variant as neutrophilic FDE, because this case is clinically and histologically distinct from previously reported cases of FDE in that the basal epidermis is intact without lichenoid disruption and there is a marked perivascular and interstitial inflammatory infiltrate with a predominance of intact neutrophils. Despite their proposal, however, the presence of this variant has not been widely recognized. In our FDE case series, a mixed inflammatory infiltrate predominantly composed of neutrophils can only occasionally be seen. This neutrophil-rich infiltrate is often found when biopsy specimens are obtained from FDE lesions on the flexural areas, as in the case reported by Agnew and Liver [35]. Thus, this may have been a function of the site of lesions and would be expected in FDE lesions developing on the flexural areas.

Diagnosis of Fixed Drug Eruption

The diagnosis of classic PFDE is generally thought to be easy for many dermatologists even after clinical resolution, based on the history of episodes after drug intake and a single or multiple round or oval, demarcated hyperpigmented lesions. However, because the clinical spectrum is quite varied as described above, the diagnosis of FDE is not as straightforward as textbooks sometimes suggest. Indeed, FDE often presents with a wide spectrum of clinical manifestations indistinguishable from those of other skin diseases, such as EM [15], SJS/TEN, cellulitis [28], paronychia [29], neutrophilic dermatosis [35], lichen planus, and parapsoriasis en plaques [32]. In these cases, the time course of appearance of the FDE lesions in relation to any drug must be examined carefully, before attributing the lesions to a particular medication. Because blister formation often occurs at an advanced stage of FDE reactions in association with systemic symptoms such as fever, physicians often encounter a great deal of difficulty in distinguishing between the multiple, bullous variant of FDE and TEN, particularly when the bullous lesions become more widespread with systemic manifestations. Because this variant does not leave typical hyperpigmentation after clinical resolution as typically seen in NPFDE, this variant can be easily misdiagnosed as TEN or bullous pemphigoid. Bullous pemphigoid can be ruled out by negative immunofluorescence for subepidermal IgG and/or C3 in the biopsied lesions. Careful history taking into account drug intake and a prior history of recurrent lesions at the same sites are essential for the precise diagnosis of FDE, but patients often ignore medications they have been taking routinely for years, such as Chinese herbal medicines, and nonmedical agents such as food.

The diagnosis of FDE is made more complex by the recent recognition that exacerbations precisely similar to those caused by the inducing drug can be triggered at the same sites not only by drugs with totally different chemical structures, but also by other nonspecific exogenous factors such as ultraviolet irradiation. Moreover, we have learned to expect FDE lesions to initially occur at previously inflamed or traumatized skin sites, such as sites of burn scars and insect bites [12, 15]. In view of the fact that a 'recall' or 'isotopic' phenomenon is typically seen in FDE lesions and that the 'isotopic'

phenomenon has also been well documented in EM, some cases with EM showing an 'isotopic' phenomenon may actually be a NPFDE variant. When the bullous lesions become rapidly widespread associated with high fever in the multiple, bullous variant, they need to be distinguished from TEN. The difference can be found in the clinical course after withdrawal of the causative drug: the bullous lesions in TEN progressively extend to involve the entire body despite drug withdrawal, while bullous FDE often resolves rapidly without the requirement for steroid administration.

