

All procedures in this study were approved by the ethics committee of The First Affiliated Hospital of Sun Yat-sen University (no [2012]017). Informed written consent was obtained from patients and controls.

Sample Collection

Serum samples were obtained from the clotted blood of each patient at every early stage at the initial diagnosis and before the introduction of treatment and from controls after centrifugation at 4°C and 2,500 rpm for 10 minutes and then stored at –80°C until tested.

Measurements of TARC, CTACK, and IL-10 Levels

Enzyme-linked immunosorbent assays were carried out in duplicate. TARC, CTACK, and IL-10 levels were detected using kits

purchased from RayBiotech, Inc (Norcross, Georgia) and according to the manufacturer's instructions.

Statistical Analysis

Differences in the levels of TARC, CTACK, and IL-10 were assessed by the Wilcoxon-Mann-Whitney nonparametric test. Correlation coefficients between various parameters were assessed using the Spearman rank correlation test. *P* values lower than .05 were considered to indicate a statistically significant difference or correlation.

Results

Demographics of Patients and Drugs Implicated in niDHRs

There were 5 patients with SJS, 8 patients with SJS-TEN overlap, and 6 patients with TEN among the 19 SJS-TEN cases. Among the culprit drugs, allopurinol (*n* = 7) and carbamazepine (*n* = 6) were the most common drugs causing SJS-TEN. However, the most commonly implicated drug to cause MPE was cephalosporins (*n* = 6; Table 1).

Serum Chemokines Levels in Patients and Healthy Subjects

The serum levels of TARC, CTACK, and IL-10 were significantly elevated in patients with niDHRs than those in the controls (*P* < .05, *P* < .001, *P* < .001, respectively; Table 2).

After comparing the different categories of niDHR, TARC levels were found to be higher in the MPE group (mean 4,440.04 pg/mL) than in the SJS-TEN group (mean 2,290.53 pg/mL) or control group (mean 2,453.28 pg/mL, *P* < .001; Fig 1). No difference in TARC levels was found between the SJS-TEN and control groups. The SJS-TEN (mean 1,816.77 pg/mL) and MPE (mean 2,468.55 pg/mL) groups exhibited significantly higher levels of CTACK compared with the control group (mean 991.81 pg/mL, *P* < .05 and *P* < .001, respectively; Fig 2). This increase was more evident in the MPE than in the SJS-TEN group (*P* < .05). A statistically significant increase in IL-10 serum levels was found for the SJS-TEN group (mean 93.67 pg/mL) and the MPE group (mean 33.16 pg/mL) compared with the control group (mean 38.82 3.58 pg/mL, *P* < .001 and *P* < .05; Fig 3). No significant difference in IL-10 levels was detected between the SJS-TEN and MPE groups.

Table 1

Demographic and clinical characteristics of patient and control groups

	SJS-TEN			MPE	Control
	SJS (n = 5)	SJS-TEN overlap (n = 8)	TEN (n = 6)	(n = 21)	(n = 21)
Age (y), median (range)	38 (18–65)			45 (19–66)	45 (18–59)
Men/women, n	10/9			10/11	11/10
Outcome (n)	A (5)	A (8)	A (4), D (2)	A (21)	A (21)
Culprit drugs, n					
Cephalosporins	0			6	
Amoxicillin	2			2	
Fluoroquinolones	1			2	
Macrolides	0			1	
Sulfonamides	1			0	
Clindamycin	0			2	
Allopurinol	7			2	
Carbamazepine	6			1	
TCM	1			2	
NSAIDs	1			1	
Methimazole	0			1	
Tetanus antitoxin	0			1	

Abbreviations: A, alive; D, dead; MPE, maculopapular exanthema; NSAIDs, nonsteroidal anti-inflammatory drugs; SJS, Stevens-Johnson syndrome; TCM, traditional Chinese medicine; TEN, toxic epidermal necrolysis.

Table 2

Serum levels of TARC, CTACK, and IL-10 in patients with nonimmediate drug hypersensitivity reactions and healthy controls

	TARC (pg/mL)	CTACK (pg/mL)	IL-10 (pg/mL)
Patients (n = 40)	3,419.02 ± 1,384	2,158.96 ± 1,879	61.90 ± 136
Controls (n = 21)	2,453.28 ± 965	991.81 ± 459	3.58 ± 2.42
Z	2.368	3.901	4.023
P value	<.05	<.001	<.001

Abbreviations: CTACK, cutaneous T-cell–attracting chemokine; IL-10, interleukin-10; TARC, thymus activation-regulated chemokine.

