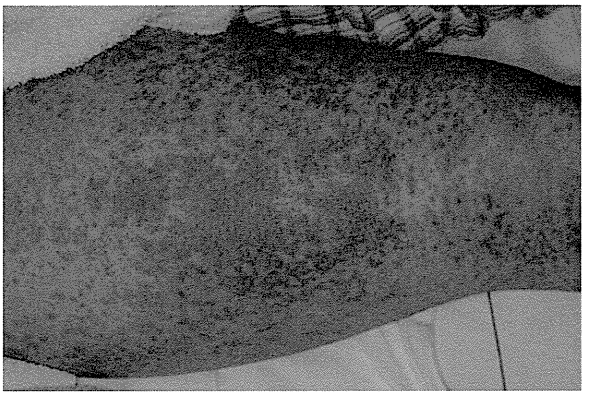

FIGURE 11.2 Purpuric rash in the thigh.

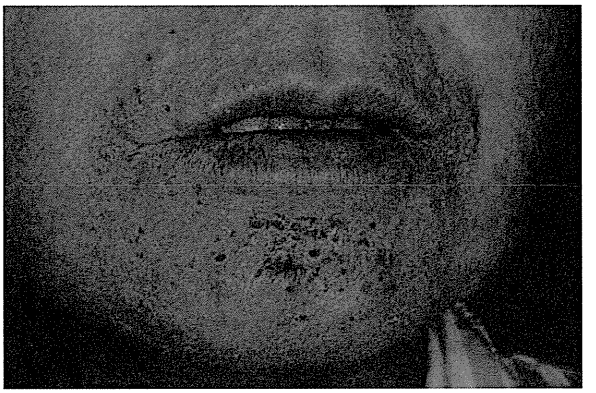

FIGURE 11.3 Perioral-infiltrated red papules and crusting.

renal failure, and dialysis may be required. Pulmonary involvement in the initial phase of DIHS/DRESS is rare, but interstitial pneumonia can occur.

Hematological abnormalities include leukocytosis, eosinophilia, and/or the appearance of atypical lymphocytes. Leukocytosis may appear after discontinuation of the causative drug and may be further aggravated for 1 to 2 weeks. Eosinophilia develops in 50 to 70% of DIHS/DRESS patients.[20,23] Although the appearance of atypical lymphocytes is commonly observed in cases of viral infection (e.g., EBV, cytomegalovirus, measles virus), viruses are not usually detected during this stage of DIHS/DRESS.[24] HHV-6 usually reactivates later.

It is conceivable that a drug allergy is responsible for these initial symptoms; therefore, clinical symptoms may vary according to the causative drug. For example, although hepatitis is common in DIHS/DRESS, renal involvement frequently occurs in allopurinol-induced DIHS/DRESS without liver

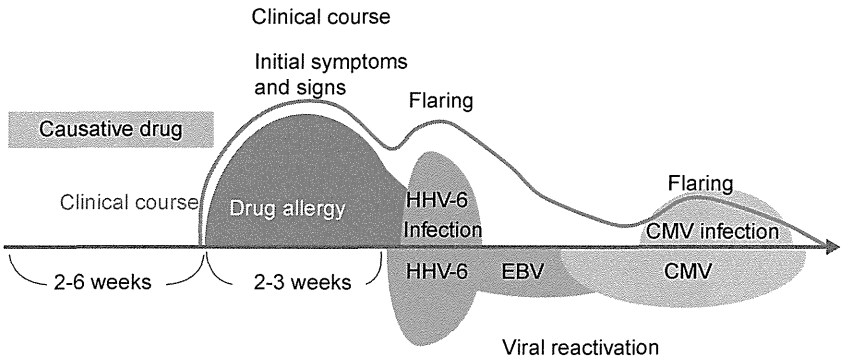

FIGURE 11.4 Relationship between clinical symptoms and viral reactivation.

involvement.[20,25] In dapson-induced DIHS/DRESS, significant hyperbilirubinemia and jaundice are observed at a high frequency, as compared to other types of drug-induced DIHS/DRESS.[26] Pulmonary involvement is often observed among patients who have minocycline-induced DIHS/DRESS.[25]

Flare-Ups and the Development of New Symptoms

In severe cases, flare-ups involving clinical signs such as fever, eruption, or hepatitis often occur after improvement of the patient's initial symptoms and more than 2 weeks after the onset of DIHS/DRESS (Figure 11.4). Recent reports have demonstrated that reactivated viruses participate in the flaring of symptoms as described in the Viral Reactivation section, below. Additionally, new symptoms may rarely develop during the course of DIHS/DRESS, such as central nervous system (CNS) disorders, myocarditis, hemophagocytic syndrome, gastrointestinal disorders, and interstitial pneumonia. Recently, it was noted that fulminant type 1 diabetes is associated with DIHS. The involvement of viruses, including HHV-6 or cytomegalovirus (CMV), has been suggested in some of these cases.

HISTOPATHOLOGY

Skin

A skin biopsy will show dense dermal infiltration of lymphocytes, as compared with an ordinary drug rash. Lymphocytes are mainly observed around dermal vessels with or without eosinophils. No epidermal change or exocytosis of a few lymphocytes with mild spongiosis is usually observed (Figure 11.5). However, apoptotic keratinocytes and vacuolar changes in basal cells are frequently observed in erythema multiforme-like eruptions.[19] When the skin manifestation shows the features of SJS/TEN, apoptotic keratinocytes and epidermal necrosis with exocytosis of lymphocytes are frequently observed (Figure 11.6).

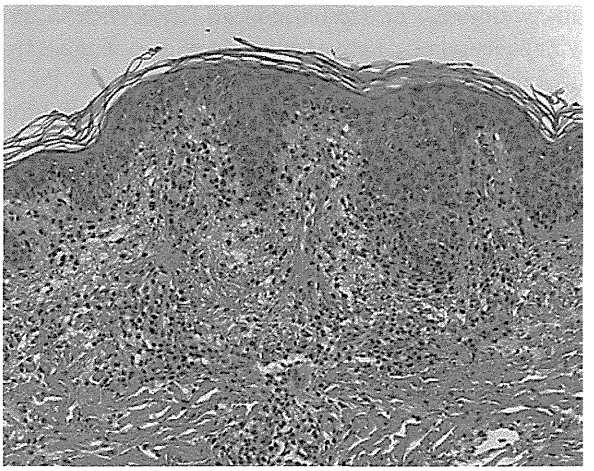

FIGURE 11.5 Histological finding of skin section from maculopapular rash.

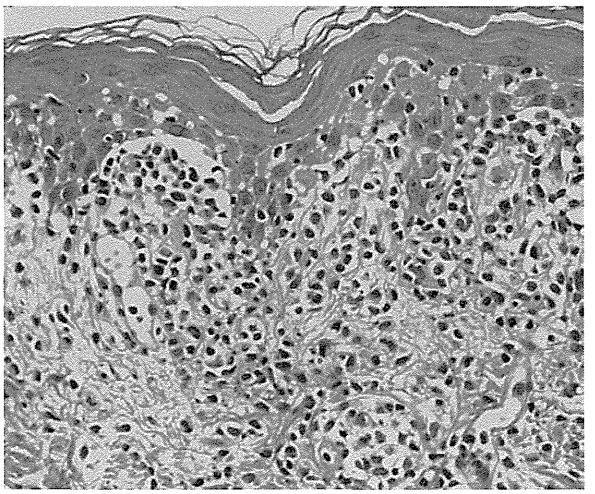

FIGURE 11.6 Histological finding of SJS-like skin lesion.

Lymph Nodes

Histopathological findings of the lymph nodes include benign lymphoid hyperplasia or features mimicking lymphoma.[1] A histological pattern resembling malignant lymphoma has been observed, mainly in cases of anticonvulsant-induced DIHS/DERSS. In these cases, the lymph nodes show hyperplasia of the reticulum cells and other elements with frequent mitoses, focal necrosis, and phagocytosis, but no Reed–Sternberg cells.[1] However, in some cases of

anticonvulsant-, antibiotic-, or dapson-induced DIHS/DRESS, the histological findings of lymphadenopathy were compatible with malignant lymphoma.[27–29] It may be difficult to assess whether lymphadenopathy is the result of drug hypersensitivity by histological examination without clinical information.