Oral rechallenge and topical provocation tests are usually performed to identify the drug responsible for the FDE. Because no systemic symptoms can usually be induced by oral challenge tests, oral challenge tests with a single therapeutic dose of the suspected drug or one tenth of the therapeutic dose can be done with relative safety. Patch tests at the site of a previous lesion yield a positive response in up to 43% of cases [36]. However, false negative results have been reported and have been attributed to the reactivity of drugs with limited penetration properties. Although patch tests are usually performed with the pure original drug on the upper back in patients with other drug eruptions, this may give misleadingly negative results in FDE. There are several reasons for these negative results. First, patients are frequently not sensitized to the original drug but to its physiologic metabolites. Since these metabolites are usually neither known nor available for patch tests, the patch tests using the original drug may give false negative results. Second, when patch tests are performed with the original drug diluted at <1% in petrolatum and/or in water, this may also give false negative results. Thus, patch tests should be performed with the drug mixed in petrolatum to 10% or diluted in water. Although positive patch test reactions can be more easily obtained when the drug diluted at >50% is used, this should not be done: the stronger delayed reactions would occur on days 3–7, indicating patch test sensitization. Third, patch tests should be performed at the previously involved sites in FDE, because patch tests on the upper back usually yield false negative results. Patch tests are particularly useful to determine the causative drugs when multiple drugs are suspected. Thus, a positive patch test reaction confined to the previously involved areas is generally regarded as strong evidence that the FDE lesions are caused by the tested drug, although it must be kept in mind that a negative test does not necessarily exclude it. Some investigators suggest that patch tests would also be useful as a screening test when multiple drugs are suspected. Patch tests with multiple drugs, however, are not advised because of increased risk of a potential patch-test sensitization. The risk is also considered to be doubled when the drug diluted at >20% is used.

The lymphocyte transformation test has been successfully used for defining the culprit drug in maculopapular drug eruptions, acute generalized exanthematous pustulosis and drug-induced hypersensitivity syndrome [37, 38], but is rarely positive in FDE [26, 27]. Positive lymphocyte transformation test reactions are rarely obtained in patients with FDE, except for those with generalized FDE, such as NPFDE. Given the above, oral challenge remains the most reliable method for establishing the causative drug in FDE.

Clinically Relevant Issues of Disease Pathogenesis

Although extensive data exist on crosstalk between the intestinal epithelium and epithelial-resident lymphocytes under basal conditions and in chronic disease [39], this relation in human epidermis has not been studied to the same degree. In this regard, we previously demonstrated that CD8+ T cells with cytotoxic potential persist between basal keratinocytes as a stable subset in the lesions over a prolonged period of time after clinical resolution [40–42]. Given the presence of these T cells before the onset of clinical symptoms, it is evident that important immunological mechanisms take place for before patients consult a physician. While the T cell compartment in mouse epidermis is exclusively composed of γδ+ T cells with invariant T cell receptors (TCRs) designated as dendritic epidermal T cells [43], major resident T cells in human epidermis express TCR-αβ [4, 40]. Although skin-resident T cells in mice are crucial for tumor immunosurveillance [44] and skin homeostasis [40, 45, 46], little is known about the physiological role of human skin-resident T cells.

Our earlier studies reported that a phenotypically homogenous population of cells that express as TCR-αβ, CD3, CD8, CD45RA, CD103, CLA and CD11b but not CD27 and CD56 were preferentially detectable in FDE lesions before the onset of the disease, but rarely in the uninvolved epidermis of FDE patients and healthy individuals [4, 5, 40, 41]. This phenotype of T cells most closely resembles that of effector memory T cells [47–49], although some differences are present: these intraepidermal CD4+ T cells constitutively express markers common to the skin-homing memory T cells such as CLA and CD103, while effector memory T cells do not. Interestingly, such accumulations of T cells with an effector memory phenotype has been reported to be found at sites of repeated pathogen entry, such as the lung [50], suggesting that these T cells may confer protective immunity, like their murine counterpart. Consistent with this suggestion, T cells with an effector memory phenotype preferentially migrate into the sites of infection, such as mucosal sites and persist for long periods of time following infection [48, 49]. However, we demonstrated that intraepidermal CD8+ T cells resident in FDE lesions are critical in the initiation of cytotoxic immune responses against surrounding keratinocytes, the opposite of what would be expected if the role of T cells with an effector memory phenotype resident in the epithelium were simply a protective response to pathogens. If the intraepidermal CD8+ T cell population with an effector memory phenotype resident in the FDE lesions is a general mediator of this type of localized tissue injury, how can the detrimental effects of these T cells on epidermal tissues be reconciled with effects such as the mediation of protection in epithelial tissues?