Correlations of Chemokines between Patient Groups and Serum CTACK Levels Paralleled with Detachment of BSA in SJS-TEN Group

Serum CTACK levels were positively correlated with TARC levels in all 40 patients with niDHRs ($r_s = 0.3422$, $P < .05$; Fig 4). No significant correlation was found between TARC and IL-10 levels or between CTACK and IL-10 levels in the niDHR group. In the niDHR subgroups, no significant correlation was found between CTACK and IL-10 levels in the SJS-TEN group or among CTACK, IL-10, and TARC levels in the MPE group.

In the SJS-TEN group, a direct correlation was observed between levels of serum CTACK and detachment of BSA (DBSA; $r_s = 0.510$, $P < .05$; Fig 5), but no correlation was observed between IL-10 level and DBSA.

Discussion

The development of niDHRs depends on genetic and environmental factors, predominated by immunologic mechanisms. In immune-mediated cutaneous diseases, chemokines play a pivotal role in the extravasation, localization, and retention of effector lymphocytes within skin tissue.^{17,18} The present study found that serum TARC, CTACK, and IL-10 levels were elevated in patients with niDHRs, indicating that these chemokines were associated with drug-induced exanthema. Interestingly, the differences were more visible for CTACK and IL-10 ($P < .001$ vs controls) than for TARC ($P < .05$ vs controls).

Different effector T cells attracted by different chemokines can induce distinctive immunologic outcomes, and MPE and SJS-TEN represented 2 distinct clinicopathologic and immunologic entities. Several studies have reported that CD4⁺ T cells, including T_H1 lymphocytes and T_H2 lymphocytes, contribute to the characteristic

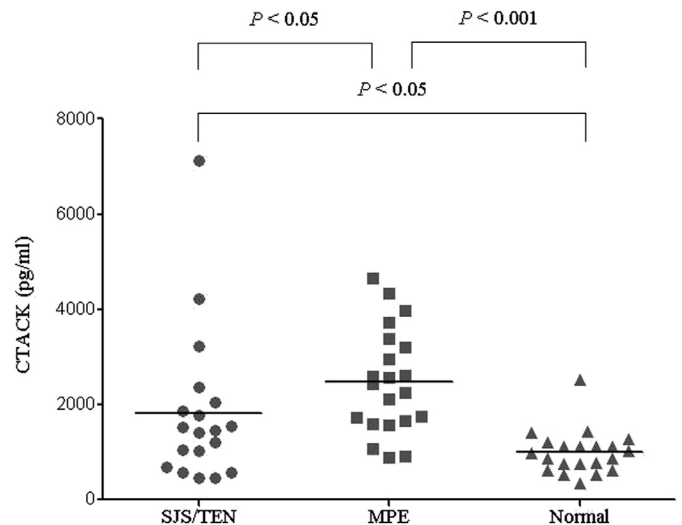


Figure 2. Serum levels of CTACK in the SJS/TEN, MPE, and control groups. Horizontal bars represent mean values. CTACK, cutaneous T-cell–attracting chemokine; MPE, maculopapular exanthema; SJS/TEN, Stevens-Johnson syndrome and toxic epidermal necrolysis.

features of MPE.² Recruitment and activation of eosinophils and enhanced IL-5 participate in the pathogenesis of MPE. TARC is a specific chemoattractant for T_H2 subtypes producing cytokines, such as IL-4, IL-5, and IL-13, which induce IgE production and eosinophil activation.¹⁹ In the present study, TARC levels were elevated in the MPE group compared with the SJS-TEN and control groups. The TARC effect on MPE might be achieved by attracting T_H2 cells and leading to the recruitment and activation of eosinophils, which contribute to erythema and itching in MPE.