Liver

Liver biopsy shows a hepatocellular pattern or mixed hepatocellular and cholestatic pattern that is commonly observed in drug-induced hepatitis. In severe hepatitis, massive necrosis or multiple disseminated necrotic foci can occur, resulting in liver failure.[1]

VIRAL REACTIVATION

HHV-6

HHV-6 is commonly reactivated 2 to 3 weeks after the onset of illness in most DIHS patients (see Figure 11.4).[24] The criteria for DIHS proposed by the Japanese Research Committee on Severe Cutaneous Adverse Reactions include the detection of HHV-6 reactivation.[2] On the other hand, in DRESS, the HHV-6 detection rate is lower than that in DIHS.[8] This difference may be explained by the differing ranges of severity between the two diseases: DIHS is regarded as a more severe form of DRESS.[2,8] A certain level of severity in patients with a drug allergy may be required for HHV-6 reactivation. HHV-6 can be detected in serum for 3 to 7 days by polymerase chain reaction (PCR) or viral culture, accompanied by viral replication in peripheral blood.[11,24] When HHV-6 DNA disappears from the serum, a significant increase in anti-HHV-6 IgG titers occurs in DIHS/DRESS (Figure 11.7).[24] A rise in anti-HHV-6 IgM is not always observed. HHV-6 DNA is detectable by PCR for a longer time in peripheral blood samples than in serum samples, even if the anti-HHV-6 IgG titer has already increased. HHV-6 reactivation sometimes

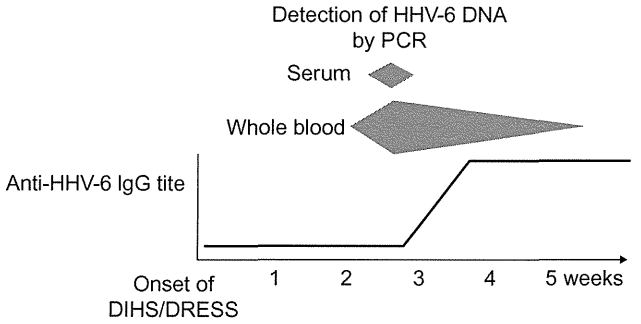

FIGURE 11.7 Detection of HHV-6 DNA and a rise of anti-HHV-6 IgG titers.

accompanies the flaring of symptoms such as fever and hepatitis.[24] The level of viral replication may influence the flaring of symptoms. Body temperature is increased during HHV-6 viremia but decreases with clearance of HHV-6 from serum. Hepatitis also relapses with HHV-6 reactivation to various degrees and is improved after the clearance of HHV-6. Thus, because HHV-6 reactivation and infection are usually self-limited in DIHS/DRESS, antiviral therapy is not required. However, the reactivation of HHV-6 can rarely cause a severe illness such as encephalitis.

Other Herpesviruses

Herpesviruses other than HHV-6, including CMV, EBV, and human herpesvirus-7 (HHV-7), may also be reactivated during DIHS/DRESS.[5,6,30] The cascade of viral reactivation initiated by HHV-6 extends to EBV and CMV (see Figure 11.7).[5,21,30] It is of interest that reactivation of these viruses has been observed in the same sequential manner as in a bone marrow transplant patient.[5] HHV-7 is detected at various periods during DIHS/DRESS[21,31] and co-reactivates with HHV-6.[30,32] In DIHS/DRESS, the reactivation of EBV and HHV-7 is thought to have no clinical relevance.

In contrast, CMV contributes to internal organ involvement and the relapse of symptoms. CMV reactivates more than 1 month after the onset of DIHS/DRESS and causes various diseases such as recurring transient fever, a skin rash, skin ulcers, hepatitis, pneumonia, and gastrointestinal bleeding.[33,34] Moreover, CMV infection might participate in the development of myocarditis or fulminant type 1 diabetes.[35,36] The detection of CMV DNA in blood has no clinical importance, as it does not indicate the existence of infectious disease. On the other hand, the detection of CMV antigenemia is useful for assessing the risk of CMV infection. In addition, positive staining for CMV antigens by immunohistochemistry can be used to make a diagnosis. Diseases caused by CMV infection such as pneumonia and gastrointestinal bleeding may lead to a fatal outcome in DIHS/DRESS.[34] Immune suppression induced by systemic corticosteroids may influence the severity of a CMV infection, although CMV can reactivate in DIHS/DRESS without systemic corticosteroids.[33] It is necessary to assess the pathological condition of a patient carefully to manage CMV infections.

VISCERAL INVOLVEMENTS AND SEQUELAE OF DIHS/DRESS

Visceral involvement may occur throughout the clinical course of DIHS/DRESS. The cause of multiorgan involvement remains unknown,[8,10,37] and these complications develop unpredictably (Table 11.2). The severity of DIHS/DRESS is commonly determined by the degree of visceral involvement. Furthermore, sequelae may occur after a disease-free interval of several months to years after resolution of the acute illness.[37]

TABLE 11.2 Visceral Involvements During the Course of DIHS/DRESS

Enterocolitis/intestinal bleeding
Fulminant type 1 diabetes mellitus
Hemophagocytic syndrome
Hepatitis/cholangitis
Limbic encephalitis/encephalopathy
Myocarditis
Nephritis
Pneumonitis/pleuritis

Visceral Involvement During the Course of DIHS/DRESS

Hepatobiliary System

Apart from the skin, the liver is the most common organ involved in DIHS/DRESS.[8] Hepatomegaly accompanied by splenomegaly is frequently observed. The presence of an underlying persistent viral infection, such as hepatitis B or hepatitis C infection, often causes a severe deterioration in liver function.[37] Although the large majority of patients recover spontaneously, massive hepatic necrosis in the setting of coagulopathy and sepsis can cause death. Prolonged prothrombin times and/or partial thromboplastin times are observed in severe cases.[38] Hepatic impairment is commonly anicteric, but if it is icteric it tends to have a poorer prognosis. The flaring of symptoms such as fever and liver dysfunction in DIHS/DRESS is closely related to HHV-6 reactivation.[24]

Renal System

Renal involvement occurs in 8 to 11% of patients with DIHS/DRESS.[8,39] Accumulated evidence from case reports suggests that severe renal insufficiency increases the risk of mortality, and mortality depends in part on the degree of renal involvement rather than hepatic involvement.[10] Renal involvement is prominent in allopurinol-induced DIHS/DRESS.[40] It is more likely to occur in patients with preexisting renal disease or those receiving diuretic therapy. Because renal function declines steadily with age, elderly patients are vulnerable to renal complications. Laboratory studies demonstrate worsening renal insufficiency, ranging from a mild elevation in serum creatinine levels to severe interstitial nephritis.[41] Granulomatous interstitial nephritis can be also noted in DIHS/DRESS.[42] Proteinuria and oliguria may also be present. No specific deposits have been detected in immunofluorescence studies of the biopsy specimens from the kidney.

