OBSERVATION

# Cytomegalovirus Disease During Severe Drug Eruptions

## *Report of 2 Cases and Retrospective Study of 18 Patients With Drug-Induced Hypersensitivity Syndrome*

*Yusuke Asano, MD; Hiroaki Kagawa, MD; Yoko Kano, MD; Tetsuo Shiohara, MD*

**Background:** Overt cytomegalovirus (CMV) disease is a serious viral infection that usually occurs in immunocompromised patients but rarely in immunocompetent patients. Cutaneous lesions, albeit rare, occur as late systemic manifestations of CMV infections and are usually fatal.

**Observations:** We describe 2 patients with drug-induced hypersensitivity syndrome (one end of a spectrum of severe drug eruptions) who subsequently developed cutaneous CMV ulcers at unusual sites, such as the trunk; this occurrence was immediately followed by gastrointestinal manifestations, which were fatal in 1 patient. To identify factors predictive of CMV disease, we retrospectively investigated the prevalence of CMV reactivation during drug-induced hypersensitivity syndrome in 18 patients. In this analysis, patients were divided into 2 groups depending on the positivity of CMV DNA in the blood.

**Conclusions:** Older and male patients with antecedent high human herpesvirus 6 DNA loads are at risk for CMV disease irrespective of corticosteroid administration. A rapid reduction in white blood cell numbers is also predictive of the onset of CMV disease.

*Arch Dermatol. 2009;145(9):1030-1036*

OVERT CYTOMEGALOVIRUS (CMV) disease, predominantly induced by reactivation of latent CMV, can be produced in an immunosuppressed host, such as organ transplant recipients, patients with AIDS, and those receiving immunosuppressive agents,[1] but rarely in immunocompetent individuals. Although CMV disease in an immunosuppressive setting usually presents as visceral disease ranging from pneumonia to various other, widely disseminated, diseases,[2] cutaneous manifestations are rare and variable; they include skin ulcerations, morbilliform eruption, purpura, vesiculobullous lesions, nodules, papular eruptions, and verrucous lesions, regardless of whether the CMV is specific or nonspecific.[3-6] These lesions occur as late systemic manifestations of CMV infections and are usually fatal.[7-9] Because the localized CMV ulceration usually seen in these patients has a predilection for the genital or perineal area,[10] cutaneous ulcers located in other areas are not usually regarded as signs of cutaneous CMV infection. In particular, the development of cutaneous CMV ulcers on the trunk is rare in the human immunodeficiency virus–negative population.[11] We describe 2 patients with drug-induced hypersensitivity syndrome (DIHS), a life-threatening multiorgan system reaction caused by a few drugs,[12-14] who subsequently developed cutaneous CMV ulcers on the trunk. This event was eventually followed by the development of gastrointestinal manifestations, which were fatal in one patient but not in the other. On the basis of the severity of complications caused by CMV reactivations, we retrospectively analyzed factors involved in the development of CMV disease in 18 patients with DIHS treated at Kyorin University Hospital.

Author Affiliations:
Department of Dermatology, Kyorin University School of Medicine, Tokyo, Japan.

## REPORT OF CASES

### CASE 1

A 74-year-old man had a 3-week history of progressively worsening generalized erythematous rashes. Seven weeks earlier, he had been prescribed mexiletine hydrochloride, 100 mg/d, for arrhythmia. On day 31 of medication use, the eruption began with erythema on his chest and gradually worsened during the next 2 weeks. Four days after the patient stopped treatment with mexiletine, the eruption rapidly spread across the

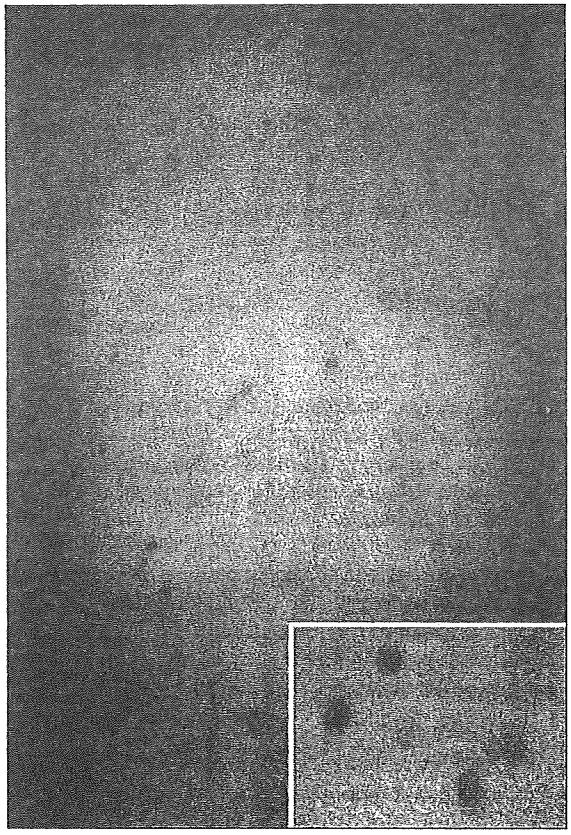

Figure 1. Multiple ulcerated erythematous papules distributed across the upper back (case 1). The inset shows a close-up view of the papules.

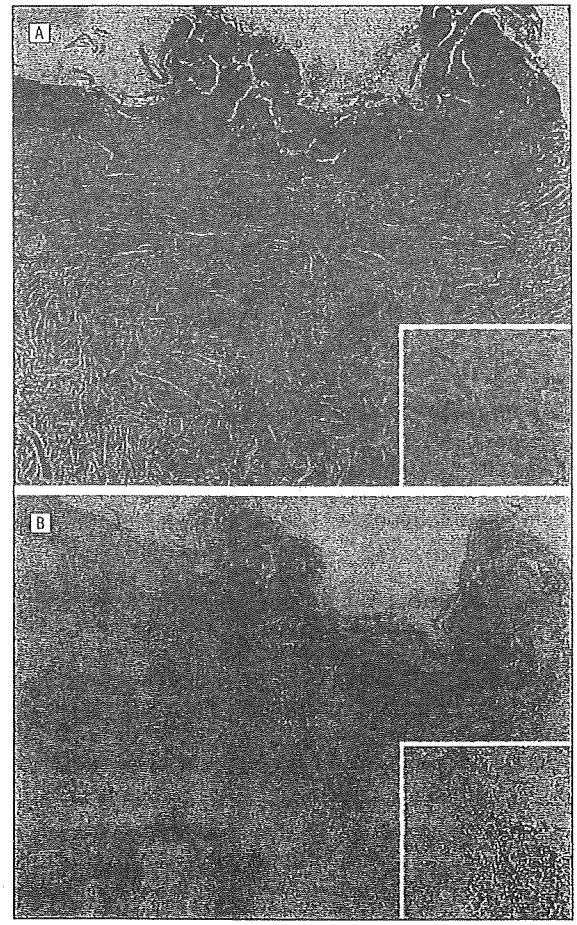


Figure 2. Histologic and immunohistochemical findings on the border of the ulcer. A, Microscopic examination shows an acanthotic epidermis adjacent to central epidermal necrosis and a superficial perivascular infiltrate of lymphocytes and neutrophils (hematoxylin-eosin, original magnification ×40). The inset shows a higher magnification (×640) of the eosinophilic intranuclear "owl's eye" inclusion surrounded by a clear halo that sharply demarcates it from the nuclear membrane. Skin biopsy of ulcerated erythematous papules on the trunk shows cytomegalic cells with a characteristic "owl's eye" intranuclear inclusion in the upper dermis. B, Immunohistochemical detection of cytomegalovirus antigens. (cytomegalovirus monoclonal antibody, original magnification ×40). The inset shows monoclonal antibody for cytomegalovirus stains infiltrating cells around the blood vessels (original magnification ×160).

trunk and extremities and was accompanied by a high-grade fever. He was admitted to the hospital with suspicion of having DIHS. Laboratory tests revealed leukocytosis (white blood cell count, 14 500/μL [to convert to $\times 10^9$ per liter, multiply by 0.001] [reference range, 3500-8000/μL]), with eosinophilia of 17% (to convert to a proportion of 1.0, multiply by 0.01) and atypical lymphocytosis of 5% (to convert to a proportion of 1.0, multiply by 0.01), and liver dysfunction. The medical history of the patient was significant for coronary artery disease since 2001, which had required percutaneous coronary intervention.

The diagnosis of DIHS was made, and oral prednisolone (50 mg/d) therapy was begun. One week after starting systemic prednisolone therapy, there was significant improvement in his condition, and the erythematous rashes faded. On hospital day 16, 2 days after the oral prednisolone dose was tapered to 40 mg/d, he developed multiple 8- to 10-mm ulcerated erythematous plaques and papules with raised borders on his trunk (**Figure 1**). The eruption began suddenly, and the papules were distributed mainly on the upper back. Skin biopsy showed cytomegalic cells with a characteristic "owl's eye" intranuclear inclusion in the upper dermis (**Figure 2**A). The CMV infection was confirmed by the use of immunohistochemical analysis (CMV monoclonal antibody) (Dako, Cambridgeshire, England) (Figure 2B). Because his ulcerations did not improve with oral prednisolone therapy, intravenous immunoglobulin (0.1 g/kg/d) was administered, with significant improvement in his ulcerations. After the identification of CMV infection in the biopsy specimens of these cutaneous ulcers, treatment with ganciclovir (200 mg/d intravenously) was added on hospital day 50, and the oral prednisolone dose was gradually tapered to prevent relapse of various symptoms of DIHS.

Although the ulcers showed gradual signs of healing, the patient noticed abdominal distention and lower back numbness, followed by abdominal pain on hospital day 47. On hospital day 61, he suddenly experienced hemorrhagic shock caused by gastrointestinal bleeding, which indicated the diagnosis of CMV enterocolitis; respiratory insufficiency and unconsciousness followed. He died of respiratory failure on day 84 of hospitalization. The clinical course and laboratory findings are summarized in

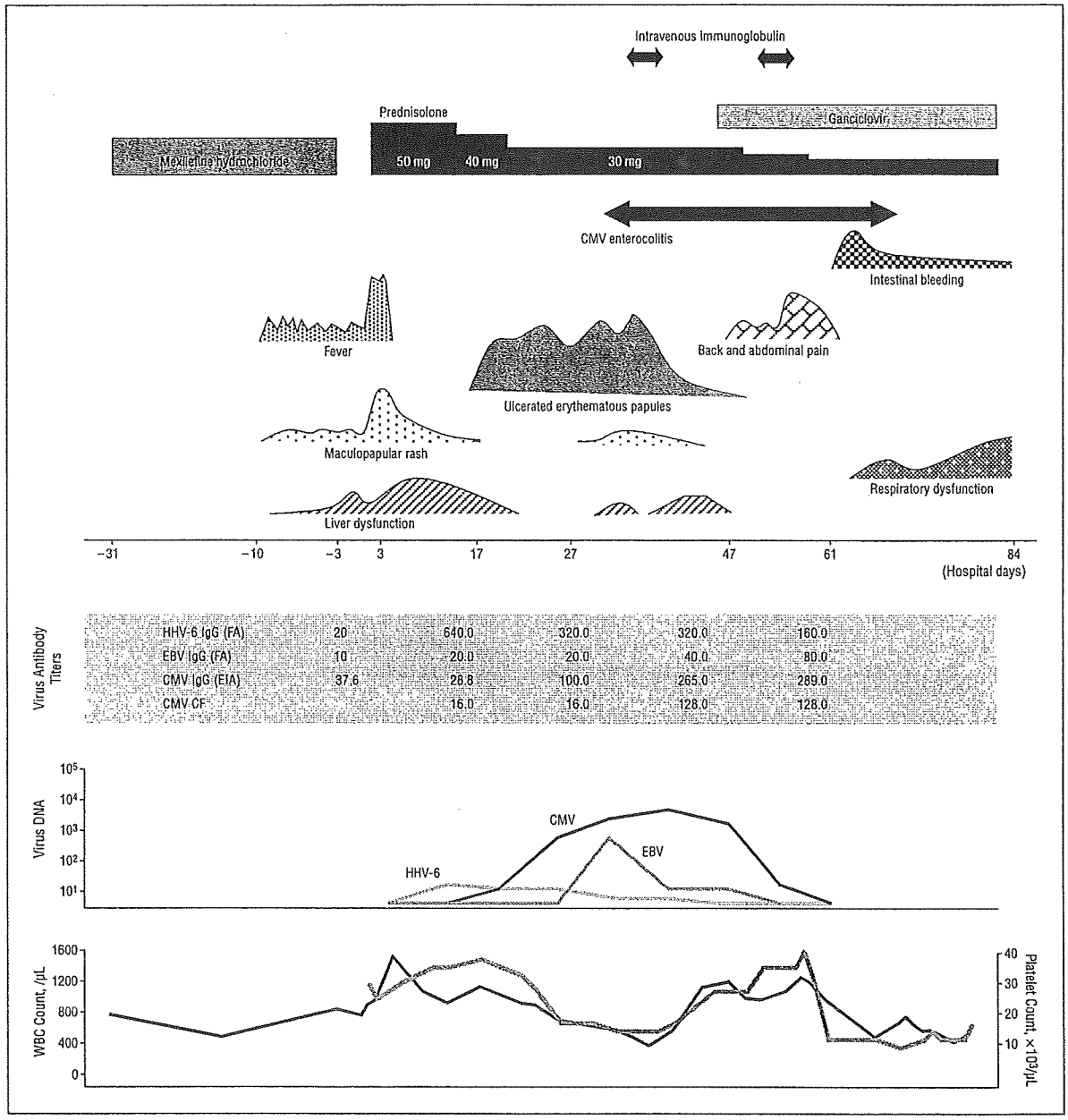


**Figure 3.** Clinical course and laboratory findings of case 1. CF indicates complement fixation; CMV, cytomegalovirus; EBV, Epstein-Barr virus; EIA, enzyme-based immunoassay; FA, fluorescent antibody technique; HHV-6, human herpesvirus 6; and WBC, white blood cell. To convert platelet count to $\times 10^9$ per liter, multiply by 1.0; WBC count to $\times 10^9$ per liter, multiply by 0.001.