Cutaneous T-cell–attracting chemokine is directly involved in T-cell recruitment to inflamed skin. CTACK attracts skin-homing T cells of the CD4⁺ and CD8⁺ subtypes, which are positive for CLA. The apoptosis of keratinocytes in SJS-TEN was caused mainly by CD8⁺ T cells that recognized MHC class I.²⁰ This study confirmed that serum CTACK levels were elevated in the SJS-TEN group, in line with a previous study reporting that CTACK gene expression was increased in bullous drug-induced skin reactions.²¹ CTACK levels

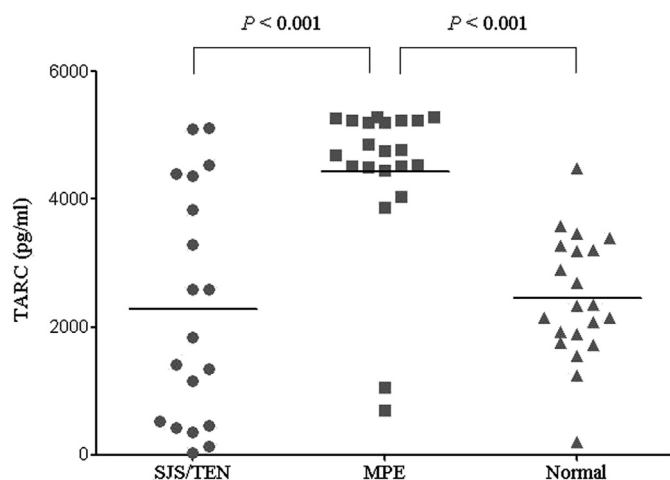


Figure 1. Serum levels of TARC in the SJS/TEN, MPE, and control groups. Horizontal bars represent mean values. MPE, maculopapular exanthema; SJS/TEN, Stevens-Johnson syndrome and toxic epidermal necrolysis; TARC, thymus activation-regulated chemokine.

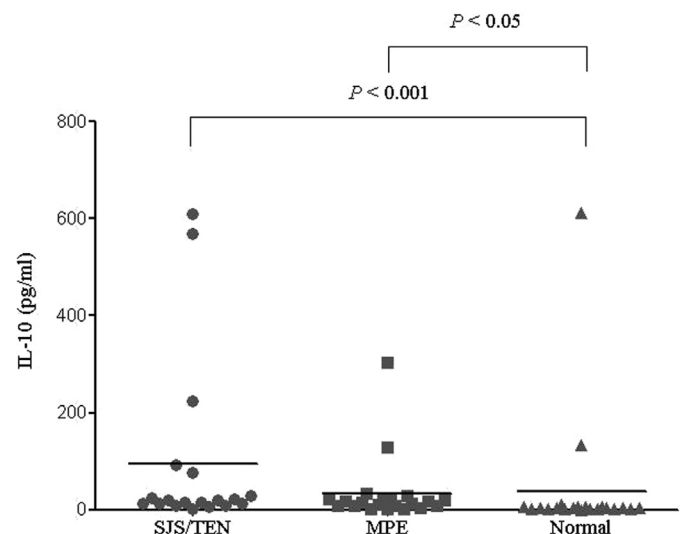


Figure 3. Serum levels of IL-10 in the SJS/TEN, MPE, and control groups. Horizontal bars represent mean values. IL-10, interleukin-10; MPE, maculopapular exanthema; SJS/TEN, Stevens-Johnson syndrome and toxic epidermal necrolysis.

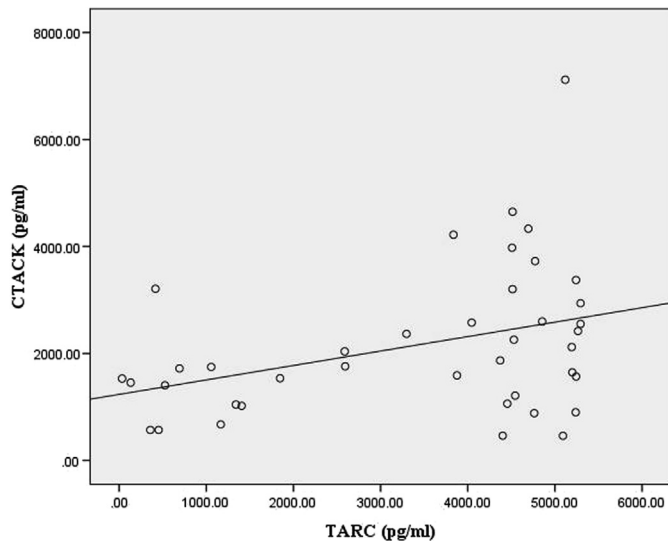


Figure 4. Positive correlation between serum TARC and CTACK levels ($r_s = 0.3422$, $P < .05$). CTACK, cutaneous T-cell-attracting chemokine; TARC, thymus activation-regulated chemokine.