Respiratory System

The prevalence of lung involvement in DIHS/DRESS is unclear because cases with less severe pulmonary involvement have been underreported, leading to

a reporting bias with only severe cases being reported.[10] Pulmonary complications include abnormal pulmonary function, acute interstitial pneumonitis, lymphocytic interstitial pneumonia, Loeffler's syndrome, and acute respiratory distress syndrome (ARDS). Clinical symptoms such as a nonproductive cough and breathlessness are highly suggestive of lung involvement. Pleuritis or pleural effusions may also occur during the course of DIHS/DRESS. Apart from the life-threatening condition of ARDS,[43] most pulmonary complications resolve without sequelae. Pulmonary infections have been documented during the course of DIHS/DRESS. Infectious pneumonia caused by *Pneumocystis jiroveci* can occasionally develop during and after resolution of DIHS/DRESS.[18]

Cardiovascular System

Cardiac involvement is a rare but life-threatening complication of DIHS/DRESS. Myocarditis associated with DIHS/DRESS is observed around 3 weeks to 4 months after onset.[44,45] Symptoms/signs suggestive of myocarditis include chest pain, unexplained tachycardia, breathlessness, low blood pressure, and symptoms of congestive heart failure, but some patients may be asymptomatic. Chest radiographs may show cardiomegaly and pleural effusions, and the electrocardiogram usually shows nonspecific ST–T wave changes or ST segment elevations, sinus tachycardia, or arrhythmias. The echocardiogram may show a significant reduction in ejection fraction;[46] cardiac catheterization reveals normal coronary arteries and a decreased ejection fraction. The appearance of the above-mentioned cardiac findings in the setting of DIHS/DRESS should raise the suspicion of drug-induced myocarditis as a complication of DIHS/DRESS. The condition may resolve or progress rapidly into fulminant heart failure.[44,45] Endomyocardial biopsy—a highly invasive, seldom performed procedure—demonstrated a mixed infiltrate of lymphocytes and eosinophils with myocyte necrosis in several cases of DIHS/DRESS.[44,46] Dilated cardiomyopathy may develop as a late sequel of DIHS/DRESS.[47] Complete atrioventricular block associated with dapsone-induced DIHS/DRESS has been reported.[48]

Central Nervous System

Neurological complications such as meningitis and encephalitis in DIHS/DRESS have been documented.[49–52] Limbic encephalitis may develop approximately 2 to 4 weeks after the onset of DIHS/DRESS; the condition is suspected if there is fever accompanied by headache, altered level of consciousness, and signs of cerebral dysfunction. Focal neurological abnormalities such as anomia, dysphagia, and hemiparesis and seizure may be present. Cerebrospinal fluid (CSF) reveals an increase in the white blood cell count and protein level. Magnetic resonance imaging (MRI) has shown bilateral lesions involving the amygdala, mesial temporal lobes, insula, and cingulate gyrus.[40] The typical MRI appearance is selective involvement of the limbic system, particularly the

mesial temporal lobes. An electroencephalogram shows diffuse slow waves, with an occasional solitary spike, in the frontal and temporal leads without periodic patterns. Considering the onset of limbic encephalitis and timing of HHV-6 reactivation in DIHS/DRESS, it is possible that limbic encephalitis might be due to HHV-6 reactivation. In a patient with allopurinol-induced DIHS/DRESS who developed encephalitis, an increase in anti-HHV-6 IgG titers and a detection of HHV-6 DNA by polymerase chain reaction in the CSF were noted.[50] However, viral DNA cannot be detectable in CSF samples from DIHS/DRESS patients after the onset of encephalitis, which may indicate secondary encephalitis or inappropriate timing of sample collection.[52] A case of a patient with DIHS/DRESS complicated by the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) and concomitant limbic encephalitis has been documented.[52]

Gastrointestinal System

Acute, life-threatening gastrointestinal bleeding may occur during the course of DIHS/DRESS and is caused by CMV infection.[33] Despite the initial belief that only HHV-6 reactivation occur in DIHS/DRESS, other herpesviruses such as EBV, HHV-7, and CMV may also reactivate during the course of the disease.[30] Gastrointestinal ulcers due to CMV infection may be misdiagnosed as steroid-induced ulcers if CMV is not considered in the differential diagnosis. Endoscopic examination reveals arterial bleeding from "punched out" gastric ulceration.[33] Gastrointestinal ulceration is often concomitant with cutaneous CMV ulcers on the shoulders and upper trunk. Histopathological examination of the biopsy specimens from the gastric mucosa and the skin shows cytomegalic cells with characteristic "owl's eye" intranuclear inclusions in the infiltrating cells. CMV infection is usually confirmed by immunohistochemical analysis using an anti-CMV monoclonal antibody.[33] Autopsy findings of a patient with severe DIHS/DRESS revealed disseminated CMV infection involving the lung, myocardium, kidney, adrenal gland, liver, pancreas, spleen, and skin.[53]

Cytomegalovirus reactivation occurs in a predictable manner during DIHS/DRESS. In most patients, CMV DNA is detected 4 to 7 weeks after the onset of DIHS/DRESS and approximately 10 days to 3 weeks after HHV-6 reactivation.[33] Scratch dermatitis and erythematous rash on the trunk, unexplained low-grade fever, and lumbar pain during the 4- to 7-week period after the onset of DIHS/DRESS are suggestive of CMV disease. A reduction in both the platelet and white blood cell counts and a decreased serum immunoglobulin level are also useful indicators of CMV reactivation. The basis for CMV reactivation is unclear, but it appears that advanced age, particularly among those older than 60 years, and patients with antecedent high HHV-6 DNA loads are at risk for overt CMV disease.[33]

Pancreatic System

DIHS/DRESS has been associated with the development of fulminant type 1 diabetes mellitus (FT1DM).[54–56] FT1DM is a subtype of type 1 diabetes

mellitus (T1DM) characterized by abrupt onset, absence of islet-related autoantibodies, and the near complete destruction of pancreatic β-cells. The initial symptoms of T1DM are vomiting and dull epigastric pain. Laboratory findings demonstrate hyperglycemia, hyperosmolarity, and metabolic acidosis, findings that are compatible with diabetic ketoacidosis. In addition, elevations in lipase and amylase consistent with acute pancreatitis have been documented at the onset of T1DM.[55]

According to a nationwide survey performed in Japan, 15 cases of FT1DM associated with DIHS/DRESS were documented between 1985 and 2010. In these cases, the mean age of FT1DM onset was 53.4 years, and the interval between the onset of DIHS/DRESS and the development of FT1DM was 39.9 days (range, 13 to 199 days). The prevalence of FT1DM in DIHS/DRESS was much greater than that found in the general Japanese population.[36] The clinical characteristics of FT1DM associated with DIHS/DRESS were similar to those unrelated to DIHS/DRESS. The etiology of FT1DM associated with DIHS/DRESS remains unknown; however, the prevalence of HLA-B62 was significantly higher in Japanese patients with FT1DM associated with DIHS/DRESS. It is likely that a genetic susceptibility may contribute to the development of FT1DM.[36] Furthermore, it seems that herpesvirus reactivation, such as HHV-6 and CMV, may contribute to the development of FT1DM in patients with DIHS/DRESS. In contrast to FT1DM, autoimmune T1DM is uncommon in patients with DIHS/DRESS. Insulin therapy should be commenced after the diagnosis of T1DM. The consequence of a missed diagnosis can be fatal.

Other Organs

Hemophagocytic syndrome (HPS) is usually associated with, and triggered by, various conditions such as viral infections (particularly EBV-related disorders), malignant tumors, or autoimmune diseases. Although rare, HPS has been noted during the course of DIHS/DRESS.[57] Reactivation of HHV-6 or EBV might be responsible for the development of HPS in DIHS/DRESS. Herpes zoster (HZ) can develop during the first 6 months after the onset of DIHS/DRESS, with HZ lesions appearing during tapering of oral corticosteroids. HZ lesions are prone to be detected on the trunk in patients with DIHS/DRESS; the cutaneous manifestations of HZ is usually mild with an uncomplicated course.[18] Telogen effluvium may develop in the absence of autoantibodies after complete recovery of DIHS/DRESS.[58]

Sequelae of DIHS/DRESS

Autoimmune sequelae can arise after resolution of DIHS/DRESS following a symptom-free interval of several months to years, with the development of autoimmune disease and/or production of autoantibodies. The link between DIHS/DRESS and autoimmune disease might be overlooked unless dermatologists perform long-term follow-up of DIHS/DRESS patients after

their complete recovery.[37] These autoimmune diseases include autoimmune T1DM,[59,60] autoimmune thyroiditis,[61,62] sclerodermoid graft-versus-host disease (GVHD)-like lesions,[61] lupus erythematosus,[63] and autoimmune polyglandular syndrome.[64] Several autoimmune diseases can develop sequentially in a single patient.[61] These cases suggest that DIHS/DRESS may trigger the development of autoimmune disease. Interestingly, some of these autoimmune diseases are similar to those seen after bone marrow transplantation.