**Figure 3**. A brief description of the sequential herpesvirus reactivation observed in this patient has been previously published.[15]

## CASE 2

An 81-year-old man was referred to Kyorin University Hospital because of a 1-week history of fever and itchy generalized erythematous rashes. One month before the eruption appeared, the patient had begun to take allopurinol, 300 mg/d, for hyperuricemia. The eruption began on the neck and chest but quickly spread across the entire body and was accompanied by a high-grade fever.

On examination, his temperature was 38.3°C and his white blood cell count was 14 200/μL, with eosinophilia of 28% without any atypical lymphocytes. Renal function tests revealed a serum creatinine level of 33.7 mg/dL (to convert to micromoles per liter, multiply by 88.4) (reference range, <22.0 mg/dL) and a serum urea nitrogen level of 34 mg/dL (to convert to millimoles per liter, multiply by 0.357) (reference range, 10-24 mg/dL). Skin examination revealed a widespread maculopapular eruption across the entire body that coalesced into purpuric erythematous

plaques on the lower legs. The face was edematous and exudative, with yellowish small crusts. The suspected diagnosis was DIHS. Allopurinol therapy was discontinued on day 1 of hospitalization. Corticosteroids were withheld because of the patient's history of hepatitis C virus infection due to blood transfusion, and he was treated with supportive therapy. Fever persisted, however, and his physical status deteriorated during the first 12 days of hospitalization. From days 12 to 25 of hospital admission, the patient received intravenous immunoglobulin (0.05 g/kg/d); his fever and skin eruptions gradually improved.

On hospital day 25, he developed a 6-mm-diameter red papule with central ulceration and urticarial erythematous lesions on his trunk. Skin biopsy of the papule taken from the trunk showed perivascular lymphohistiocytic infiltration in the dermis; cytomegalovirus antigens were detected in mononuclear cells in the upper dermis by means of immunohistochemical analysis. On the same day, his hemoglobin level suddenly decreased from 9.0 to 6.9 g/dL (to convert to grams per liter, multiply by 10.0). Emergency endoscopic examination revealed gastric "punched-out" ulcers (**Figure 4**). Endoscopic clipping and blood transfusion were performed immediately. On the basis of these findings, cutaneous CMV infection probably associated with CMV gastritis was diagnosed. Treatment with ganciclovir, 200 mg/d, was started. Cutaneous CMV ulcers and gastrointestinal bleeding improved after 2 weeks; the leukocyte CMV load decreased from $3.2 \times 10^3$ per $10^6$ leukocytes to undetectable levels, and no CMV antigenemia was detected 1 month after hospital admission.

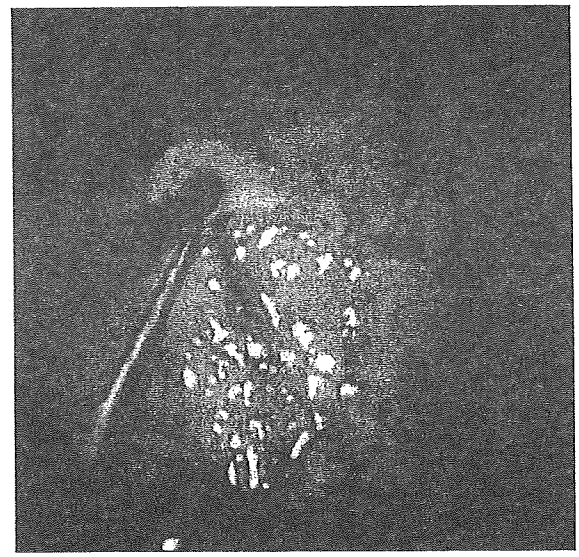

**Figure 4.** Arterial bleeding from "punched-out" gastric ulcerations on endoscopic examination (patient 2).

## RETROSPECTIVE ANALYSIS

On the basis of the severity of complications caused by CMV reactivation, we reasoned that a retrospective analysis should be performed of patients who met the full criteria for DIHS.[14] Between January 1, 2002, and December 31, 2006, 18 patients (10 men and 8 women; age range, 24-81 years; mean [SD] age, 50.6 [4.2] years) who developed DIHS and were treated at Kyorin University Hospital were enrolled in this study. This study was approved by the institutional review board at Kyorin University School of Medicine. Eighteen patients with DIHS were CMV seropositive and were examined for CMV load in sequential blood samples by means of polymerase chain reaction (PCR) assay at biweekly intervals for 10 weeks after onset; this was performed as part of a standard operating procedure for detecting sequential herpesvirus reactivation at the Department of Dermatology, Kyorin University School of Medicine, to analyze the temporal appearance of and complications caused by CMV reactivation. Overall, 6 of 18 patients (33%) tested were positive for CMV DNA in the blood by means of quantitative PCR assay, whereas 12 remained quantitatively PCR assay negative during surveillance, and none of these patients had evidence of CMV disease. The 6 patients in whom CMV DNA was detected at least once were included in the definition of "CMV DNA–positive DIHS." These patients were compared with the 12 patients in whom CMV DNA was not detected, defined as "CMV DNA–negative DIHS." On average, treatment with systemic prednisolone was initiated 3 to 5 days after the onset of symptoms suggestive of DIHS; the initial dose of prednisolone used was 0.8 to 1.0 mg/kg/d. The prednisolone dose was tapered to 50% in 6 weeks and to zero in 3 months gradually after full control was achieved. Results were analyzed for statistical significance by the use of the paired *t* test and Microsoft Excel (Microsoft Corporation, Redmond, Washington). Differences between groups were considered significant at $P < .05$.

The results of this retrospective analysis are given in **Table 1** and **Table 2**. Patients with CMV DNA–positive DIHS had an older mean [SD] age at onset than did those with CMV DNA–negative DIHS (62.0 [7.8] vs 45.4 [4.2] years), although not significant, and a male predominance. Human herpesvirus 6 (HHV-6) reactivations were detected by means of PCR assay 3 to 4 weeks after DIHS onset in both groups. However, HHV-6 DNA loads detected were significantly higher in CMV DNA–positive DIHS than in CMV DNA–negative DIHS ($1.5 \times 10^4$ vs $7.5 \times 10^1$, $P = .01$). No significant difference was detected in the interval between onset and HHV-6 reactivation. In 4 of the 6 patients with CMV DNA–positive DIHS, CMV DNA was initially detected 4 to 5 weeks after onset, but in 2 patients, the onset of CMV DNAemia occurred at 7 weeks. All of the patients with CMV DNA–positive DIHS were symptomatic, ranging from a low-grade fever to lumbago on detection of CMV DNA in the blood. Four patients in the CMV DNA–positive DIHS group (67%) were receiving immunosuppressive treatment with prednisolone, and 5 patients in the CMV DNA–negative DIHS group (42%) were receiving prednisolone. These results indicate that both groups included patients whose disease at presentation was sufficiently severe to warrant systemic corticosteroids. The median interval between initiation of prednisolone therapy and the onset of CMV disease was 22 days (range, 14-49 days) in the CMV DNA–positive group. Gastrointestinal CMV disease was observed in the 2 cases presented herein only immediately after detection of CMV DNA in the blood. Quantitation of PCR assay demonstrated a relationship be-

**Table 1. Characteristics of Patients With CMV DNA–Positive and CMV DNA–Negative DIHS**

| Patient No./Sex/ Age, y | Underlying Disease | Causative Drug | Duration Between Onset and HHV-6 Reactivation, wk | HHV-6 Load, Maximum[a] | Systemic Corticosteroids, mg/d | Treatment With IVIg | Alterations in Anti-CMV Antibody | CMV Load, Maximum[b] | Detection of CMV Antigenemia |
|---|---|---|---|---|---|---|---|---|---|
| **CMV DNA–Positive DIHS** | | | | | | | | | |
| 1/M/74 | Arrhythmia | Mexiletine hydrochloride | 3 | $4.4 \times 10$ | 50 | + | 28.8→289.0 | $3.4 \times 10^3$ | + |
| 2/M/81 | Hyperuricemia | Allopurinol | 3 | $2.9 \times 10^3$ | 0 | + | 16.8→42.2 | $4.0 \times 10^2$ | + |
| 3/M/68 | Epileptic fits | Phenobarbital | 3 | $1.0 \times 10^2$ | 60 | + | 58.4→380.0 | $2.9 \times 10^3$ | + |
| 4/M/53 | Cerebral infarct | Phenytoin | 3 | ND | 0 | + | 8→64[b] | $4.8 \times 10$ | Not tested |
| 5/M/68 | Rheumatoid arthritis | Salazosulfapyridine | 3 | $4.5 \times 10^3$ | 50 | + | 12.5→156.0 | $3.2 \times 10^3$ | + |
| 6/F/28 | Epilepsy | Carbamazepine | 3 | $9.4 \times 10^1$ | 70 | + | 41.4→580.0 | $2.7 \times 10^3$ | + |
| **CMV DNA–Negative DIHS** | | | | | | | | | |
| 1/M/30 | Psychotic disorder | Carbamazepine | 3 | $2.8 \times 10$ | 40 | + | NA | NA | NA |
| 2/F/54 | Epileptic fits | Phenobarbital | 3 | ND | 0 | – | NA | NA | NA |
| 3/M/24 | Epilepsy | Phenobarbital | 3 | ND | 60 | + | NA | NA | NA |
| 4/F/68 | Epilepsy | Carbamazepine | 4 | $3.0 \times 10^5$ | 60 | – | NA | NA | NA |
| 5/M/39 | Psychotic disorder | Carbamazepine | 3 | ND | 60 | – | NA | NA | NA |
| 6/M/49 | Psychotic disorder | Carbamazepine | 3 | $4.1 \times 10$ | 0 | – | NA | NA | NA |
| 7/F/52 | Epileptic fits | Carbamazepine | 4 | ND | 0 | – | NA | NA | NA |
| 8/M/70 | Neuralgia | Carbamazepine | 3 | $8.6 \times 10$ | 0 | – | NA | NA | NA |
| 9/F/28 | Psychotic disorder | Carbamazepine | 3 | $2.4 \times 10$ | 0 | – | NA | NA | NA |
| 10/F/47 | Brain tumor | Phenytoin | 3 | $2.4 \times 10$ | 30 | – | NA | NA | NA |
| 11/F/36 | Psychotic disorder | Carbamazepine | 3 | ND | 0 | – | NA | NA | NA |
| 12/F/48 | Psychotic disorder | Carbamazepine | 3 | $6.3 \times 10^2$ | 0 | – | NA | NA | NA |

Abbreviations: CMV, cytomegalovirus; DIHS, drug-induced hypersensitivity syndrome; HHV-6, human herpesvirus 6; IVIg, intravenous immunoglobulin; NA, not applicable; ND, not detected; +, yes; –, no.
[a] Virus DNA copies per $10^6$ leukocytes.
[b] Complement fixation test.