also were elevated in patients with MPE in the present study. These results indicated that CTACK was involved in the pathogenesis of niDHRs by attracting CLA^+ skin-homing T cells, perhaps CLA^+CD4^+ T cells in MPE and CLA^+CD8^+ T cells in SJS-TEN. The standard to judge SJS, SJS-TEN overlap, and TEN is DBSA. The presentation of higher levels of CTACK in TEN than in SJS or SJS-TEN overlap (Fig 5) and the positive correlation between CTACK levels and DBSA emphasized the vital role of CTACK, the main source of keratinocytes, in assessing severity of SJS-TEN.

Interleukin-10 is produced relatively late after the activation of T cells compared with other cytokines.²² IL-10 may act as a down-regulator of the lesional pathway and exert strong anti-inflammatory activity against excessive tissue inflammation. In the present study, the increased expression of IL-10 in patients with niDHRs suggests an immune defense mechanism to inhibit

inflammation in drug reactions. There were 3 patients with SJS-TEN with highest levels of IL-10 who also showed high levels of CTACK (Figs 2 and 3), suggesting that IL-10 may play a role in the feedback of the severity of SJS-TEN. However, the other biologic activity of IL-10 is as a chemotactic for $CD8^+$ T cells.¹⁵ The elevated serum levels of IL-10 in patients with SJS-TEN in the present study suggest the recruitment of $CD8^+$ T lymphocytes to the blister fluid, implicating IL-10 functioning in attracting $CD8^+$ T cells. Although SJS-TEN was much more severe than MPE, IL-10 levels displayed no statistical significance between the 2 groups, which could be the result of the chemotactic activity of IL-10 for exacerbating $CD8^+$ T-cell migration.

Under normal conditions, the expression of CTACK, but not TARC, by human keratinocytes has been reported.²³ During cutaneous inflammation, CTACK can be superinduced by the proinflammatory cytokine tumor necrosis factor- α and this effect can be augmented by TARC.²⁴ In particular, several studies have proved that tumor necrosis factor- α produced by keratinocytes is involved in the immune mechanisms of niDHRs.^{2,15,25} The authors found a positive correlation between TARC and CTACK levels in patients with niDHRs, suggesting that TARC and CTACK may work in a sequential way, with TARC acting initially followed by CTACK being overexpressed in the tumor necrosis factor- α environment. The ability of TARC to amplify CTACK production might contribute to the result of significantly higher CTACK levels in the MPE group than in the SJS group in the present study.

In summary, the present data suggest that chemokines TARC, CTACK, and IL-10 are implicated in the pathogenesis of niDHRs. Different profiles of chemokines were presented for diverse cutaneous drug reactions. It would be interesting to investigate CTACK as a possible marker for evaluating the severity of SJS-TEN and how the 2 aspects of IL-10 interact in the pathogenesis of SJS-TEN.

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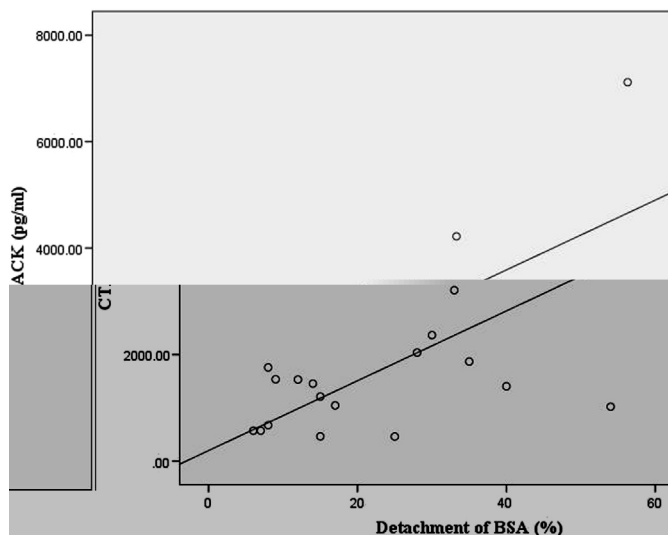


Figure 5. Positive correlation between serum CTACK levels and detachment of BSA ($r_s = 0.510$, $P < .05$). BSA, body surface area; CTACK, cutaneous T-cell-attracting chemokine.

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