The development of autoimmune T1DM is uncommon in DIHS/DRESS. Various autoantibodies including anti-glutamic acid decarboxylase (GAD) and anti-islet cell antibodies are present in autoimmune T1DM. The coexistence of autoimmune T1DM and autoimmune thyroiditis has been observed in a patient with DIHS/DRESS. In this case, various autoantibodies, including anti-GAD, anti-thyroid peroxidase, anti-thyroglobulin, anti-nuclear, and anti-SSA, were detected.[59]

Graves' disease, characterized by anti-thyroid stimulating hormone receptor autoantibodies, may develop after the resolution of DIHS/DRESS.[59] The interval between discontinuation of the causative drug and onset of Graves' disease is approximately 2 to 4 months. Painless thyroiditis and Hashimoto's thyroiditis—characterized by anti-thyroid peroxidase and anti-thyroglobulin antibodies—may also develop after resolution of DIHS/DRESS. Anti-thyroid antibodies have been detected in asymptomatic patients without functional alteration of the thyroid gland following the resolution of DIHS/DRESS.[18] Rarely, sclerodermoid GVHD-like lesions may appear 3 to 4 years after DIHS/DRESS following the development of autoimmune thyroiditis.[61] Lupus erythematosus can also develop in DIHS/DRESS.[62]

DIFFERENTIAL DIAGNOSIS

Differentiating DIHS/DRESS from viral eruptions can be challenging, as DIHS/DRESS can resemble viral infections (Table 11.3). Thus, careful history taking, including examination of drug history, and a thorough physical examination are important for making the correct diagnosis. The diagnosis of DIHS/DRESS is usually not difficult if there is a long period of drug intake (e.g., anticonvulsants). Many patients have been initially misdiagnosed with a viral

TABLE 11.3 Differential Diagnosis

Atopic dermatitis
Infectious mononucleosis
Kawasaki disease
Measles
Pseudolymphoma
Serum sickness-like reaction

illness, such as infectious mononucleosis or measles; however, viral infections may be identified by the lack of eosinophilia and/or hypoimmunoglobulinemia in the laboratory findings. Infectious mononucleosis develops most commonly in teenagers or young adults, while DIHS/DRESS occurs in middle-aged and elderly individuals with a long period of administration of specific drugs. Kawasaki disease can be excluded by using the established diagnostic criteria and laboratory testing. The serum sickness-like reaction is characterized by the presence of urticarial lesions and the absence of internal organ involvement. The clinical manifestations of DIHS/DRESS can be indistinguishable from atopic erythroderma with bacterial infection, but hepatic and/or renal involvement is not characteristic of this condition. In a substantial number of patients with DIHS/DRESS, small pustules on the face and trunk are observed at an early stage that may resemble acute generalized exanthematous pustulosis (AGEP); however, duration of drug intake is less than a week and internal organ involvement is uncommon in AGEP. Pseudolymphoma needs to be differentiated because lymphadenopathy is frequently observed at an early stage of DIHS/DRESS. Pseudolymphomas have been reported to develop in association with drugs such as phenytoin and carbamazepine.[65] A diagnosis of drug-induced pseudolymphoma is usually based on histologic findings and clinical presentation, ranging from solitary nodules to multiple infiltrative papules or plaques without evidence of extracutaneous lymphoma and resolution of the skin eruption after drug cessation.

To identify the drug responsible for the development of DIHS/DRESS, *in vivo* and *in vitro* testing such as patch tests and lymphocyte transformation tests (LTTs) are available. Positive LTT reactions are obtained if more than 4 weeks have elapsed after the onset of DIHS/DRESS, and strong positive reactions may be seen even a year after discontinuation of the causative drug.[66]

MANAGEMENT AND TREATMENT

Early recognition of clinical symptoms associated with DIHS/DRESS is essential in improving patient outcomes. Although the management of DIHS/DRESS is not well established, prompt cessation of the culprit drug is necessary. Because a dramatic deterioration of clinical symptoms, such as fever, skin rashes, and facial edema, often develops 3 to 4 days after withdrawal of the causative drug, a suspicion of underlying infection may be raised. However, empirical treatment with antibiotics or nonsteroidal anti-inflammatory drugs should be avoided as these agents often worsen the early stage of DIHS/DRESS.[67] In contrast to toxic epidermal necrolysis, factors that predict the outcome of DIHS/DRESS have not been identified; however, advanced age, allopurinol- or minocycline-induced DIHS/DRESS, and the presence of comorbid conditions appear to be poor prognostic factors.[8,25]

Patients with moderate disease often recover within 3 to 4 weeks if given supportive care without systemic corticosteroids.[18] Oral corticosteroids are the

mainstay of treatment, despite the absence of randomized control trials confirming its efficacy. Given that herpesvirus reactivation is implicated in DIHS/DRESS, the corticosteroid dose should be given carefully. The usual prednisolone dosage is 0.5 to 1.0 mg/kg/day. After commencement of oral corticosteroids, fever and skin rashes usually subside within a week. It is necessary to taper the dose down gradually to avoid the re-emergence of symptoms. The mean duration of corticosteroid treatment is 6 to 8 weeks.[67]

The risk of CMV reactivation is significantly higher in DIHS/DRESS patients under corticosteroid treatment than those managed without corticosteroid therapy. Thus, physicians should be vigilant for signs of CMV disease in these patients. Given the high mortality rate associated with gastrointestinal CMV ulceration in patients with DIHS/DRESS, early intervention with emergency endoscopic clipping and blood transfusion is usually required. If CMV antigenemia is present during the course of DIHS/DRESS, administration of ganciclovir is recommended to continue until CMV antigenemia is absent in order to avoid CMV disease progression.

Various infections have been noted in patients undergoing corticosteroid treatment in the acute early phase of DIHS/DRESS, including herpesvirus disease and *Pneumocystis jiroveci* pneumonia. Infections are frequent and most appear within 3 months of oral corticosteroid therapy. For this reason, a minimum of 3 months of follow-up is recommended after resolution of DIHS/DRESS.[18]

The development of infections in DIHS/DRESS during corticosteroid treatment may be analogous to the pathomechanism of immune reconstitution inflammatory syndrome (IRIS).[67,68] Infections in the early stage of DIHS/DRESS such as herpes zoster, CMV disease, and *Pneumocystis jiroveci* pneumonia are similar to the illnesses in acquired immune deficiency syndrome patients with IRIS after highly active antiretroviral therapy.[67]

It has been shown that intravenous immunoglobulin (IVIG) compensates for the decreased immunoglobulin concentration, provides anti-inflammatory effects, and regulates the immune response in autoimmune diseases. Beneficial effects of IVIG treatment have been observed in a patient with DIHS/DRESS;[69] however, a separate report documented severe adverse events resulting from IVIG that required systemic corticosteroid therapy.[70]

CONCLUSION

DIHS and DRESS show a wide range of clinical manifestations and laboratory findings. The association between severe visceral involvement and herpesvirus reactivation has been clarified, and the pathomechanisms have been partially elucidated. A better understanding of the interplay between drug allergy and viral reactivations will allow more efficient management of this disease. Furthermore, the immunological aspects of DIHS/DRESS may serve as excellent tools for investigating the autoimmune diseases after viral infections.

ACKNOWLEDGMENT

This work was supported by the Research Project on Measures for Intractable Diseases; a matching fund subsidy from the Ministry of Health, Labour and Welfare (to M.T. and T.S.); and by the Ministry of Education, Culture, Sports, Science and Technology (to M.T., T.S., and Y.K.).