**Table 2. Statistical Data**

| | CMV DNA Positive (n=6) | CMV DNA Negative (n=12) | *P* Value |
|---|---|---|---|
| Age, mean (SD), y | 62.0 (7.8) | 45.4 (4.2) | .06[a] |
| Sex, M:F, No. | 5:1 | 5:7 | .15[b] |
| HHV-6 viral load, mean[c] | $1.5 \times 10^4$ | $7.5 \times 10^1$ | .01[a] |

Abbreviations: CMV, cytomegalovirus; HHV-6, human herpesvirus 6.
[a] Unpaired *t* test.
[b] Fisher exact test.
[c] Virus DNA copies per $10^6$ leukocytes.

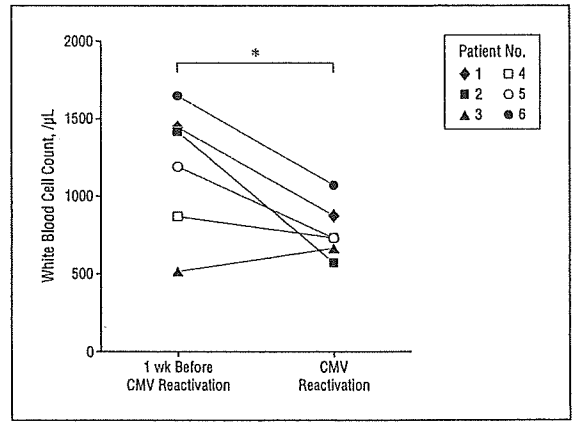


**Figure 5.** Alterations in white blood cell counts 1 week before and at the time of cytomegalovirus reactivation in patients with cytomegalovirus DNA–positive drug-induced hypersensitivity syndrome. The patient numbers correspond to those in Table 1. *$P$=.04 (paired $t$ test). To convert white blood cell count to $\times 10^9$ per liter, multiply by 0.001.

tween CMV load and disease severity: the highest level of CMV DNA ($3.4 \times 10^3$ genomes per $10^6$ leukocytes) was detected in case 1, when the patient developed fatal gastrointestinal CMV disease. White blood cell counts at the time of CMV reactivation were significantly lower than were those 1 week before CMV reactivation in CMV DNA-positive DIHS (**Figure 5**).

## COMMENT

Because it is widely believed that cutaneous CMV disease arises from reactivation of a local latent virus or by autoinoculation in periorificial areas by fecal, urinary, or salivary shedding of CMV,[10] CMV ulcers limited to unusual sites, such as the trunk, will go unrecognized unless a search is made to identify relevant pathogens. These cases indicate the need for monitoring of CMV reactivation even in immunocompetent patients, particularly when unexplained ulcers suddenly develop in patients with DIHS, and suggest that a high index of suspicion and early intervention may decrease morbidity, as in case 2. These cases raise the question of why CMV ulcers were exclusively located on the trunk and shoulders, where CMV ulcers are rarely seen, but not in the anogenital sites. Although comprehensive explanations are unavailable,

we suggest the presence of unrelated preexisting factors that contribute to CMV reactivation in these lesions, which may increase the risk of ulceration. In view of the previous observations that anogenital ulcers frequently develop after erosive lesions associated with herpes simplex virus infection in human immunodeficiency virus–infected patients,[16] a preceding herpesvirus reactivation may play a pivotal role in CMV reactivation. Given the potential interaction of HHV-6 with other herpesviruses in DIHS and the ability to activate Epstein-Barr virus, CMV, and human papillomavirus,[17,18] it is attractive to suppose that the preceding HHV-6 reactivation may have induced CMV reactivation, with the result of the sequential development of CMV ulcers at a particular anatomical location. Indeed, the present sequential analyses of patients with DIHS by means of real-time PCR revealed a good correlation between the degree of preceding HHV-6 DNAemia and a clinically significant CMV reactivation as evidenced by CMV antigenemia or a dramatic increase in leukocyte CMV loads. Other studies[19] in the setting of bone marrow transplantation also demonstrated that CMV reactivation is consistently preceded by HHV-6 reactivation. If so, patients with DIHS, in which HHV-6 reactivation is commonly seen, would be at risk for subsequent CMV disease.

Another intriguing question about these cases is which factors are responsible for the development of CMV ulcers, which usually occur in the setting of immunosuppression. The risk factors associated with CMV disease are those that affect cell-mediated immunity because cell-mediated immunity is the critical host defense for preventing CMV reactivation. Could the widespread reactivation of CMV be a mere complication of treatment with oral prednisolone? In fact, in case 1, overt CMV disease developed approximately 2 weeks after initiation of oral prednisolone therapy. However, after careful consideration of the timing of overt CMV ulcers, we noted that the CMV ulcers developed soon after tapering the dose of oral prednisolone. Although it is clear that long-term immunosuppression due to therapy with prednisolone will place a patient at risk for CMV reactivation, the present patient (case 1) had been taking prednisolone for only 2 weeks before the onset of CMV ulcers: such short-term prednisolone therapy is unlikely to be the major cause of CMV disease. Indeed, recent investigators[20] found no significant adverse outcomes associated with short-term use of prednisolone in advanced human immunodeficiency virus infection. Alternatively, this patient may have had a background of quiescent CMV disease before the onset of DIHS. Cytomegalovirus infection may have already been established before the onset, although it had not been clinically recognized.

Unrecognized CMV infection in immunocompromised patients may often lead to exacerbation of their underlying diseases and even death, as with patient 1. Because gastrointestinal CMV diseases, in particular, are unpredictable and often take a rapidly fatal course in these settings, a high index of suspicion and early recognition are needed for efficient management of patients who undergo immunosuppressive therapy. Despite the documentation of a range of cutaneous and gastrointestinal manifestations, the synchronous occurrence of skin ulcers and gastrointestinal ulcers as shown in patients 1 and 2 herein has rarely been reported. The present cases indicate that cutaneous ulcers that occur in an unusual site as a relatively late systemic manifestation of DIHS would usually portend a fatal course. According to the retrospective study, CMV disease or CMV reactivation would occur during a predictable time course: in most patients with CMV DNA–positive DIHS, CMV DNA was detected during a critical 4- to 5-week period after onset, when patients often receive immunosuppressive agents and are at risk for infection. Consistent with this observation, Seishima et al[21] noted that CMV reactivation in all 7 patients with DIHS detected on days 32 to 51 after onset, a time that corresponded to 10 to 21 days after HHV-6 reactivation; however, in these patients, no fatal CMV disease developed, and risk factors for fatal disease were not provided. The results of the retrospective study indicate that aged, particularly older than 60 years, and male patients with DIHS are at risk for overt CMV disease approximately 4 to 5 weeks after onset and that a rapid reduction in white blood cell counts may be a useful predictor of CMV disease. Thus, we, for the first time, to our knowledge, provide clinical factors predictive of the onset of fatal gastrointestinal CMV disease in settings that are not overtly immunocompromised.

Given the extremely high mortality rate of this infection, CMV disease, in particular, gastrointestinal CMV disease, must be ruled out in patients with DIHS, who developed inconspicuous ulcers 4 to 5 weeks after the onset of DIHS, coincident with a rapid reduction in white blood cell counts. Whether earlier treatment with ganciclovir would have altered the fate of these patients with CMV reactivation is unclear because previous articles[22] described that many immunocompromised patients who showed cutaneous ulcers as a late systemic manifestation died within 2 weeks after onset. Nevertheless, early consideration and identification of CMV reactivation by means of PCR analyses are crucial for the establishment of the diagnosis in otherwise difficult-to-treat ulcers and for the improvement of the prognosis.

Accepted for Publication: February 11, 2009.
Correspondence: Yusuke Asano, MD, Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa Mitaka, Tokyo 181-8611, Japan (yskasn @kyorin-u.ac.jp).
Author Contributions: All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Asano, Kano, and Shiohara. *Acquisition of data:* Asano, Kagawa, Kano, and Shiohara. *Analysis and interpretation of data:* Asano, Kano, and Shiohara. *Drafting of the manuscript:* Asano. *Critical revision of the manuscript for important intellectual content:* Asano, Kagawa, Kano, and Shiohara. *Statistical analysis:* Kano and Shiohara. *Obtained funding:* Kano and Shiohara. *Administrative, technical, and material support:* Kano. *Study supervision:* Shiohara.
Financial Disclosure: None reported.
Funding/Support: This study was supported in part by a research grant-in-aid for scientific research from the Ministry of Education, Sports, Science, and Culture of

Japan; by a health and labor sciences research grant from the Ministry of Health, Labor, and Welfare of Japan; and by the Japanese Research Committee on Severe Cutaneous Adverse Reaction.

Role of the Sponsors: The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; or in the preparation, review, or approval of the manuscript.

## REFERENCES

1. Sissons JG, Carmichael AJ. Clinical aspects and management of cytomegalovirus infection. *J Infect.* 2002;44(2):78-83.
2. Streblow DN, Orloff SL, Nelson JA. Acceleration of allograft failure by cytomegalovirus. *Curr Opin Immunol.* 2007;19(5):577-582.
3. Bhawan J, Gellis S, Ucci A, Chang TW. Vesiculobullous lesions caused by cytomegalovirus infection in an immunocompromised adult. *J Am Acad Dermatol.* 1984;11(4, pt 2):743-747.
4. Walker JD, Chesney TM. Cytomegalovirus infection of the skin. *Am J Dermatopathol.* 1982;4(3):263-265.
5. Weigand DA, Burgdorf WH, Tarpay MM. Vasculitis in cytomegalovirus infection. *Arch Dermatol.* 1980;116(10):1174-1176.
6. Horn TD, Hood AF. Clinically occult cytomegalovirus present in skin biopsy specimens in immunosuppressed hosts. *J Am Acad Dermatol.* 1989;21(4, pt 1):781-784.
7. Pariser RJ. Histologically specific skin lesions in disseminated cytomegalovirus infection. *J Am Acad Dermatol.* 1983;9(6):937-946.
8. Lesher JL Jr. Cytomegalovirus infections and the skin. *J Am Acad Dermatol.* 1988;18(6):1333-1338.
9. Colsky AS, Jegasothy SM, Leonardi C, Kirsner RS, Kerdel FA. Diagnosis and treatment of a case of cutaneous cytomegalovirus infection with a dramatic clinical presentation. *J Am Acad Dermatol.* 1998;38(2, pt 2):349-351.
10. Daudén E, Fernández-Buezo G, Fraga J, Cardeñoso L, García-Díez A. Mucocutaneous presence of cytomegalovirus associated with human immunodeficiency virus infection: discussion regarding its pathogenetic role. *Arch Dermatol.* 2001;137(4):443-448.
11. Choi Y-L, Kim J-A, Jang K-T, et al. Characteristics of cutaneous cytomegalovirus infection in non-acquired immune deficiency syndrome, immunocompromised patients. *Br J Dermatol.* 2006;155(5):977-982.
12. Suzuki Y, Inagi R, Aono T, Yamanishi K, Shiohara T. Human herpesvirus 6 infection as a risk factor for the development of severe drug-induced hypersensitivity syndrome. *Arch Dermatol.* 1998;134(9):1108-1112.
13. Tohyama M, Yahata Y, Yasukawa M, et al. Severe hypersensitivity syndrome due to sulfasalazine associated with reactivation of human herpesvirus 6. *Arch Dermatol.* 1998;134(9):1113-1117.
14. Shiohara T, Iijima M, Ikezawa Z, Hashimoto K. The diagnosis of DRESS syndrome has been sufficiently established on the basis of typical clinical features and viral reactivations. *Br J Dermatol.* 2007;156(5):1083-1084.
15. Kano Y, Hiraharas K, Sakuma K, Shiohara T. Several herpesviruses can reactivate in a severe drug-induced multiorgan reaction in the same sequential order as in graft-versus-host disease. *Br J Dermatol.* 2006;155(2):301-306.
16. Toome BK, Bowers KE, Scott GA. Diagnosis of cutaneous cytomegalovirus infection: a review and report of a case. *J Am Acad Dermatol.* 1991;24(5, pt 2):860-867.
17. Katsafanas GC, Schirmer EC, Wyatt LS, Frenkel N. In vitro activation of human herpesviruses 6 and 7 from latency. *Proc Natl Acad Sci U S A.* 1996;93(18):9788-9792.
18. Wang F-Z, Larsson K, Linde A, Ljungman P. Human herpesvirus 6 infection and cytomegalovirus-specific lymphoproliferative responses in allogeneic stem cell transplant recipients. *Bone Marrow Transplant.* 2002;30(8):521-526.
19. Takemoto Y, Takatsuka H, Wada H, et al. Evaluation of CMV/human herpes virus 6–positivity in bronchoalveolar lavage fluids as early detection of acute GVHD following BMT: evidence of a significant relationship. *Bone Marrow Transplant.* 2000;26(1):77-81.
20. Mayanja-Kizza H, Jones-Lopez E, Okwera A, et al; Uganda-Case Western Research Collaboration. Immunoadjuvant prednisolone therapy for HIV-associated tuberculosis: a phase 2 clinical trial in Uganda. *J Infect Dis.* 2005;191(6):856-865.
21. Seishima M, Yamanaka S, Fujisawa T, Tohyama M, Hashimoto K. Reactivation of human herpesvirus (HHV) family members other than HHV-6 in drug-induced hypersensitivity syndrome. *Br J Dermatol.* 2006;155(2):344-349.
22. Fleischmann M, Milpied B, Dréno B, et al. Cutaneous cytomegalovirus ulceration in AIDS: diagnosis by in situ hybridization and response to treatment [article in French]. *Ann Dermatol Venereol.* 1992;119(11):877-879.