These findings are not easily reconciled, unless one assumes that these intraepidermal T cells would be expected to play a previously unrecognized role in protection against pathogens under quiescent nonpathological conditions, but could nevertheless mediate localized epidermal damage through collaboration with cells of the acquired immune system such as CD4+ T cells. In support of this notion, Gebhardt

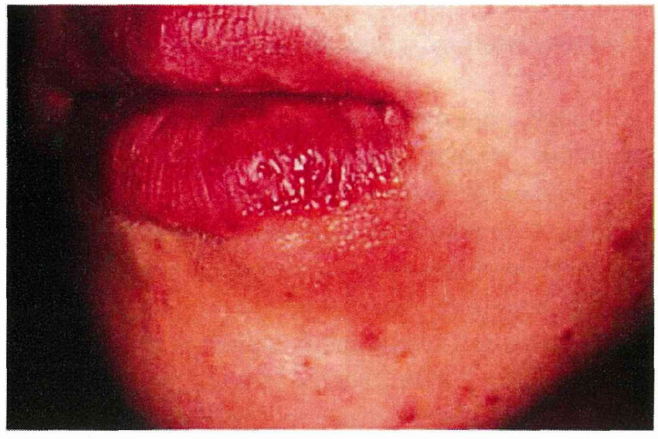

Fig. 3. A typical FDE lesion developed at exactly the same sites as the patient's previous herpes simplex lesions.

et al. [51] provided evidence to indicate that a unique memory T cell subset with the phenotype common to our intraepidermal CD8+ T cells remains resident in the skin after acute infection with herpes simplex virus (HSV) and provides enhanced local immunity during infection with HSV. Virus-specific CD8+ T cells have also been shown to accumulate near sensory nerve endings in genital skin during subclinical HSV-2 reactivation [52]. According to our scenario, CD8+ T cells with such an effector memory phenotype and skin-homing potential can initially be called to seed to the skin upon infection with HSV or other nonspecific stimuli, persist in these peripheral tissues for long periods of time following infection, and are noted for immediate expression of effector function, which mediate pathogen clearance. As shown in our previous studies [5, 41], such intraepidermal T cells can produce large amounts of cytokines, such as IFN-γ, in response to antigen without proliferative responses, a finding consistently observed in T cells with the effector memory phenotype [53]. The lack of an antigen-induced proliferative response by the intraepidermal CD8+ T cells in human skin despite the retention of a strong effector cytokine response within the lesional epidermis is consistent with these T cells having a protective role in mediating effector responses. Consistent with this scenario, the intraepidermal CD8+ T cells are not constitutively cytolytic, but, once activated via the CD3/TCR complex, display a strong cytolytic activity against NK-sensitive or NK-resistant tumor cells and cultured keratinocytes [41]. Support for this scenario also comes from our observations that the vast majority of patients with FDE are asymptomatic HSV-seropositive individuals without a known history of recognized herpetic lesions [54], and that FDE lesions often develop at the sites where herpes simplex repeatedly recurs, after complete resolution of HSV lesions. Furthermore, in some patients, HSV lesions appear to evolve into newly developed FDE lesions (fig. 3). Recent studies have also raised the possibility that the sustained functional T cell responses by HSV may provide protection not only against HSV but also bacterial infections [55, 56]. Thus, the T cell populations resident in peripheral tissues, which face repeated infection by microbial