REFERENCES

1. 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* 1996;**15**:250–7.
2. Shiohara T, Iijima M, Ikezawa Z, et al. The diagnosis of a DRESS syndrome has been sufficiently established on the basis of typical clinical features and viral reactivations. *Br J Dermatol* 2007;**156**:1083–4.
3. Kardaun SH, Sidoroff A, Valeyrie-Allanore L, et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist?. *Br J Dermatol* 2007;**156**:609–11.
4. Ushigome Y, Kano Y, Hirahara K, et al. Human herpesvirus 6 reactivation in drug-induced hypersensitivity syndrome and DRESS validation score. *Am J Med* 2012;**125**:e9–10.
5. Kano Y, Hiraharas K, Sakuma K, et al. 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**:301–6.
6. Picard D, Janela B, Descamps V, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS): a multiorgan antiviral T cell response. *Sci Transl Med* 2010;**2**:46–62.
7. Shear NH, Spielberg SP. Anticonvulsant hypersensitivity syndrome. *In vitro* assessment of risk. *J Clin Invest* 1988;**82**:1826–32.
8. Cacoub P, Musette P, Descamps V, et al. The DRESS syndrome: a literature review. *Am J Med* 2011;**124**:588–97.
9. Aihara M. Pharmacogenetics of cutaneous adverse drug reactions. *J Dermatol* 2011;**38**:246–54.
10. Shiohara T, Kano Y, Takahashi R, Mizukawa Y. Drug-induced hypersensitivity syndrome: recent advances in the diagnosis, pathogenesis and management. *Chem Immunol Allergy* 2012;**97**:122–38.
11. 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**:1113–7.
12. Descamps V, Valance A, Edlinger C, et al. Association of human herpesvirus 6 infection with drug reaction with eosinophilia and systemic symptoms. *Arch Dermatol* 2001;**137**:301–4.
13. Shiohara T, Kano Y. A complex interaction between drug allergy and viral infection. *Clin Rev Allergy Immunol* 2007;**33**:124–33.
14. Mardivirin L, Descamps V, Lacroix A, et al. Early effects of drugs responsible for DRESS on HHV-6 replication *in vitro*. *J Clin Virol* 2009;**46**:300–2.
15. Pompeu YA, Stewart JD, Mallal S, et al. The structural basis of HLA-associated drug hypersensitivity syndromes. *Immunol Rev* 2012;**250**:158–66.
16. Takahashi R, Kano Y, Yamazaki Y, et al. Defective regulatory T cells in patients with severe drug eruptions: timing of the dysfunction is associated with the pathological phenotype and outcome. *J Immunol* 2009;**182**:8071–9.

17. Descamps V, Avenel-Audran M, Valeyrie-Allanore L, et al. Saliva polymerase chain reaction assay for detection and follow-up of herpesvirus reactivation in patients with drug reaction with eosinophilia and systemic symptoms (DRESS). *JAMA Dermatol* 2013;**149**(5):565–9.
18. Ushigome Y, Kano Y, Ishida T, Hirahara K, Shiohara T. Short- and long-term outcomes of 34 patients with drug-induced hypersensitivity syndrome in a single institution. *J Am Acad Dermatol* 2013;**68**(5):721–8.
19. Walsh S, Diaz-Cano S, Higgins E, et al. Drug reaction with eosinophilia and systemic symptoms: is cutaneous phenotype a prognostic marker for outcome? A review of clinicopathological features of 27 cases. *Br J Dermatol* 2013;**168**:391–401.
20. Chen YC, Chiu HC, Chu CY. Drug reaction with eosinophilia and systemic symptoms: a retrospective study of 60 cases. *Arch Dermatol* 2010;**146**:1373–9.
21. Tohyama M, Hashimoto K. New aspects of drug-induced hypersensitivity syndrome. *J Dermatol* 2011;**38**:222–8.
22. Hashimoto K, Tohyama M, Shiohara T. Drug reactions with eosinophilia and systemic symptoms(DIHS/DRESS). In: Revuz J, Roujeau JC, Kerdel FA, Valeyrie-Allanore L, editors. *Life-Threatening Dermatoses and Emergencies in Dermatology*. Berlin: Springer-Verlag; 2009. p. 97–102.
23. Criado PR, Criado RF, Avancini Jde M, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS): a review of current concepts. *An Bras Dermatol* 2012;**87**:435–49.
24. Tohyama M, Hashimoto K, Yasukawa M, et al. Association of human herpesvirus 6 reactivation with the flaring and severity of drug-induced hypersensitivity syndrome. *Br J Dermatol* 2007;**157**:934–40.
25. Eshki M, Allanore L, Musette P, et al. Twelve-year analysis of severe cases of drug reaction with eosinophilia and systemic symptoms: a cause of unpredictable multiorgan failure. *Arch Dermatol* 2009;**145**:67–72.
26. Agrawal S, Agarwalla A. Dapsone hypersensitivity syndrome: a clinico-epidemiological review. *J Dermatol* 2005;**32**:883–9.
27. Saltzstein SL, Ackerman LV. Lymphadenopathy induced by anticonvulsant drugs and mimicking clinically pathologically malignant lymphomas. *Cancer* 1959;**12**:164–82.
28. Schnetzke U, Bossert T, Scholl S, et al. Drug-induced lymphadenopathy with eosinophilia and renal failure mimicking lymphoma disease: dramatic onset of DRESS syndrome associated with antibiotic treatment. *Ann Hematol* 2011;**90**:1353–5.
29. Rim MY, Hong J, Yo I, et al. Cervical lymphadenopathy mimicking angioimmunoblastic T-cell lymphoma after dapsone-induced hypersensitivity syndrome. *Korean J Pathol* 2012;**46**:606–10.
30. Seishima M, Yamanaka S, Fujisawa T, et al. Reactivation of human herpesvirus (HHV) family members other than HHV-6 in drug-induced hypersensitivity syndrome. *Br J Dermatol* 2006;**155**:344–9.
31. Morito H, Kitamura K, Fukumoto T, et al. Drug eruption with eosinophilia and systemic syndrome associated with reactivation of human herpesvirus 7, not human herpesvirus 6. *J Dermatol* 2012;**39**:669–70.
32. Yagami A, Yoshikawa T, Asano Y, et al. Drug-induced hypersensitivity syndrome due to mexiletine hydrochloride associated with reactivation of human herpesvirus 7. *Dermatology* 2006;**213**:341–4.
33. Asano Y, Kagawa H, Kano Y, et al. Cytomegalovirus disease during severe drug eruptions: report of 2 cases and retrospective study of 18 patients with drug-induced hypersensitivity syndrome. *Arch Dermatol* 2009;**145**:1030–6.

34. Sano S, Ueno H, Yamagami K, et al. Isolated ileal perforation due to cytomegalovirus reactivation during management of terbinafine hypersensitivity. *World J Gastroenterol* 2010;**16**:3339–42.
35. Sekiguchi A, Kashiwagi T, Ishida-Yamamoto A, et al. Drug-induced hypersensitivity syndrome due to mexiletine associated with human herpes virus 6 and cytomegalovirus reactivation. *J Dermatol* 2005;**32**:278–81.
36. Onuma H, Tohyama M, Imagawa A, et al. High frequency of HLA B62 in fulminant type 1 diabetes with the drug-induced hypersensitivity syndrome. *J Clin Endocrinol Metab* 2012;**97**:E2277–81.
37. Kano Y, Ishida T, Hirahara K, et al. Visceral involvements and long-term sequelae in drug-induced hypersensitivity syndrome. *Med Clin North Am* 2010;**94**:743–59.
38. Newell BD, Moinfar M, Mancini AJ, et al. Retrospective analysis of 32 pediatric patients with anticonvulsant hypersensitivity syndrome (ACHSS). *Pediatr Dermatol* 2009;**26**:536–46.
39. Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. *N Engl J Med* 1994;**331**:1272–85.
40. Kano Y, Shiohara T. The Variable clinical picture of drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS) in relation to the eliciting drug. *Immunol Allergy Clin North Am* 2009;**29**:481–501.
41. Augusto J-F, Sayegh J, Simon A, et al. A case of sulphasalazine-induced DRESS syndrome with delayed acute interstitial nephritis. *Nephrol Dial Transplant* 2009;**24**:2940–2.
42. Eguchi E, Shimazu K, Nishiguchi K, et al. Granulomatous interstitial nephritis associated with atypical drug-induced hypersensitivity syndrome induced by carbamazepine. *Clin Exp Nephrol* 2012;**16**:168–72.
43. Roca B, Calvo B, Ferrer D. Minocycline hypersensitivity reaction with acute respiratory distress syndrome. *Intensive Care Med* 2003;**29**:338.
44. Bourgeois GP, Cafardi JA, Groysman V, et al. Fulminant myocarditis as a late sequel of DRESS: two cases. *J Am Acad Dermatol* 2011;**65**:889–90.
45. Shaughnessy KK, Bouchard SM, Mohr MR, et al. Minocycline-induced drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome with persistent myocarditis. *J Am Acad Dermatol* 2010;**62**:315–8.
46. Teo RY, Tay YK, Tan CH, et al. Presumed dapson-induced drug hypersensitivity syndrome causing reversible hypersensitivity myocarditis and thyrotoxicosis. *Ann Acad Med Singapore* 2006;**35**:833–6.
47. Lo MH, Huang CF, Chang LS, et al. Drug reaction with eosinophilia and systemic symptoms syndrome associated myocarditis: a survival experience after extracorporeal membrane oxygenation support. *J Clin Pharm Ther* 2013;**38**:172–4.
48. Zhu KJ, He FT, Jin N, et al. Complete atrioventricular block associated with dapson therapy: a rare complication of dapson-induced hypersensitivity syndrome. *J Clin Pharm Ther* 2009;**34**:489–92.
49. Fujino Y, Nakajima M, Inoue H, et al. Human herpesvirus 6 encephalitis associated with hypersensitivity syndrome. *Ann Neurol* 2002;**51**:771–4.
50. Masaki T, Fukunaga A, Tohyama M, et al. Human herpes virus 6 encephalitis in allopurinol-induced hypersensitivity syndrome. *Acta Derm Venereol* 2003;**83**:128–31.
51. Descamps V, Collot S, Houhou N, et al. Human herpesvirus-6 encephalitis associated with hypersensitivity syndrome. *Ann Neurol* 2003;**53**:280.
52. Sakuma K, Kano Y, Fukuhara M, et al. Syndrome of inappropriate secretion of antidiuretic hormone associated with limbic encephalitis in a patient with drug-induced hypersensitivity syndrome. *Clin Exp Dermatol* 2008;**33**:287–90.