〔ウイルス　第59巻　第1号，pp.23-30，2009〕

総　説

# 3. 薬剤性過敏症症候群とHHV-6の再活性化について

藤山　幹子，橋本　公二
愛媛大学大学院医学系研究科感覚皮膚医学

薬剤性過敏症症候群は，発熱と多臓器障害を伴い遷延する薬疹である．抗けいれん薬，アロプリノール，サラゾスルファピリジン，ジアフェニルスルフォン，メキシレチン，ミノサイクリンが原因となる．その大きな特徴は，発症後10日から30日の間のある時期に，HHV-6の再活性化を伴うことにある．HHV-6の再活性化は，血液，血清中のHHV-6 DNAの検出と著明なIgG抗体価の上昇で確認される．HHV-6の再活性化に際して，発熱と肝障害を認めることが多い．薬剤性過敏症症候群は，薬剤アレルギーとHHV-6感染症の複合した病態である．

## はじめに

薬疹は主に薬剤アレルギーにより生じ，さまざまな型の発疹を示す．原因薬剤を中止することで軽快することが多いが，原因薬剤の中止のみでは軽快せず適切な治療を行わなければ致死的経過をたどることもある重症型の薬疹がある．

薬剤性過敏症症候群（drug-induced hypersensitivity syndrome：DIHS）は，そのような重症の薬剤アレルギーの一つである．原因薬剤は，抗けいれん薬が最も多く，けいれんやてんかんに対して処方されるフェニトイン，カルバマゼピン，フェノバルビタール，ゾニサミドがその原因となる．フェニトイン，カルバマゼピンなどにより生じる重症の薬剤アレルギーは，薬剤が使用されるようになった1950年ころから報告されていた[1-3]．そのひとつは，Stevens-Johnson症候群や中毒性表皮壊死症といわれる，高熱と皮膚の熱傷様の剥離を主要症状とする薬剤アレルギーであり，もうひとつは，初期には皮膚症状は通常のありふれた薬疹のようでありながら全身に拡大して，発熱と種々の臓器障害を伴い重篤となる薬剤アレルギーであった[4]．

後者の薬剤アレルギーは，個々の症例で症状に幅があるものの，基本的には皮疹が必発で，発熱，肝障害，血液障害，リンパ節腫脹を種々の程度で伴う[4,5]．血液障害は，白血球増多，好酸球増多，異型リンパ球の出現が特徴とされる．重篤な肝障害のために致死的な経過をたどることがあり，それも重症型といわれるゆえんであるが，さらには経過が特徴的である．通常の薬疹と異なり，このタイプの薬疹では，原因薬剤を中止後も症状が増悪する．その後ピークを超えて軽快傾向を示し始めると，再び症状が悪化し（再燃），すべての症状が沈静化するまでに数週を要する．また，消化管出血，肺炎，敗血症など種々の合併症を生じてくることもある．これらの合併症は，従来は偶発的な合併症として，あるいはステロイド薬による副作用やその免疫抑制に基づく日和見感染として見過ごされていたものであり，注意して経過を見るとその頻度は高い．

抗けいれん薬によるこれらの薬疹は，1990年代よりanticonvulsant hypersensitivity syndromeといわれてきた[6,7]．また，興味深いことに同様の薬剤アレルギーは，アロプリノール，サラゾスルフピリジン，ジアフェニルスルフォン，メキシレチン，ミノサイクリンでも認められ（**表1**），それぞれ，allopurinol hypersensitivity[8]，サラゾスルファピリジンによる伝染性単核球症様薬疹[9,10]などの病名で報告されていた．原因薬剤を問わず臨床経過が共通するため，フランスの薬疹を専門とするグループより，drug rash with eosinophilia and systemic symptoms（DRESS）[11]という新しい病名が提唱されたが，あまりに症状の重症度に幅があるため，Stevens-Johnson症候群や中毒性表皮壊死症のような独立した一つの疾患概念とすることには疑問をとなえる向きもあった．

連絡先
〒791-0295
愛媛大学大学院医学系研究科感覚皮膚医学
TEL：089-960-5350
FAX：089-960-5352
E-mail: tohm@m.ehime-u.ac.jp

表1 薬剤性過敏症症候群の原因薬剤

| | 商品名 | 適応症 |
|---|---|---|
| 抗けいれん薬 | | てんかん、けいれん |
| カルバマゼピン | テグレトールなど | 上記に加え、躁病、統合失調症、三叉神経痛 |
| フェニトイン | アレビアチンなど | |
| フェノバルビタール | フェノバールなど | |
| ゾニサミド | エクセグラン | |
| アロプリノール | ザイロリックなど | 高尿酸血症、痛風 |
| サラゾスルファピリジン | サラゾピリン、アザルフィジンENなど | 潰瘍性大腸炎、慢性関節リウマチ |
| ジアフェニルスルフォン | レクチゾール | ハンセン病、好中球性皮膚疾患、天疱瘡など |
| 塩酸メキシレチン | メキシチールなど | 不整脈、糖尿病性神経障害にともなう痛みやしびれ |
| 塩酸ミノサイクリン | ミノマイシンなど | 種々の細菌感染症 |

## 薬剤性過敏症症候群の概念の確立

薬剤性過敏症症候群がひとつの疾患概念として確立されたのは，ヒトヘルペスウイルス6（HHV-6）の再活性化を伴うことが明らかになったことによる．DRESSが提唱されたのと同じ頃，我々および杏林大学皮膚科の塩原らのグループは，それぞれサラゾスルファピリジン，アロプリノールによる前述の薬剤アレルギーの患者において，HHV-6が関与していることを見いだした[12, 13]．われわれの経験した症例では，発症後13日目の血液からHHV-6が分離され，その後抗HHV-6 IgG抗体価が上昇した．

これをきっかけとし，我々は，HHV-6とこれらの薬剤アレルギーとの関係について検討を行った[14]．従来hypersensitivity syndromeと呼ばれてきた薬剤アレルギーに明確な診断基準はなく，発熱，皮疹，臓器障害があれば診断されていた．そこで，皮疹の他に発熱あるいは少なくとも一つの臓器障害を伴っている薬剤アレルギーの症例の血清を集め，HHV-6 IgG抗体価の測定を行った．原因薬剤は，薬剤性過敏症症候群の原因薬剤に限った．その結果，これの薬剤アレルギーの中には，発症後数週遅れて顕著なHHV-6 IgG抗体価の上昇を来す一群があることが明らかとなった（図1）．抗体価は，発症後10日から30日の間に上昇しており，発症時の再活性化ではなく，発症後しばらくしてからの再活性化と考えられた．またHHV-6の抗体価の上昇を認めた群では，抗体価の上昇を伴わなかった群と比較して，発熱の期間が長く，白血球増多や異型リンパ球の出現といった血液障害が顕著であり，肝障害を伴うのみならず，症状の再燃を認め，経過が遷延する傾向にあった．次に，血清中のHHV-6 DNAの検討を行ったところ，HHV-6 IgG抗体価の上昇した62例中の18例で血清中にHHV-6 DNAが検出され，この全ての症例でHHV-6 DNAの検出と同じときに発熱と肝障害の再燃がみられることがわかった（表2）．以上の結果から，これまで説明のつかなかった症状の「再燃」がHHV-6の再活性化により生じていることが明らかとなった．こうして，薬剤アレルギーとウイルス感染の複合した病態が存在することが認識されるようになった．

2000年ころより，主に皮膚科領域で，HHV-6の再活性化を伴うhypersensitivity syndromeの症例が多数報告され，重症薬疹の一型として広く認められるようになったことから，厚生労働省の重症薬疹の研究班により，HHV-6の再活性化を認める，限られた薬剤による薬剤アレルギーをdrug-induced hypersensitivity syndrome（DIHS）と呼称することが提案され，診断基準が作成された（表3）[15]．

## 薬剤性過敏症症候群の典型的経過

薬剤性過敏症症候群では，原因薬剤を2週から6週間内服した後に発症する（図2）．臨床上よく経験する抗生物質による薬疹が，投与開始後5日から14日目までに出現することと比較すると，この内服期間の長さは特異である．

症状の始まりは，発熱あるいは発疹である．同時のこともあるが，どちらかが先行することも多い．発疹は，麻疹や風疹でみられるような比較的小さい紅斑が多発して出現し，次第にくっつきあって広い範囲の紅斑となっていく．顔面にも紅斑や浮腫を認める．

次第にリンパ節腫脹も出現してくる．頚部に触知される症例がほとんどであるが，全身の表在リンパ節が腫脹することもある．

血液検査を行うと，肝機能障害が認められる．ただし，アロプリノールが原因の場合には，肝機能障害を欠き，腎障害のみを認めることがある．

皮疹，血液障害や肝障害は，発症後1週から2週目ころにかけて時期は異にするがそれぞれピークを迎え，その後

表 2　血清中 HHV-6 DNA の検出とそのときに認められた臨床症までの再燃

| No. | Age/ sex | Causative drug | Days after onset | HHV-6 DNA Copy number (copies/ml) | HHV-6 IgG titer | Flaring of symptoms |
|---|---|---|---|---|---|---|
| 1 | 44/M | Carbamazepine | 12 | 0 | 80 | |
| | | | 13 | 3300 | 80 | |
| | | | 16 | 2400000 | 80 | Fever (day 16-18) |
| | | | 17 | 1600000 | 80 | |
| | | | 18 | 4300 | 320 | Hepatitis (day 18, ALT 404) |
| | | | 19 | 0 | 10240 | |
| 2 | 22/M | Phenobarbital Zonisamide | 15 | 0 | 20 | |
| | | | 22 | 310000 | 20 | Hepatitis (day 24, ALT 1200) |
| | | | 29 | 0 | 5120 | |
| 3 | 66/M | Mexiletin | 20 | 0 | <20 | |
| | | | 24 | 1200 | <20 | Hepatitis (day 27, ALT 505) |
| | | | 27 | 73000 | 20 | |
| | | | 32 | 0 | 640 | |
| 4 | 72/M | phenytoin | 12 | 6700 | 40 | |
| | | | 14 | 2800 | 40 | Fever (day 14-17) |
| | | | 15 | 57000 | 80 | |
| | | | 18 | 0 | 10240 | |
| 5 | 55/F | Carbamazepine | 12 | 60000 | 20 | |
| | | | 14 | 51000 | 80 | Hepatitis (day 15, ALT 519) |
| | | | 18 | 0 | 640 | |
| 6 | 88/F | Carbamazepine | 14 | 16000 | 80 | Fever (day 13-18) |
| | | | 16 | 40000 | 80 | |
| | | | 20 | 270 | 10240 | |
| | | | 23 | 0 | 10240 | |
| 7 | 59/F | Carbamazepine | 13 | 0 | 40 | |
| | | | 15 | 1100 | 40 | Fever (day 15-19), skin rash |
| | | | 16 | 14000 | 40 | |
| | | | 20 | 980 | 1280 | Hepatitis (day 20, ALT 210) |
| | | | 23 | 0 | 1280 | |
| 8 | 45/M | Allopurinol | 9 | 0 | 80 | |
| | | | 11 | 5100 | 80 | |
| | | | 12 | 12000 | 80 | Hepatitis (day 12, ALT 365) |
| | | | 17 | 0 | 10240 | |
| 9 | 47/F | Phenytoin | 23 | 0 | 40 | |
| | | | 24 | 3400 | 40 | |
| | | | 25 | 7800 | 40 | |
| | | | 26 | 1000 | 40 | |
| | | | 28 | 0 | 640 | Hepatitis (day 28, ALT 97) |
| 10 | 55/M | Phenytoin | 13 | 0 | 80 | |
| | | | 19 | 6600 | 80 | Fever (day 18-21) |
| | | | 23 | 0 | 1280 | Hepatitis (day 22, ALT 280), skin rash |
| 11 | 28/M | Salazosulfapyridine | 4 | 0 | 160 | |
| | | | 11 | 6200 | 160 | Hepatitis (day 13, ALT 250) |
| | | | 21 | 0 | 1280 | |
| 12 | 52/F | Allopurinol | 19 | 0 | 20 | |
| | | | 25 | 6000 | 20 | Fever (day 22-27) |
| | | | 32 | 0 | 2560 | |
| 13 | 49/F | Carbamazepine | 14 | 6000 | 20 | Fever (day 14) |
| | | | 28 | 0 | 1280 | |
| 14 | 39/F | Allopurinol | 22 | 0 | <20 | |
| | | | 24 | 200 | 40 | |
| | | | 27 | 2900 | 40 | Fever (day 25) |
| | | | 34 | 0 | 1280 | Hepatitis (day 31, ALT 666) |
| 15 | 40/F | Mexiletin | 11 | 0 | 80 | |
| | | | 14 | 750 | 80 | Fever (day 14-16) |
| | | | 19 | 0 | 1280 | Hepatitis (day 17, ALT 143) |
| 16 | 30/M | Carbamazepine | 17 | 0 | <20 | |
| | | | 21 | 310 | 20 | |
| | | | 24 | 0 | 1280 | Hepatitis (day 26, ALT 729) |
| 17 | 78/M | Allopurinol | 9 | 0 | 160 | |
| | | | 13 | 300 | 160 | Fever (day 11-14), |
| | | | 18 | 0 | 1280 | Hepatitis (day 16, ALT 850) |
| 18 | 51/F | Carbamazepine | 11 | 0 | 160 | |
| | | | 23 | 120 | 5120 | Hepatitis (day 23, ALT 200) |
| | | | 26 | 0 | 20480 | |