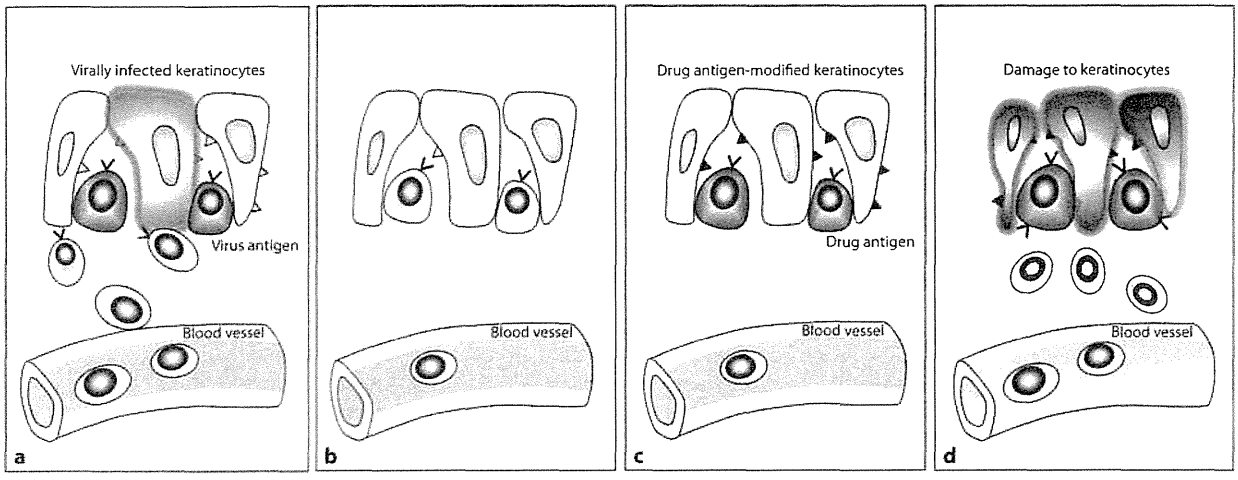

Fig. 4. Hypothetical model from the effector site of the antiviral immune responses to FDE lesions. **a** Migration of antiviral CD8+ T cells with the effector memory phenotype. **b** Retention of these intraepidermal T cells in the epidermis for protection against virus infection. **c** Acquisition of cross-reactivity to drug antigens on these intraepidermal T cells. **d** Recruitment of CD4+ T cells, including regulatory T cells, into the lesions.

pathogens, such as intraepidermal T cells, could play a key role in mediating T cell-dependent protective immunity against the pathogens.

If the intraepidermal CD8+ T cells are indeed mediators for protection against HSV and other microbial pathogens, what is the explanation for their activation by drug antigen? In this regard, there is now sufficient evidence to indicate that a large proportion of antigen-specific self-HLA restricted T cells are also directed toward infectious agents, particularly herpesviruses [57–59]. According to these studies, these cross-reactive T cells can recognize self- and nonself HLA molecules while maintaining a strong antiviral immune response by recruiting non-cross-reactive TCRs to control the virus [58, 60, 61]. In view of our observations that FDE lesions often appear at the sites of recent HSV and herpes zoster lesions (including zoster sine herpete), together with the recent data on the cross-reactivity of T cells with the effector memory phenotype, it is reasonable to speculate that virus-specific CD8+ T cells, once drug antigen is cross-recognized, can be activated to kill surrounding keratinocytes, resulting in localized epidermal injury (fig. 4). When the harmful consequences of such accidental activation of intraepidermal T cells by drug antigen become dominant, FDE would ensue.

Conclusion

It has long been speculated but not clearly shown that viruses are involved in the development of allergic diseases, such as FDE. The hypothesis that FDE lesions can

be induced by a virus-driven process is attractive. FDE that reliably mimics in part features of severe drug eruptions often associated with viral infections would represent a useful disease model in helping us understand the mechanisms of how allergic diseases can be induced by the virus-driven process. Unraveling the complex dual roles of intraepidermal CD8+ T cells with the effector memory phenotype resident in the FDE lesions should also provide valuable insight toward the development of therapies to prevent excessive immunopathology, while maintaining efficient antiviral immune responses.

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Brief Report—Endocrine Research

High Frequency of HLA B62 in Fulminant Type 1 Diabetes with the Drug-Induced Hypersensitivity Syndrome

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Context: Fulminant type 1 diabetes (FT1D) is a subtype of type 1 diabetes characterized by an extremely abrupt onset. FT1D cases associated with the drug-induced hypersensitivity syndrome (DIHS) have recently been reported.

Objective: The clinical characteristics of FT1D associated with DIHS were investigated in this study.

Methods: Case reports of FT1D associated with DIHS in Japanese subjects were collected and analyzed by means of a questionnaire to the authors. A nationwide questionnaire survey was administered to dermatology specialists, concerning the frequency of FT1D associated with DIHS.