53. Arakawa M, Kakuto Y, Ichikawa K, et al. Allopurinol hypersensitivity syndrome associated with systemic cytomegalovirus infection and systemic bacteremia. *Intern Med* 2001;**40**:331–5.
54. Sekine N, Motokura T, Oki T, et al. Rapid loss of insulin secretion in a patient with fulminant type 1 diabetes mellitus and carbamazepine hypersensitivity syndrome. *JAMA* 2001;**285**:1153–4.
55. Sommers LM, Schoene RB. Allopurinol hypersensitivity syndrome associate with pancreatic exocrine abnormalities and new-onset diabetes mellitus. *Arch Intern Med* 2002;**162**:1190–2.
56. Chiou CC, Chung WH, Hung SI, Yang LC, Hong HS. Fulminant type 1 diabetes mellitus caused by drug hypersensitivity syndrome with human herpesvirus 6. *J Am Acad Dermatol* 2006;**54**(Suppl. 2):S14–7.
57. Descamps V, Bouscarat F, Laglenne S, et al. Human herpesvirus 6 infection associated with anticonvulsant hypersensitivity syndrome and reactive haemophagocytic syndrome. *Br J Dermatol* 1997;**137**:605–8.
58. Wongkitisophon P, Chanprapaph K, Pattanakaemakorn P, et al. Six-year retrospective review of drug reaction with eosinophilia and systemic symptoms. *Acta Derm Venereol* 2012;**92**:200–5.
59. Brown RJ, Rother KI, Artman H, et al. Minocycline-induced drug hypersensitivity syndrome followed by multiple autoimmune sequelae. *Arch Dermatol* 2009;**145**:63–6.
60. Ozaki N, Miura Y, Sakakibara A, et al. A case of hypersensitivity syndrome induced by methimazole for Graves' disease. *Thyroid* 2005;**15**:1333–6.
61. Kano Y, Sakuma K, Shiohara T. Sclerodermoid graft-versus-host disease-like lesions occurring after drug-induced hypersensitivity syndrome. *Br J Dermatol* 2007;**156**:1061–3.
62. Minegaki Y, Higashida Y, Ogawa M, et al. Drug-induced hypersensitivity syndrome complicated with concurrent fulminant type 1 diabetes mellitus and Hashimoto's thyroiditis. *Int J Dermatol* 2013;**52**:355–7.
63. Aota N, Hirahara K, Kano Y, Fukuoka T, Yamada A, Shiohara T. Systemic lupus erythematosus presenting with Kikuchi–Fujimoto's disease as a long-term sequelae of drug-induced hypersensitivity syndrome, a possible role of Epstein–Barr virus reactivation. *Dermatology* 2009;**218**:275–7.
64. Van den Driessche A, Eenkhoom V, Van Gaal L, De Block C. Type 1 diabetes and autoimmune polyglandular syndrome: a clinical review. *Neth J Med* 2009;**67**:376–87.
65. Callot V, Roujeau JC, Bagot M, et al. Drug-induced pseudolymphoma and hypersensitivity syndrome: two different clinical entities. *Arch Dermatol* 1996;**132**:1315–21.
66. Kano Y, Hirahara K, Mitsuyama Y, Takahashi R, Shiohara T. Utility of the lymphocyte transformation test in the diagnosis of drug sensitivity: dependence on its timing and type of drug eruption. *Allergy* 2007;**62**:1439–44.
67. Shiohara T, Kurata M, Mizukawa Y, Kano Y. Recognition of immune reconstitution syndrome necessary for better management of patients with severe drug eruption and those under immunosuppressive therapy. *Allegol Int* 2010;**59**:333–43.
68. Leohloenya R, Meintjes G. Dermatologic manifestations of the immune reconstitution inflammatory syndrome. *Dermatol Clin* 2006;**24**:549–70.
69. Kito Y, Ito T, Tokura Y, Hashizume H. High-dose intravenous immunoglobulin monotherapy for drug-induced hypersensitivity syndrome. *Acta Derm Venereol* 2012;**92**:100–1.
70. Joly P, Janela B, Tetart F, et al. Poor benefit/risk balance of intravenous immunoglobulins in DRESS. *Arch Dermatol* 2012;**148**:543–4.

The Pharmacogenomics Journal (2013) 13, 60–69

www.nature.com/tpj

ORIGINAL ARTICLE

A whole-genome association study of major determinants for allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients

M Tohkin[1,8], N Kaniwa[1,8], Y Saito[1,8], E Sugiyama[1,8], K Kurose[1,8], J Nishikawa[1,8], R Hasegawa[1,8], M Aihara[2,8], K Matsunaga[3,8], M Abe[3,8], H Furuya[4,8], Y Takahashi[5,8], H Ikeda[5,8], M Muramatsu[6,8], M Ueta[7,8], C Sotozono[7,8], S Kinoshita[7,8], Z Ikezawa[2,8] and the Japan Pharmacogenomics Data Science Consortium[9]

[1]*Department of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan;* [2]*Department of Environmental Immunodermatology, Yokohama City University Graduate School of Medicine, Yokohama, Japan;* [3]*Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan;* [4]*Department of Neurology, Neuro-Muscular Center, National Oomuta Hospital, Oomuta, Japan;* [5]*Shizuoka Institute of Epilepsy and Neurological Disorders, National Epilepsy Center, Shizuoka, Japan;* [6]*Department of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan and* [7]*Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan*

Correspondence:
Dr M Tohkin, Department of Medicinal Safety Science, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan.
E-mail: tohkin@phar.nagoya-cu.ac.jp

[8]Members of the Japan Severe Adverse Reaction (JSAR) research group.
[9]The members of the Japan Pharmacogenomics Data Science Consortium are listed in the Appendix.