（文献 14 より引用）

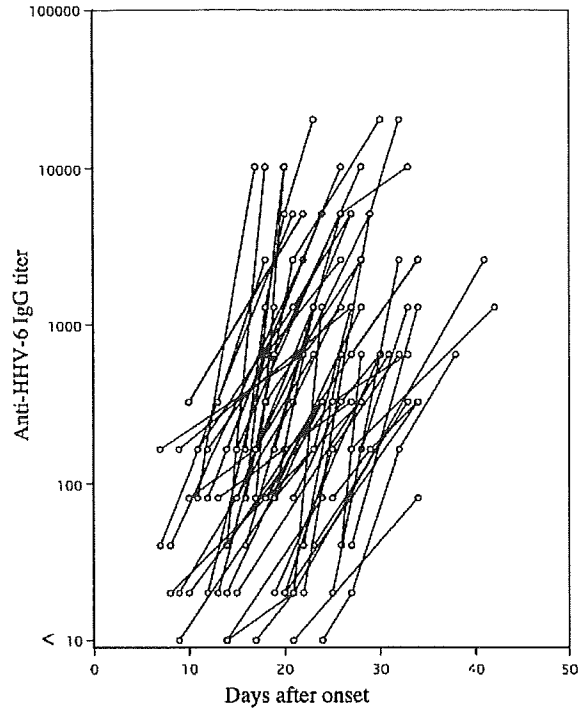


**図1 HHV-6 IgG 抗体価の上昇**

38例の薬疹患者でHHV-6 IgG抗体価の4倍以上の上昇を認めた．図は，抗体価の変動した部分のみを表している．抗体価の上昇は，発症後10日目以降に始まり，30日目までに上昇した．（文献14より引用）

**表3 薬剤性過敏症症候群の診断基準**

主要所見

1. 限られた薬剤投与後に遅発性に生じ、急速に拡大する紅斑。しばしば紅皮症に移行する。
2. 原因薬剤中止後も2週間以上遷延する
3. 38度以上の発熱
4. 肝機能障害
5. 血液学的異常：a,b,cのうち一つ以上
   a. 白血球増多(11000/$mm^3$以上)
   b. 異型リンパ球の出現（5％以上）
   c. 好酸球増多（1500/$mm^3$以上）
6. リンパ節腫脹
7. HHV-6の再活性化

典型DIHS：1~7全て

非典型DIHS：1~5全て、ただし4に関しては、その他の重篤な臓器障害をもって代えることができる。

HHV-6の再活性化による再燃が生じる．主に発熱と肝障害の再燃として認められるが，再活性化したウイルスの量と，おそらくは個々の免疫反応の違いにより，程度は様々である．HHV-6のウイルス血症が数日で終息するのと同時に，発熱や肝障害の再燃も数日で軽快することが多い．

HHV-6の再活性化による臨床症状の再燃のあとは，比較的速やかに軽快する症例が多いようである．しかし，引き続いてサイトメガロウイルスの再活性化等が関与すると思われる発熱や皮疹の再燃を認めることもある．これが，薬剤性過敏症症候群の典型的経過である．

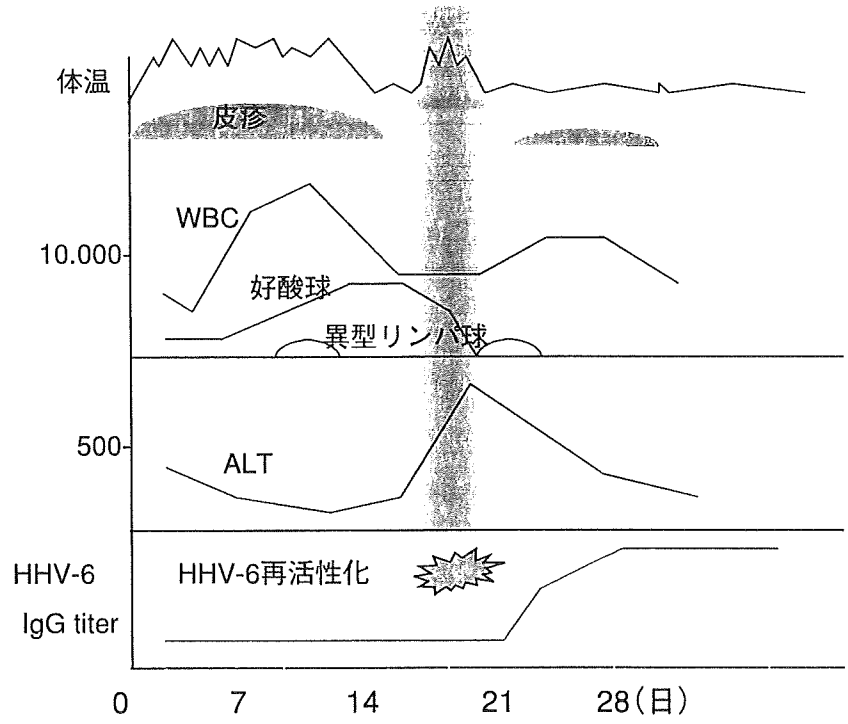


**図2　薬剤性過敏症症候群の典型的経過**
原因薬剤を2週から6週間内服後に，発熱と発疹で発症する．薬剤を中止しても症状は増悪し，リンパ節腫脹や白血球増多，好酸球増多，異型リンパ球が認められるようになる．肝障害も伴う．症状がピークを越えて軽快傾向となるころに，HHV-6の再活性化を生じ，発熱や肝障害の再燃を認める．HHV-6のIgG抗体価がその後急激に上昇する．

### 薬剤性過敏症症候群における HHV-6再活性化の関与した病態

薬剤性過敏症症候群でHHV-6の再活性化の認められる期間は比較的短く，血清中にHHV-6 DNAが検出されるのは通常数日である．そのときに，発熱や肝障害の再燃を生じるのは，前述のとおりである．HHV-6 IgG抗体価の上昇とともに，急速に血清中のHHV-6 DNAは消失し，十分にウイルス排除の免疫が働いていると考えられる．HHV-6の再活性化において認められる肝障害の再燃も，この免疫学的機序に基づく可能性がある．ウイルスの増殖が少ないときには，抗体価の上昇は認められるが，適切な時期の血清を検査してもHHV-6 DNAが検出されず，症状の再燃はほとんど認められない．

HHV-6の再活性化によって生じる発熱と肝障害の再燃は，特別な治療を要さずに軽快するが，HHV-6の再活性化の時期に一致して重篤な合併症が生じることもある．主に二つが知られており，中枢神経障害と劇症1型糖尿病である．中枢神経障害，特に脳炎では，初発症状は，けいれん，意識障害見当識障害，短期記憶障害とされ，髄液中にHHV-6DNAが証明されることにより診断される．HHV-6により移植後に生じる脳炎と比較すると発症頻度は高くはないと思われるが，死亡例も報告されている．MRIでは，海馬，側頭葉，辺縁系の異常所見を認める[16,17]．しかし，HHV-6のウイルス血症と同時ではなく遅れて中枢神経障害を生じた症例もあり[18]，この障害がウイルスの増殖に基づくものか，免疫学的機序に基づくものか，今後検討が必要である．

また，劇症1型糖尿病の報告は，年々増加の傾向にある[19-21]．劇症1型糖尿病は，膵臓のインスリンを産生するβ細胞が急激に破壊され，突然の高血糖を来す病態である．ウイルス感染に関連して発症する一群が存在することが知られており，このような症例では，抗glutamic acid decarboxylase (GAD) 抗体や抗インスリン自己抗体（IAA）などの自己抗体が検出されない[22]．最近，狩野が薬剤性過敏症症候群に合併して1型糖尿病を発症した13症例の報告を解析しているが[23]，自己抗体を認めたのは2例のみであった．13例のすべてがHHV-6の再活性化に一致して発症しているわけではないが，HHV-6の再活性化と同時に発症が認められる症例があることも確かであり，今後さらなる検討が望まれる．また，移植後のHHV-6の再活性化に関連した劇症1

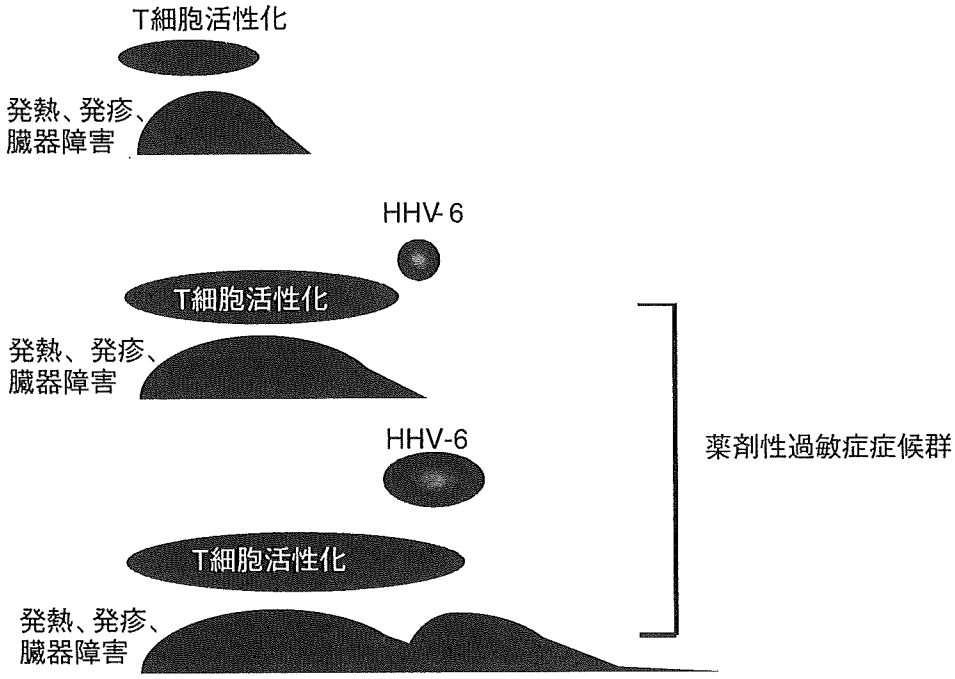


**図3 T細胞の活性化とHHV-6の再活性化**
同じ薬剤が原因となり生じた薬剤アレルギーでも，薬剤の中止によりすみやかに軽快する場合には，HHV-6の再活性化を認めない．発症後しばらくたってからHHV-6の増殖が始まったときに，T細胞の活性化が持続していると，HHV-6の増殖は増強される．増殖の程度が強いと血清中にHHV-6 DNAが検出され，症状の再燃が生じる．

型糖尿病は調べえた限りでは報告がなく，ウイルスに対する免疫反応の違いがこの疾患の発症に起因する可能性がある．

### 薬剤性過敏症症候群におけるHHV-6の再活性化の機序

薬剤性過敏症症候群におけるHHV-6の再活性化の機序はまだよくわかっていない．興味深いのは，HHV-6の再活性化を生じるには，診断基準にあるすべての症状がそろうことが必要ということである．発疹を欠く薬剤性過敏症症候群は今のところ存在せず，また発熱と発疹はみられても，その他が肝機能障害のみ，あるいは血液障害のみ場合には，HHV-6の再活性化を生じる確立は非常に低くなる．これらすべてが揃った臨床像は，麻疹やデング熱とも類似しているが，これらの疾患でも発症の数週後にHHV-6の再活性化が確認されることが報告されている[24,25]のは，非常に興味深い．免疫抑制以外の，ある種の免疫反応が，HHV-6の再活性化に必要であることが示唆される．

また，薬剤性過敏症症候群におけるHHV-6の再活性化は，移植後に認められるHHV-6の再活性化と比較されることが多い．Kitamuraらは，末梢血幹細胞移植か臍帯血幹細胞移植をうけた15人の末梢血において，HHV-6 DNAの定量を経時的に行っている[26]．15例中10例でGVHDと考えられる皮疹を生じており，うち8例で皮疹と同時期にHHV-6 DNAの増加を検出しているが，皮疹の生じなかった5例においてはHHV-6 DNAの増加が確認できたのは1例のみであった．幹細胞移植後のHHV-6の再活性化と臨床症状を検討した報告は多数あり，de Pagterらの2008年のレビューによると，18の報告のうち6つにおいてGVHDとHHV-6の再活性化との関連が示唆されている[27]．皮疹の出現時期でみると，薬剤性過敏症症候群では発症時から皮疹を認めるのに対し，移植後ではHHV-6の再活性化とともに皮疹が出現し，皮疹とHHV-6の関与についてははっきりしない．しかし，免疫状態に目を転じると，移植後のHHV-6の再活性化は，免疫再構築のTリンパ球の増殖をベースとして生じている可能性があり[27]薬剤性過敏症症候群では，薬剤によって活性化されたT細胞の存在がHHV-6の増殖に関与している可能性がある．薬剤性過敏症症候群と，移植後の免疫状態は類似しているのかもしれない．

薬剤アレルギーにおいて，薬剤に対するT細胞の活性化が強く長い状態が続いたときにHHV-6の増殖が生じると考えれば，図3のように病態を捉えることが可能である．しかし薬剤性過敏症症候群において，HHV-6の増殖が発症後数週たってから生じる理由を説明することはできない．

最近，塩原らのグループは，薬剤性過敏症症候群の急性期に制御性T細胞が増殖することを見いだし，制御性T細胞により抗ウイルス免疫が抑制されることがHHV-6の再活性化を誘導するのではないかと考察している[28]．

### HHV-6以外のウイルスの関与

薬剤性過敏症症候群では，HHV-6以外のヘルペスウイルスの再活性化も認められる[29,30]．HHV-7，EBウイルス，サイトメガロウイルスについて，DNAレベルあるいは抗体価の上昇によって再活性化が確認されるが，臨床症状を伴うのは，主にサイトメガロウイルスである．HHV-6に続いて再活性化を生じ，サイトメガロウイルス感染症としての皮膚潰瘍，消化管障害，肺炎を生じることがある．サイトメガロウイルスの関与が疑われる心筋炎の報告もあり[31]，予後を左右する重要な因子となっている．また，サイトメガロウイルスの再活性化が感染症とはなっていなくても，皮疹の再燃が生じることはしばしば経験される．病態によっては，抗ウイルス薬による治療を必要とするが，サイトメガロウイルスの関与に気づかれないことも多く，現時点では薬剤性過敏症症候群の治療における盲点となっている．今後は，このウイルスについても検討を進めていく必要があると考えられる．

### 参考文献


1) Silber IB, Epstein JW. The treatment of chorea with phenylethylhydantoin: a study of 28 cases. Arch Pediatr 51:373-82, 1934.
2) Chaiken BH, Goldberg BI, Segal JP. Dilantin hypersensitivity: report of a case of hepatitis with jaundice, pyrexia, and exfoliative dermatitis. N Engl J Med 242: 897-8, 1950.
3) Tennis P, Stern RS. Risk of serious cutaneous disorder after initiation of use of phenytoin, carbamazepine, or sodium valproate: a record linkage study. Neurology 49:542-6, 1997.
4) Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. N Engl J med 331:1272-85, 1994.
5) Callot V, Roujeau JC, Baqot M, Wechsler J, Chosidow O, Souteyrand P, Morel P, Dubertret L, Avril MF, Revuz J. Drug-induced pseudolymphoma and hypersensitivity syndrome. *Arch Dermatol* 1996; 132: 1315-21.
6) Shear NH, Spielberg SP. Anticonvulsant hypersensitivity syndrome. In vitro assessment of risk. J Clin Invest. 82:1826-32, 1998.
7) Vittorio CC, Muglia JJ. Anticonvulsant hypersensitivity syndrome. Arch Intern Med. 155:2285-90, 1995.
8) Singer JZ, Wallace SL. The allopurinol hypersensitivity syndrome. Arthritis and Rhematism 29:82-7, 1986.
9) Iwatsuki K, Tsugiki M, Tagami H, Yamada M. Infectious mononucleosis-like manifestations. An adverse reaction to sulfasalazine. Arch Dermatol. 120:964-5, 1984.
10) 伯野めぐみ,菊池新,井出瑛子他　伝染性単核球症様症状を呈したSalazosulfapyridine (Salazopyrin®)による薬疹の1例　皮膚科の臨床 36: 1219-23, 1994
11) Bocquet H, Bagot M, Roujeau JC. Drug-induced pseudolymphoma and drug hypersensitivity syndrome (Drug Rash with Eosinophilia and Systemic Symptoms: DRESS). Semin Cutan Med Surg. 15:250-7, 1996.
12) Tohyama M, Yahata Y, Yasukawa M, Inagi R, Urano Y, Yamanishi K, Hashimoto K. Severe hypersensitivity syndrome due to sulfasalazine associated with human herpesvirus 6. *Arch Dermatol* 1998; 134: 1113-7.
13) Suzuki Y, Inagi R, Aono T, Yamanishi K, Shiohara T. Human herpesvirus 6 infection as arisk factor for the development of severe drug-induced hypersensitivity syndrome. *Arch Dermatol* 1998; 134: 1108-12.
14) Tohyama M, Hashimoto K, Yasukawa M, Kimura H, Horikawa T, Nakajima K, Urano Y, Matsumoto K, Iijima M, Shear NH. Association of human herpesvirus 6 reactivation with the flaring and severity of drug-induced hypersensitivity syndrome. Br J Dermatol. 157:934-40, 2007.
15) 橋本公二　Stevens-Johnson症候群，toxic epidermal necrolysis (TEN) とhypersensitivity syndromeの診断基準および治療指針の研究　厚生科学特別研究事業　平成17年度総括研究報告, 2005.
16) Fujino Y, Nakajima M, Inoue, H, Kusuhara T, Yamada T. Human Herpesvirus 6 encephalitis associated with hypersensitivity syndrome. Ann Neurol 51:771-4, 2002.
17) 伊東貴雄，大石知瑞子，千葉厚郎，作田学，佐久間恵一，塩原哲夫．フェノバルビタールによるdrug-induced hypersensitivity syndromeに続発した辺縁系脳炎の1例．臨床神経学 45: 495-501, 2005.
18) Masaki T, Fukunaga A, Tohyama M, Koda Y, Okuda S, MaedaN, Kanda F, Yasukawa M, Hashimoto K, Horikawa T, Ueda M. Human herpes virus 6 encephalitis in allopurinol-induced hypersensitivity syndrome. Acta Derm Venereol 83: 128-31, 2003.
19) Sekine N, Motokura T, Oki T, Umeda Y, Sasaki N, Hayashi M, Sato H, Fujita T, KanekoT, Asano Y, Kikuchi K. Rapid loss of insulin secretion in a patient with fulminant type 1 diabetes mellitus and carbamazepine hypersensitivity syndrome. JAMA. 285: 1153-4, 2001.
20) Seino Y, Yamauchi M, Hirai C, Okumura A, Kondo K, Yamamoto M, Okazaki Y. A Case of fulminant Type 1 diabetes associated with mexiletine hypersensitivity syndrome. Diabet Med 2004; 21: 1156-7.
21) Chiou CC, Chung WH, Hung SI, Yang LC, Hong HS. Fulminant type 1 diabetes mellitus caused by drug hypersensitivity syndrome with human herpesvirus 6 infection. J Am Acad Dermatol. 54(2 Suppl):S14-7, 2006.
22) Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y. A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. Osaka IDDM Study Group. N Engl J Med 342: 301-7, 2000.
23) 狩野葉子　DIHS (drug-induced hypersensitivity syndrome) に合併する劇症1型糖尿病　臨皮　63(5

増)：18-21, 2009.
24) Suga S, Yoshikawa T, Asano Y, Nakashima T, Kobayashi I, Yazaki T. Activation of human herpesvirus-6 in children with acute measles. J Med Virol 38: 278-82, 1992.
25) Balachandra K, Chimabutra K, Supromajakr P, Wasi C, Yamamoto T, Mukai T, Okuno T, Yamanishi K. High rate of reactivation of human herpesvirus 6 in children with dengue hemorrhagic fever. J Infect Dis 170: 746-8, 1994.
26) Kitamura K, Asada H, Iida H, Fukumoto T, Kobayashi N, Niizeki H, Morii T, Kimura H, Miyagawa S. Relationship among human herpesvirus 6 reactivation, serum interleukin 10 levels, and rash/graft-versus-host disease after allogeneic stem cell transplantation. J Am Acad Dermatol 58: 802-9, 2008.
27) de Pagter PJ, Schuurman R, Meijer E, van Baarle D, Sanders EAM, Boelens JJ. Human herpesvirus type 6 reactivation after haematopoietic stem cell transplantation. J Clin Virol 43:361-6, 2008.
28) Aota N, Shiohara T. Viral connection between drug rashes and autoimmune diseases: How autoimmune responses are generated after resolution of drug rashes. Autoimmune review 2009. (in press)
29) Seishima M, Yamanaka S, Fujisawa T, Tohyama M, Hashimoto K. Br J Dermatol 155: 344-9, 2006.
30) Kano Y, Hirahara K, Sakuma K, Shiohara T. Several herpesvirused can reactivate in a severe drug-induced multiorgan reaction in the same sequential order as in graft-versus-host disease. Br J Dermatol 155:301-6, 2006.
31) Sekiguchi A, Kashiwagi T, Ishida-Yamamoto A, Takahashi H, Hashimoto Y, Kimura H, Tohyama M, Hashimoto K, Iizuka H. Drug induced hypersensitivity syndrome due to mexiletine associated with human herpes virus 6 and cytomegalovirus reactivation. J Dermatol. 32: 278-81, 2005.

# Drug-induced hypersensitivity syndrome and HHV-6 reactivation

**Mikiko TOHYAMA, Koji HASHIMOTO**

Department of Dermatology, Ehime University Graduate School of Medicine

Drug-induced hypersensitivity syndrome (DIHS) is an adverse reaction with clinical signs of fever, rash, and internal organ involvement. The culprit drugs of DIHS are limited to several drugs such as carbamazepine, phenytoin, phenobarbital, zonisamide, allopurinol, salazosulfapyridine, diaphenylsulphone, and mexiletine. The association of HHV-6 reactivation with DIHS has been known. Flaring of symptoms such as fever and hepatitis is closely related to HHV-6 reactivation. A combination of immunologic reaction to a drug and HHV-6 reactivation results in the severe course of DIHS.

# IL-17 and IL-22 mediate IL-20 subfamily cytokine production in cultured keratinocytes *via* increased IL-22 receptor expression

*Mikiko Tohyama, Yasushi Hanakawa, Yuji Shirakata, Xjuju Dai, Lujun Yang, Satoshi Hirakawa, Sho Tokumaru, Hidenori Okazaki, Koji Sayama and Koji Hashimoto*

Department of Dermatology, Ehime University Graduate School of Medicine, Shitsukawa, Toon-city, Ehime, Japan

IL-20 cytokine subfamily members, including IL-19, IL-20, and IL-24, are highly expressed in psoriatic skin lesions. Here, we demonstrate that psoriasis mediators IL-17 and IL-22 synergistically induce the production of IL-20 subfamily proteins in cultured human keratinocytes. Interestingly, expression of the IL-22 receptor (IL-22R) also increased in epidermal lesions *versus* normal skin. IL-22R over-expression using an adenoviral vector to mimic psoriatic conditions in cultured keratinocytes significantly enhanced IL-17- and IL-22-induced production of IL-20 subfamily cytokines. Furthermore, IL-17 and IL-22 coordinately enhanced MIP-3α, IL-8, and heparin-binding EGF-like growth factor (HB-EGF) production, depending on the amount of IL-22R expression. Additionally, because IL-20 and IL-24 share the IL-22R with IL-22, the function of IL-20 and IL-24 was also increased. IL-20 and IL-24 have effects similar to that of IL-22; IL-24 showed more potent expression than IL-20. A combination of IL-24 and IL-17 increased the production of MIP-3α, IL-8, and HB-EGF, as did a combination of IL-22 and IL-17. These data indicate that increased IL-22R expression in epidermal keratinocytes contributes to the pathogenesis of psoriasis through enhancing the coordinated effects of IL-22 and IL-17, inducing the production of the IL-20 subfamily, chemokines, and growth factors.