Received 12 January 2011; revised 10 August 2011; accepted 11 August 2011; published online 13 September 2011

Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) are severe, cutaneous adverse drug reactions that are rare but life threatening. Genetic biomarkers for allopurinol-related SJS/TEN in Japanese were examined in a genome-wide association study in which Japanese patients ($n=14$) were compared with ethnically matched healthy controls ($n=991$). Associations between 890 321 single nucleotide polymorphisms and allopurinol-related SJS/TEN were analyzed by the Fisher's exact test (dominant genotype mode). A total of 21 polymorphisms on chromosome 6 were significantly associated with allopurinol-related SJS/TEN. The strongest association was found at rs2734583 in *BAT1*, rs3094011 in *HCP5* and GA005234 in *MICC* ($P=2.44\times10^{-8}$; odds ratio = 66.8; 95% confidence interval, 19.8–225.0). rs9263726 in *PSORS1C1*, also significantly associated with allopurinol-related SJS/TEN, is in absolute linkage disequilibrium with *human leukocyte antigen-B*5801*, which is in strong association with allopurinol-induced SJS/TEN. The ease of typing rs9263726 makes it a useful biomarker for allopurinol-related SJS/TEN in Japanese.
The Pharmacogenomics Journal (2013) **13**, 60–69; doi:10.1038/tpj.2011.41; published online 13 September 2011

Keywords: allopurinol; Stevens–Johnson syndrome; toxic epidermal necrolysis; human lymphocyte antigen; single nucleotide polymorphism; genome-wide association study

Introduction

Allopurinol is a xanthine oxidase inhibitor that prevents the production of uric acid to reduce plasma uric acid levels to a normal range. It is the most frequently used anti-hyperuricemic agent in the world due to its long-term pharmacological effect.[1] However, allopurinol is also one of the most frequent causes of a variety of delayed severe cutaneous adverse drug reactions (SCARs).[2] According to spontaneous reports of severe adverse drug reactions to the Ministry of Health, Labor, and Welfare of Japan, allopurinol-related SCARs accounted for about 11% of all reported SCAR cases in Japan in 2008.[3] Allopurinol-related SCARs include the drug-induced hypersensitivity syndrome, Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).[4] SJS/TEN are characterized by high fever, malaise and rapid development of blistering exanthema, with macules and target-like lesions, accompanied by mucosal involvement.[5] Even though the incidence of SJS/TEN is extremely low, the mortality rate of TEN can be as high as 26%.[5] Therefore, SJS/TEN is a serious problem in allopurinol therapy, in spite of the ideal anti-hyperuricemic effect of allopurinol.

Although previous works have suggested that the development of SJS/TEN depends on an immune mechanism involving a drug-dependent cytotoxic cell response against epidermal cells,[5,6] the pathophysiology of SJS/TEN remains largely unknown. Susceptibility to such idiosyncratic reactions is thought to be genetically determined, and familial predisposition to allopurinol-induced SJS/TEN has been reported.[6] Therefore, the exploratory studies for genetic risk factors related to SJS/TEN are needed. A strong association has been observed between allopurinol-induced SCAR and the human lymphocyte antigen (*HLA*) allele B variant (*HLA-B*5801*) in the Han Chinese in Taiwan[7] and in the Thai population.[8] These studies showed that the *HLA-B*5801* allele is present in all patients with allopurinol-induced SCAR (51/51 of Han Chinese and 27/27 of Thai patients) and in only 12–15% of tolerant patients (20/135 and 7/54, respectively). The odds ratio (OR) was 580 (95% confidence interval , 34–9781; $P = 4.7 \times 10^{24}$) for the Han-Chinese data[7] and 348.3 (95% confidence interval, 19.2–6336.9; $P = 1.61 \times 10^{13}$) for the Thai study.[8] Although the association was confirmed in both Caucasian and Japanese subjects,[9,10] the OR in the Han-Chinese and Thai populations were much higher than those in the Caucasian (OR = 80) and Japanese (OR = 40) groups. These reports indicated that *HLA-B*5801* is the valid genetic biomarker for allopurinol-induced SJS/TEN in various ethnic groups, but the mechanisms by which *HLA-B*5801* is specifically involved in allopurinol-induced SJS/TEN progression and the strength of the association showed ethnic differences are unknown.

Currently, genotyping by high-density array scanning of the whole genome allows discovery of previously unsuspected genetic risk factors that influence the pathogenesis of serious adverse drug reactions.[11–13] Genome-wide association studies (GWASs) provide opportunities to uncover polymorphisms that influence susceptibility to allopurinol-induced SJS/TEN free of mechanistic hypotheses. Therefore, in addition to *HLA-B* typing as shown in our previous study,[10] we further conducted a retrospective pharmacogenetic case–control study using whole-genome single nucleotide polymorphism (SNP) data from high-density DNA microarrays in order to identify new and effective genetic biomarkers for allopurinol-related SJS/TEN in Japanese patients.

Materials and methods

Recruitment of study subjects

A total of 141 Japanese SJS/TEN patients from unrelated families were recruited from July 2006 to April 2010 from participating institutes of the Japan Severe Adverse Reactions (JSAR) research group and through a nationwide blood-sampling network system in Japan for SJS/TEN onset patients, operated by the National Institute of Health Sciences.[10] In all, 121 of these patients were diagnosed as defined SJS or TEN by JSAR research group's dermatological experts based on diagnostic criteria[4] that are currently used in Japan. Information was collected using a standardized case report form that includes medical records, co-administrated drug records, disease progress and involvement of systemic complications, as well as SJS/TEN treatment. Among the 141 SJS/TEN patients, 20 were diagnosed as probable SJS due to atypical or mild symptoms. TEN and SJS were defined as mucocutaneous disorders characterized by extensive erythema, blisters, epidermal detachment, erosions, enanthema and high fever. SJS was defined as skin detachment of 10% or less of the body surface area, and TEN as skin detachment of more than 10%, excluding staphylococcal scaled skin syndrome.[5] In all enrolled cases defined as SJS or TEN, allopurinol was regarded as the drug responsible for SJS or TEN if the onset of SJS/TEN symptoms occurred within the first 2 months of allopurinol exposure. For the retrospective pharmacogenetic case–control study, 991 healthy, ethnically matched subjects in the Tokyo metropolitan area were used as the control group. Healthy subjects were used as the control group instead of allopurinol-tolerant patients because the incidence of SJS/TEN is extremely low (0.4–6 per million per year).[3]

The ethics committees of the National Institute of Health Sciences, each participating institute of the JSAR research group and the Japan Pharmacogenomics Data Science Consortium (JPDSC) approved this study. Written informed consent was obtained from all cases and ethnically matched controls.

Whole-genome genotyping of SNPs

Genome-wide genotyping of the 14 allopurinol-related SJS/TEN patients and 991 ethnically matched controls was conducted using the Illumina Human 1M-Duo BeadChip (Illumina, San Diego, CA, USA), which contained 11 63 218 SNPs. SNPs were discarded from case–control association analysis if they exhibited a minor allele frequency <0.001 in the control group (2 37 890 SNPs), a call rate <0.95 for each SNP (32 640 SNPs) or a P-value <0.001 in the test of Hardy–Weinberg equilibrium among controls (2 368 SNPs). These quality control steps removed a total of 2 72 897 SNPs. All samples had a call rate for each microarray above 0.99. Sample duplicates and hidden relatedness were investigated on the basis of pairwise identity-by-state analysis via PLINK;[14] however, there was no duplicate or hidden relatedness in the samples. This quality-control procedure ensured reliable genotyping data.

HLA genotyping and TaqMan genotyping of SNPs on chromosome 6

HLA A, *B* and *Cw* types were determined using sequencing-based methods, as described previously.[10] Representative SNPs of 6p21 (rs2734583, rs3099844, rs9263726 and rs3131643) were re-genotyped using TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA, USA) (ID; C__27465749_10, C__27455402_10, C__30352071_10, C__26778946_20) according to the manufacturer's instruction using 5 ng of genomic DNA. We did not genotype rs9267445 and rs1634776 because TaqMan SNP genotyping assays for these SNPs were not available. Measurement of the linkage disequilibrium (LD) coefficient was performed using

the *HLA* types and 6p21 SNPs of the 141 Japanese SJS/TEN cases and an additional 65 Japanese individuals (non-SJS/TEN patients). The LD coefficient was calculated as previously described.[15,16]

Association analysis

Genome-wide SNPs data from allopurinol-related SJS/TEN cases and ethnically matched controls were used for association analysis using the Fisher's exact test based on the dominant genotype mode and minor allele frequencies of each SNP. Because there are no homozygotes of minor alleles of SNPs, which have significantly related to allopurinol-related SJS/TEN except rs3099844 and rs3131643 in 'Case group', other association analysis models such as trend test (Cochran–Armitage analysis) or recessive model analysis were not applied in this study. All association analyses were carried out with PLINK.[14] *P*-values were corrected for multiple testing according to the Bonferroni's correction. *P*-values $<5.62\times10^{-8}$ were regarded as statistically significant.

Results

Characteristics of study subjects

A total of 14 allopurinol-treated Japanese patients, who were diagnosed with definite SJS/TEN were recruited for the whole-genome association study (IDs 1–14 in Table 1). Patients, IDs 1, 2, 3, 9, 10, 13 and 14 were reported in our previous paper.[10] After the GWAS, an additional four allopurinol-treated Japanese SJS/TEN patients were recruited for *HLA* typing (IDs 15–18). Therefore, a total of 18 allopurinol-treated Japanese SJS/TEN patients participated in the study (Table 1). In all, 12 of 18 patients were male and 6 were female, and the average age was 72.3 ± 10.0 (mean $\pm$ s.d.) years. In all, 12 of 18 cases showed systemic complications of liver and/or renal dysfunction, and most patients had high fever. The average period of SJS/TEN onset after allopurinol treatment was 21.7 ± 11.9 days. Drug-induced lymphocyte stimulation tests were examined in 13 of 18 patients to determine the causative agent; however, in these tests, only two cases (IDs 1 and 5) were positive for allopurinol and only one (ID 16) was positive for oxipurinol, a metabolite of allopurinol. The patient (ID 1) who was positive for the drug-induced lymphocyte stimulation test for allopurinol was also positive for other co-administrated drugs (Table 1). On the other hand, patients who received a patch test showed positive reactions for allopurinol although only two patients were examined (ID 4, 10). The patient who was patch test positive for allopurinol (ID 4) was also patch test positive for other co-administrated drugs (Table 1). Four patients (ID 1, 2, 4 and 14) were co-administrated non-steroidal anti-inflammatory drugs, four (ID 7, 8, 11 and 15) were co-administrated angiotensin II receptor antagonists and three (ID 4, 7 and 17) were co-administrated statin anti-hyperlipemic agents.

Whole-genome association study of major determinants for allopurinol-related SJS/TEN

A total of 14 allopurinol-related SJS/TEN patients (IDs 1–14), who were diagnosed with definite SJS/TEN, and 991 ethnically matched controls, were genotyped with the use of the Illumina Human 1M-Duo BeadChip containing 1 163 218 SNPs. A series of quality-control steps resulted in the elimination of 272 897 polymorphisms. For each SNP, Fisher's exact tests were performed to compare the dominant genotype distributions and minor allelic frequencies in the allopurinol-related SJS/TEN patients (the case group) versus those in the ethnically matched healthy control group. The resulting *P*-values were adjusted with the Bonferroni's correction ($P<5.62\times10^{-8}$). The distribution of *P*-values from the Fisher's exact tests (dominant genotype mode) along each chromosome indicated that 21 SNPs were significantly associated with the cases, all of which were located on the chromosome 6: 6p21.3, 6p22.1 and 6p21.1 (Figures 1a and b). The quantile–quantile (Q–Q) plot for the distribution of *P*-values showed that observed *P*-values matched the expected *P*-values over the range of $0<-\log_{10}(p)<4.0$ (Figure 2). A departure was observed at the extreme tail ($-\log_{10}(p)>4.0$) of the distribution of test statistics for the allopurinol-related Japanese SJS/TEN, suggesting that the identified associations are likely due to true variants rather than potential biases such as genotyping error. These SNPs, with their associated genes, are described in Table 2. As is observed in all SNPs in Table 2, minor allele frequencies in the controls were quite small, ranging around 0.5–0.6%. The genotypic distributions of the case and control groups are identical among groups with the same *P*-value, suggesting that these SNPs might be linked. These SNPs also have ORs that are much higher than the ORs of SNPs commonly observed in sporadic cancer and other complex diseases, suggesting they are of higher penetrance. For example, the most significant SNPs (rs2734583, rs3094011 and GA005234) had an OR of 66.8 (95% confidence interval, 19.8–225.0), and the twentieth most significant SNPs (rs9263827 and rs1634776) had an OR of 60.9 (95% confidence interval, 18.3–202.5). Most SNPs in Table 2 are associated with known or predicted genes; of these, 13 are in known genes. Three SNPs (rs17190526, rs9263726 and rs2233945) were found in *PSORS1C1* (psoriasis susceptibility 1 candidate 1), which is considered as one of the potential psoriasis genes.[17–19] The *CCHCR1* (coiled coil α helical rod protein 1), which is a regulator of keratinocyte proliferation or differentiation and is over-expressed in keratinocytes in psoriatic lesions,[20–23] contained four SNPs (rs9263745, rs130077, rs9263781 and rs9263785). *HCP5* (HLA complex P5), which is involved in hypersensitivity to abacavir,[24–26] had three SNPs (rs3094011, rs3099844 and rs31431643). *TCF19* (transcription factor 19), which is a potential trans-activating factor that might play an important role in the transcription of genes required for the later stages of cell cycle progression,[27] contained two SNPs (rs9263794 and rs10448701). Two SNPs (rs9263796 and rs9263800) were also found in *POU5F1* (POU class 5 homeobox; alternative names for Oct4). *BAT1* (HLA-B

Table 1 Summary of clinical characteristics of Japanese patients with allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis

								Co-administered drugs	
Patient ID[a]	*ADR type*	*Sex/age (years)*	*Highest BT (°C)*	*Total area of blistering skin (%)*	*Systemic complications*	*DLST to allopurinol (PT)*	*Period of onset (days) by allopurinol*	*Drug name*	*DLST result/period of onset*
1	SJS	F/53	38.1	0.5	liver dysfunction renal dysfunction	+	26	loxoprofen clarithromycin	+/9 days +/26 days
2	TEN	M/58	37.1	15	neutropenia liver dysfunction	–	ca 10 days	loxoprofen levofloxacin	–/1 day –/1 day
3	SJS	M/77	unknown	unknown	none	not tested	16	none	
4	TEN	F/72	>37	20	none	–(PT+)	16	pitavastatin lansoprazole salicylamide, acetaminophen, caffeine, promethazine, methylenedisalicylate serrapeptase loxoprofen acetaminophen	–/16 days –/179 days –(PT+)/8 days –/1 day –/8 days (PT+)/8 days
5	TEN	M/82	39	35	none	+	52	none	
6	SJS	M/67	1	1	liver dysfunction	not tested	14	none	
7	SJS	M/76	38.8	unknown	GI tract disturbance liver dysfunction renal dysfunction	not tested	<26 days	losartan furosemide carbon atorvastatin	not tested/8 days not tested/3 days not tested/7 days not tested/8 days
8	SJS	M/83	>38	10	renal dysfunction	–	20	amlodipine olmesartan medoxomil	not tested/very long not tested/very long
9	TEN	M/75	>38	20	neutropenia liver dysfunction renal dysfunction	–	6	none	
10	SJS	M/75	38.4	6	neutropenia liver dysfunction renal dysfunction	–(PT+)	14	none	
11	SJS	M/74	37.8	8	neutropenia liver dysfunction renal dysfunction	–	38	cefazolin Furosemide Sodium polystyrene sulfonate olmesartan medoxomil	not tested/1 day not tested/53 day not tested/51 day not tested/59 day
12	SJS	M/67	38.9	2	liver dysfunction	not tested	17	none	
13	SJS	F/81	39.2	0.5	renal dysfunction	–	28	spironolactone	–/24 days
14	SJS	M/83	39	0	respiratory involvement	–	29	diclofenac	–/1 day
15	TEN	F/73	38	10	liver dysfunction renal dysfunction	–	27	valsartan epoetin β	–/18 days –/2 days
16	SJS	M/53	40	5	liver dysfunction	–(oxipurinol +)	19	none	
17	SJS	F/86	38	0	liver dysfunction renal dysfunction	–	30	rosuvastatin	–/43 days
18	TEN	F/66	37.8	15	none	not tested	2	none	

Abbreviations: ADR, adverse drug reaction; BT, body temperature; DLST; drug-induced lymphocyte stimulation test; F, female; M, male; PT, patch test; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

[a]Patients ID 1–14 were applied for whole genome analysis. ID 1–18 were for the *HLA* typing and the analysis of linkage disequilibrium.
Patients IDs 1, 2, 3, 9, 10, 13, and 14 were reported in our previous paper.[10]