Key words: Chemokine · Cytokine · Dermatitis · Epithelial cells · Inflammation

## Introduction

Psoriasis is a common chronic inflammatory skin disease, and typical lesions are well-circumscribed red plaques covered by a silvery white scale. Histologically, hyperkeratosis and epidermal hyperplasia with suprapapillary epidermal thinning are observed, with neutrophilic microabscesses and the disappearance of the granular layer. Vasodilation and infiltration of leukocytes are also seen in papillary dermis.

Epidermal keratinocytes from psoriatic lesions produce abundant chemokines, growth factors, and antimicropeptides, such as human beta-defensin 2 (HBD2) and LL-37 [1–5]. Chemokines produced by epidermal keratinocytes are important in recruiting inflammatory cells to the skin, and the chemokine expression pattern in psoriasis is different from that in atopic dermatitis, another common chronic inflammatory skin disease [3, 4]. In particular, expression of MIP-3α and IL-8 is strongly up-regulated in psoriatic epidermis compared with atopic dermatitis; these chemokines recruit T lymphocytes and neutrophils, respectively. Over-expression of several growth factors, such as heparin-binding EGF-like growth factor (HB-EGF), TGF-α, epiregulin, and amphiregulin, has been reported, and may contribute to the epidermal hyperproliferation in psoriasis [6–9].

Cytokines produced by Th17 cells, including IL-17 and IL-22, are generally accepted to be involved in the development of psoriasis [10]. IL-17 acts directly on keratinocytes and regulates the

Correspondence: Dr. Mikiko Tohyama
e-mail: tohm@m.ehime-u.ac.jp

 

production of MIP-3α, IL-8, and HBD2 [5, 11, 12], whereas IL-22 regulates keratinocyte differentiation [5, 13–15]. In addition, treatment of reconstructed skin with IL-22 induces epidermal hyperproliferation and the disappearance of the granular layer, characteristic features of psoriasis [5, 13, 15]. While the IL-17 receptor is highly and widely expressed in normal human tissue, expression of the receptor for IL-22 is relatively limited. Receptors for IL-22 are the IL-22 receptor (IL-22R) and the IL-10 receptor 2 (IL-10R2) [16]. Although IL-10R2 is expressed in a variety of normal human tissues, including immune cells, IL-22R mRNA is detected only in the pancreas, small intestine, colon, liver, lung, and skin, but not in immune cells [15, 17–20]. These findings indicate that skin is among the limited target tissues of IL-22.

IL-22 belongs to the IL-10 family, which also includes IL-19, IL-20, IL-24, IL-26, IL-28, and IL-29 [21]. Although the amino acid sequences of these cytokines are homologous to that of IL-10, their functions differ. IL-19, IL-20, and IL-24, the so-called IL-20 subfamily, have similar functions to IL-22 [13]. The IL-20 subfamily signals through IL-22R/IL-20R2 and/or the IL-20R1/IL-20R2 complex [22, 23], and the IL-20 subfamily and IL-22 induce STAT3 phosphorylation. Recently, STAT3 phosphorylation in epidermal keratinocytes has been implicated in the development of psoriasis [24]. Thus, the over-expression of IL-19, IL-20, and IL-24 in psoriatic skin lesions [17, 25, 26] may play an important role in the pathogenesis of this disease. The production of IL-20 subfamily in monocytes has been characterized by many groups [19, 27–29]. Furthermore, the regulation of IL-19, IL-20, and IL-24 expression in keratinocytes was also investigated [13, 17, 25]. Recently, the effect of Th17 cytokine in producing these mediators was suggested. In bronchial epithelial cells, IL-17 induces the production of IL-19 [20]. Sa *et al.* demonstrated that IL-22 increased IL-20 mRNA expression in epidermal keratinocytes [13]. Here, we demonstrate that Th17 cytokines induce IL-19, IL-20, and IL-24 production in keratinocytes. This production was especially enhanced by stimulation with IL-17 and IL-22 in combination. Additionally, we found that IL-22R expression was elevated in psoriatic epidermis. When IL-22R expression increased, the response to a combination of IL-17 and IL-22 was strengthened, producing the IL-20 subfamily, and the responses for IL-20 and IL-24 were also augmented *via* IL-22R.

## Results

### IL-19, IL-20, and IL-24 expression in psoriatic epidermis

Previous reports demonstrated that IL-19, IL-20, and IL-24 mRNA expression increased in psoriatic skin lesions compared with normal

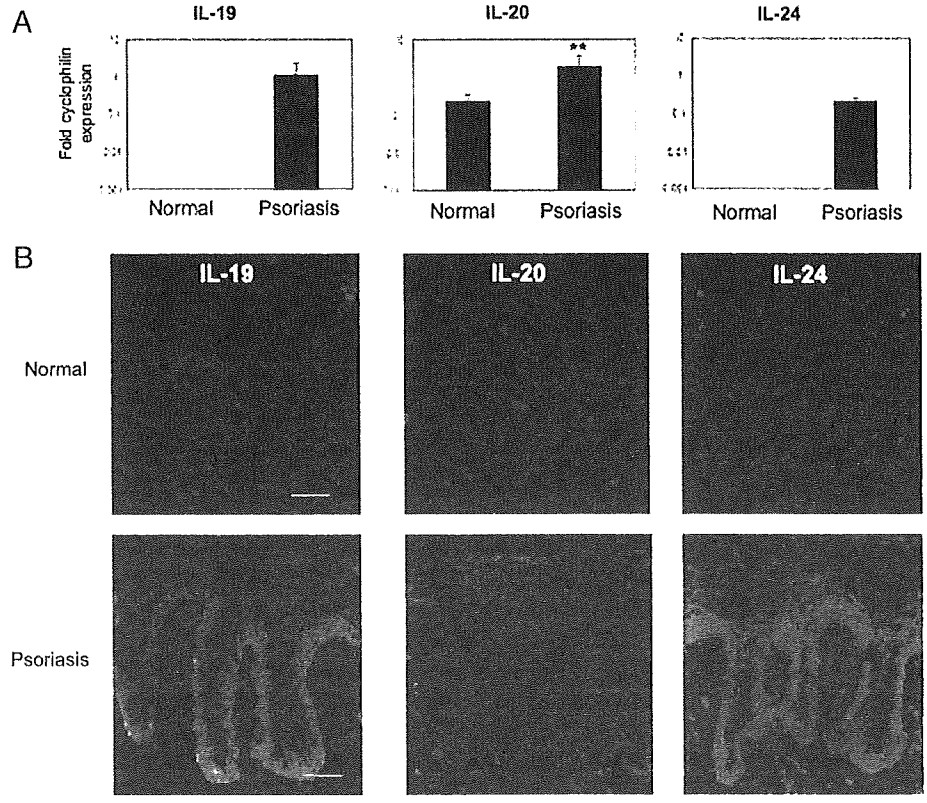


**Figure 1.** IL-19, IL-20, and IL-24 expression in normal and psoriatic epidermis. IL-19, IL-20, and IL-24 expression levels were determined by real-time RT-PCR and immunostaining. (A) Total RNA was extracted from the epidermis of nine normal and 11 psoriatic skin samples. The expression of IL-19, IL-20, and IL-24 was analyzed by real-time RT-PCR, with cyclophilin as house keeping control. Data show mean ± SD. $^{**}p<0.01$. (B) Frozen sections of four normal and six psoriatic skin samples were stained with antibodies against IL-19, IL-20, and IL-24. Scale bar, 50 μm.

 

skin [17, 25, 26]. In these studies, total RNA was extracted from whole skin. However, this method results in the inclusion of RNA from dermal cells (*e.g.* fibroblasts, endothelial cells) and inflammatory cells. Therefore, we extracted total RNA from normal and psoriatic epidermis after first heat-separating the epidermis from the dermis. Heat separation does not influence the stability of RNA [9]. IL-19, IL-20, and IL-24 mRNA expression levels were then analyzed *via* real-time RT-PCR. After 40 PCR cycles, IL-20 mRNA was detected in normal epidermis, whereas IL-19 and IL-24 were not (Fig. 1A). In contrast, psoriatic epidermis showed up-regulated IL-19, IL-20, and IL-24 mRNA expression.

IL-19, IL-20, and IL-24 protein expression was analyzed *via* immunostaining. Normal epidermis expressed IL-19, IL-20, and IL-24 (Fig. 1B). Pretreatment of antibodies with their corresponding recombinant cytokines blocked this reactivity (data not shown). IL-20$^+$ cells were also observed in the upper dermis; they were also stained using macrophage-specific antibodies (data not shown), as described previously [30]. IL-24$^+$ cells in the epidermis were identified as Langerhans cells based on CD1a co-expression (data not shown). In psoriatic skin, IL-19, IL-20, and IL-24 expression increased significantly throughout the entire epidermis. These results suggest that psoriatic keratinocytes show increased IL-20 subfamily protein production.

## IL-17 enhanced IL-19, IL-20, and IL-24 mRNA expression in cultured keratinocytes

We examined the effects of IL-17 on cultured keratinocytes, which express IL-17 receptor mRNA at a level almost equal to that of normal epidermis (data not shown). In cultured keratinocytes, IL-20 and IL-24 mRNA, but not IL-19 mRNA were detected in the steady state. IL-17 treatment induced time-dependent increases in IL-19, IL-20, and IL-24 mRNA expression (Fig. 2).

## IL-22 enhanced IL-19, IL-20, and IL-24 mRNA expression in cultured keratinocytes

To evaluate the effect of IL-22 on IL-20 subfamily production in cultured epidermal keratinocytes, we first examined the expression of receptors for this cytokine. IL-22 receptors include both IL-22R and IL-10R2. The expression of IL-22R, but not of IL-10R2, was suppressed in cultured keratinocytes, as previously reported (Fig. 3A) [13]. Next, we examined whether IL-22R expression level was modulated by the differentiated state of keratinocytes. Keratinocytes were cultured with a high concentration of calcium, a differentiation-inducing factor, and IL-22R mRNA expression was analyzed. However, the IL-22R mRNA expression level was not increased, compared with undifferentiated keratinocytes (data not shown). Thus, we used an adenoviral vector (Ax) carrying IL-22R (AxIL22R) to increase expression in cultured keratinocytes. When cultured keratinocytes were infected with AxIL22R for 48 h, increased IL-22R expression was confirmed by Western blotting (Fig. 3B) and flow cytometry (Fig. 3C). Moreover, we detected IL-22R expression beginning 24 h after adenovirus vector infection, and the infection resulted in the constant expression of IL-22R, depending on the MOI. In keratinocytes infected with AxIL22R, IL-20, IL-24, and IL-22 induced STAT3 phosphorylation at 15 min and 24 h after stimulation (Fig. 3D).

In control keratinocytes, IL-22 enhanced the mRNA expression of IL-20 and IL-24 after 1 h, but did not increase IL-19 expression (Fig. 4). In keratinocytes infected with AxIL22R, IL-22 stimulation also induced a marked increase in IL-20 and IL-24 mRNA expression, which peaked at 1 h for IL-20 and at 3 h for IL-24, and was sustained for 24 h. IL-19 mRNA expression was also detectable between 3 and 12 h after stimulation. The vector control, AxLacZ, was not able to increase IL-20 subfamily expression (data not shown).

## IL-17 and IL-22 coordinately enhanced IL-19, IL-20, and IL-24 production in cultured keratinocytes

The effect of various cytokines on the production of the IL-20 subfamily was examined. Keratinocytes infected with AxIL22R or AxLacZ were treated with IFN-γ, IL-1α, TNF-α, IL-17, or IL-22, which are involved in the pathogenesis of psoriasis. IL-19, IL-20, and IL-24 proteins in culture medium were analyzed by ELISA (Fig. 5A). IL-19 and IL-24 were

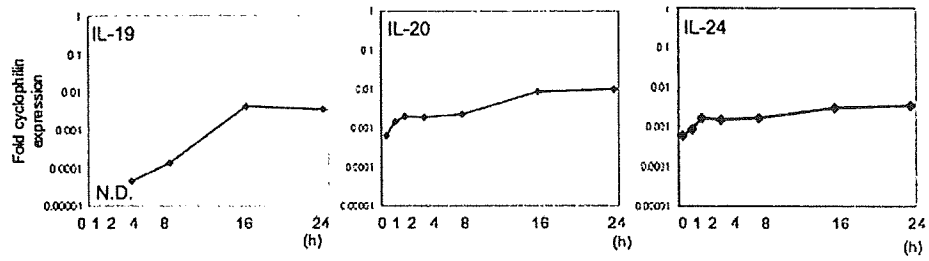


**Figure 2.** IL-19, IL-20, and IL-24 mRNA expression in cultured keratinocytes stimulated with IL-17. IL-17 (10 ng/mL) was added to culture media containing keratinocytes. Total RNA was extracted at various time points up to 24 h after stimulation. IL-19, IL-20, and IL-24 expression levels were determined by real-time RT-PCR, with cyclophilin as house keeping control. Representative data are shown from two independent experiments. N.D. indicates not detectable.

 

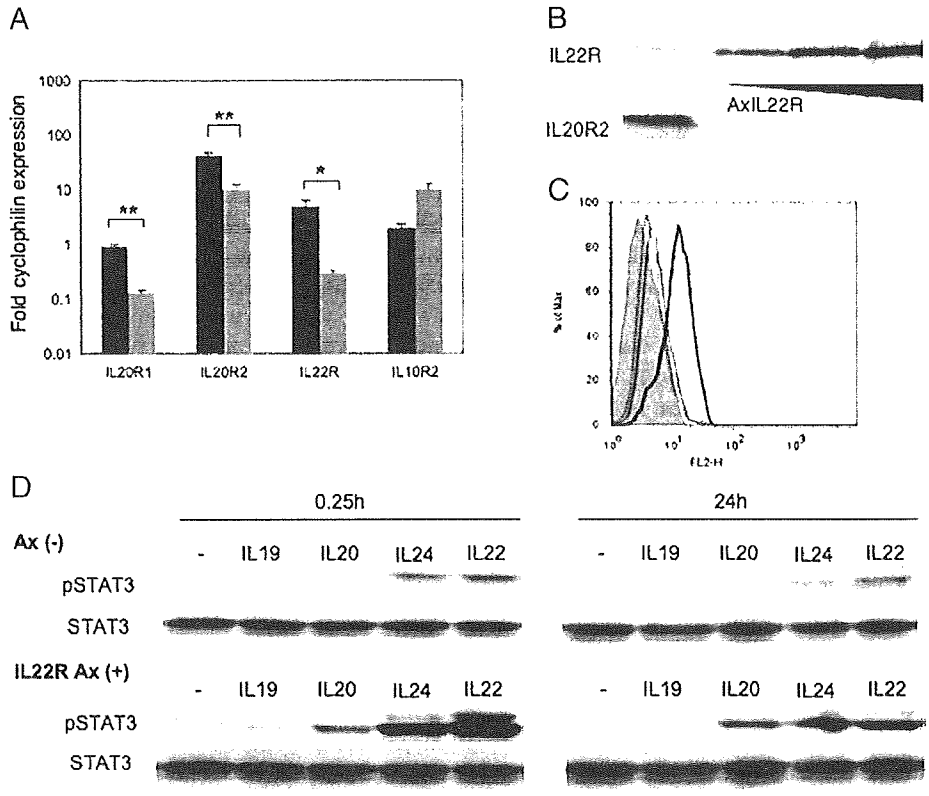


**Figure 3.** IL-20R1, IL-20R2, IL-22R, and IL-10R2 expression in cultured epidermal keratinocytes. (A) Normal epidermis was obtained from nine subjects and cultured keratinocytes were from eight other normal subjects. IL-20R1, IL-20R2, IL-22R, and IL-10R2 expression levels were determined by real-time RT-PCR, with cyclophilin as house keeping control, and compared between normal epidermis (black bar) and cultured keratinocytes (gray bar). Data show mean ± SD. $^{*}p<0.05$; $^{**}p<0.001$. (B) Western blot analysis of IL-20R1, IL-20R2, and IL-22R protein expression in cultured keratinocytes. (C) IL-22R expression levels were analyzed by flow cytometry (isotype control Ab: shaded area; anti-IL-22R Ab: thin line). Infection of AxIL22R at an MOI of 5 for 48 h increased IL-22R expression in keratinocytes (thick line), but not AxLacZ (dotted line). Data are representative of two independent experiments. (D) Uninfected control cultures or keratinocytes infected with AxIL22R were treated with 10 ng/mL IL-19, IL-20, IL-24, and IL-22. STAT-3 phosphorylation at 15 min or 24 h after stimulation was determined by Western blot. Data are representative of three independent experiments.

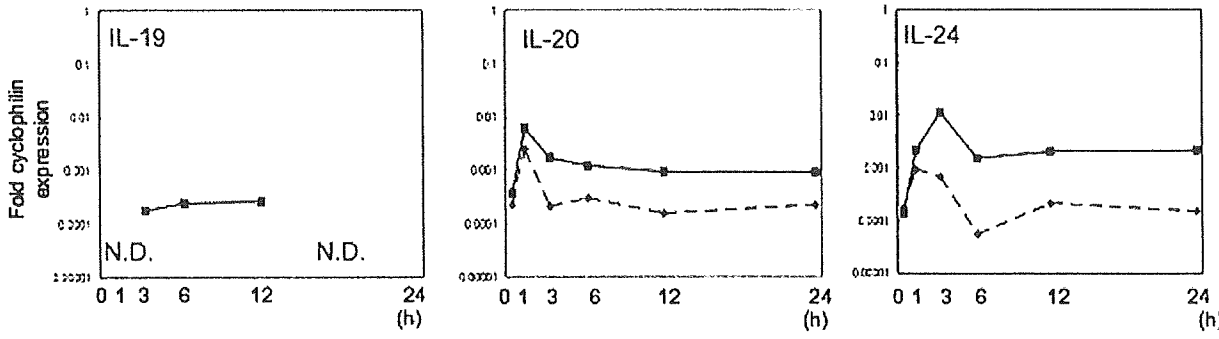


**Figure 4.** IL-19, IL-20, and IL-24 mRNA expression in cultured keratinocytes stimulated with IL-22. IL-22 (10 ng/mL) was added to culture media containing keratinocytes transduced with AxIL22R (black line) or uninfected control keratinocytes (dotted line). Total RNA was extracted at various time points up to 24 h after stimulation. IL-19, IL-20, and IL-24 expression levels were determined by real-time RT-PCR, with cyclophilin as house keeping control. Data are representative of two independent experiments. N.D. indicates not detectable.

not detected in the culture medium of keratinocytes treated with IFN-γ, TNF-α, or IL-1α. On the other hand, IL-20 was detected at low levels in the culture medium without stimulation, and the production was slightly increased by TNF-α and IL-1α treatment. Notably, IL-19, IL-20, and IL-24 were all produced by stimulation with IL-17. In addition,

 

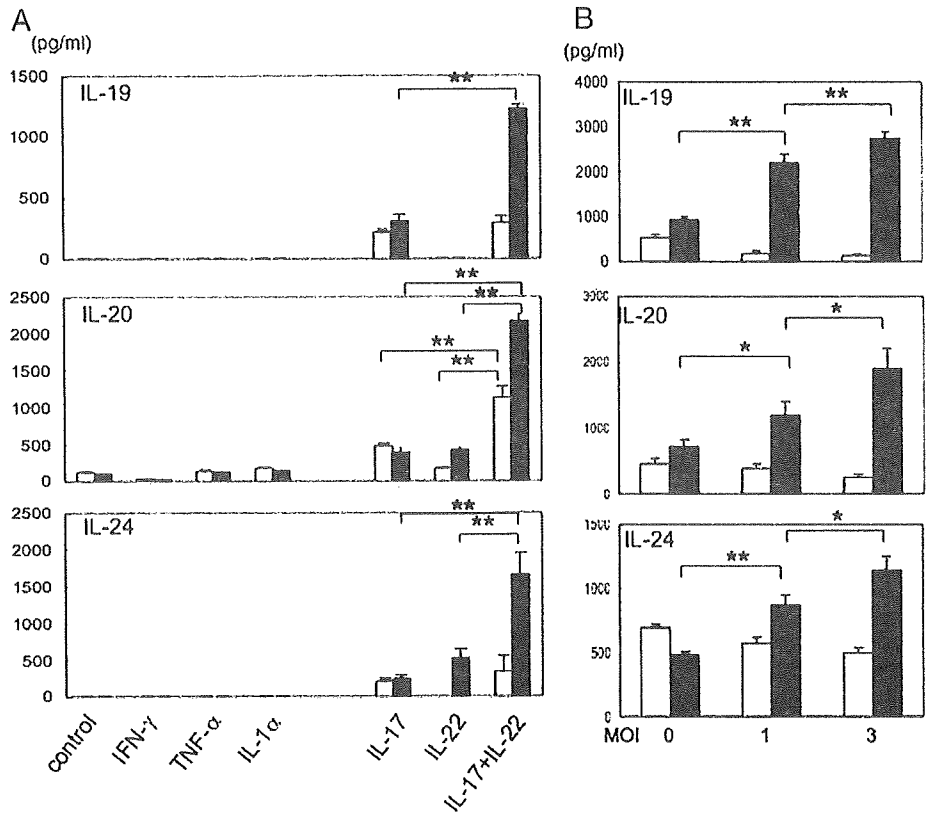


**Figure 5.** IL-19, IL-20, and IL-24 production in cultured keratinocytes. (A) Cultured keratinocytes infected with AxIL22R (black bar) or AxLacZ (white bar) at an MOI of 1 were treated with 50 IU/mL of IFN-γ, 10 ng/mL of IL-1α, TNF-α, IL-17, or IL-22 for 48 h. (B) Cultured keratinocytes were infected with AxIL22R (black bar) or AxLacZ (white bar) at an MOI of 1 or 3 for 24 h. After washing, cells were co-treated with 10 ng/mL IL-17 and 10 ng/mL IL-22 for 48 h. (A, B) IL-19, IL-20, and IL-24 in culture medium were measured by ELISA. Data show mean ± SD ($n = 3$). Data are representative of three independent experiments. $^{*}p<0.05$; $^{**}p<0.01$.

increased IL-22R expression markedly augmented IL-22-stimulated IL-20 and IL-24 production. Moreover, a combination of IL-17 and IL-22 dramatically enhanced production of all of these cytokines (Fig. 5A), which was dependent on IL-22R expression (Fig. 5B).

## Epidermal keratinocytes of psoriasis lesion skin express higher than normal levels of IL-22R

Because IL-20 subfamily production increased in response to IL-22R over-expression, we examined IL-22R expression in psoriatic epidermis. IL-22R mRNA levels increased significantly in psoriatic epidermis compared with normal epidermis, whereas mRNA levels for IL-20R1, IL-20R2, and IL-10R2 were similar to those of normal epidermis (Fig. 6A). Immunostaining revealed only weak IL-22R signals throughout the entire epidermis of normal skin. However, fluorescence staining intensity increased in psoriatic epidermis (Fig. 6B). Significant staining was primarily observed in the upper layer.

## A combination of IL-17 and IL-22 mediated chemokine and growth factor production *via* increased IL-22R

We postulated that increased IL-22R expression might augment signal transduction by IL-22, and strengthen the coordinated effect of IL-22 and IL-17, other than by production of the IL-20 subfamily. Previous reports demonstrated that IL-17 induced HBD2 mRNA expression [5, 12] and that a combination of IL-17 and IL-22 markedly enhanced mRNA expression [31]. In our study, increased IL-22R augmented HBD2 mRNA expression stimulated by IL-17 and IL-22 in combination (Fig. 7A). Similarly, IL-22 treatment induced enhanced MIP-3α and IL-8 mRNA expression in cultured keratinocytes infected with AxIL22R, and the combination of IL-17 and IL-22 strongly enhanced their mRNA expression.

We further analyzed the production of growth factors. HB-EGF mRNA expression was enhanced by IL-22 stimulation in cultured keratinocytes expressing IL-22R, and co-stimulation with IL-17 and IL-22 markedly augmented the mRNA expression (Fig. 7A). In contrast, although TGF-α mRNA expression was also enhanced by IL-22 treatment, no combination effect was observed.

 