Results: In 15 case reports, the mean age at onset of FT1D was 53.4 yr and the mean time for its development from the onset of DIHS was 39.9 d. A higher frequency of human leukocyte antigen (HLA) B62, but not of HLA DR was found in FT1D with DIHS than that for cases without DIHS ($P < 0.001$). The reactivation of herpes virus 6 and cytomegalovirus was detected in 11 and four cases, respectively. Among 746 patients with DIHS in the nationwide survey, four developed FT1D during a 3-yr period. The frequency of FT1D in DIHS (0.54%) was much higher than that in the general Japanese population (0.010%).

Conclusions: The clinical characteristics of FT1D with DIHS were similar to those without DIHS except for the high frequency of HLA B62, which may be involved in the pathogenesis of FT1D with DIHS. Because the frequency was much higher than that in the general Japanese population, FT1D should be kept in mind when DIHS develops. **(*J Clin Endocrinol Metab* 97: E0000–E0000, 2012)**

Fulminant type 1 diabetes (FT1D) is a subtype of type 1 diabetes (T1D) characterized by an extremely rapid onset, absence of islet-related autoantibodies, and the nearly complete destruction of pancreatic β-cells (1, 2). FT1D is not rare in the Asian population. Approximately 20% of cases of ketosis-onset T1D in Japan

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Abbreviations: CMV, Cytomegalovirus; DIHS, drug-induced hypersensitivity syndrome; EBV, Epstein-Barr virus; FT1D, fulminant type 1 diabetes; HbA1c, glycated hemoglobin; HHV, human herpes virus; HLA, human leukocyte antigen.

results from FT1D and 7% in Korea. Although genetic and environmental factors, such as human leukocyte antigen (HLA) and viral infections, are associated with FT1D, its pathogenesis is unclear (3).

The drug-induced hypersensitivity syndrome (DIHS) is characterized by severe multi-organ hypersensitivity, with clinical signs that include a rash, fever, lymphadenopathy, leukocytosis, and acute hepatitis (4). The hypersensitivity reaction is triggered by exposure to a limited number of drugs. DIHS has been characterized by the reactivation of human herpes virus (HHV)-6 at 2–4 wk after the onset of DIHS (4). DIHS has been suggested to be a complex pathological condition involving both immunological reactions to a drug and virus reactivation.

Some cases of FT1D have been reported to be associated with DIHS (5, 6), suggesting that the pathogenesis of FT1D could be shared with that of DIHS. However, the clinical features in the FT1D with DIHS remain unknown. To address this question, we analyzed the clinical characteristics of FT1D with DIHS by collecting case reports for Japanese subjects. We also administered a nationwide questionnaire survey to dermatology specialists concerning the frequency of FT1D with DIHS.

Materials and Methods

A literature search in the published articles or the proceedings of the meetings for cases of the abrupt onset of T1D associated with DIHS in the Japanese population was conducted. Of 16 case reports dealing with FT1D with DIHS, 15 were affirmed to be valid cases. Inclusion criteria for FT1D were 1) ketosis or ketoacidosis within 1 wk after the onset of hyperglycemic symptoms; 2) urinary C-peptide excretion below 10 μg/d, fasting serum C-peptide below 0.3 ng/ml (0.10 nmol/liter), or serum C-peptide below 0.5 ng/ml (0.17 nmol/liter) after glucagon injection or meal load soon after disease onset; and 3) plasma glucose level above 16.0 mmol/liter (>288 mg/dl) and glycated hemoglobin (HbA1c) below 8.9% at the first visit (2). Basically, FT1D was diagnosed by the attending physician or was based on literature data or answers to the questionnaire. DIHS was diagnosed by dermatology specialists based on the diagnostic criteria for DIHS, the presence of cutaneous eruptions, and systemic symptoms such as liver dysfunction, fever, or hematological abnormalities (leukocytosis, eosinophilia, and/or the appearance of atypical lymphocytes) subsequent to the recent initiation of a specific drug treatment (7). A 4-fold or greater increase in anti-HHV-6 IgG antibody titer was defined as significant (8).

To estimate the frequency of FT1D with DIHS, a nationwide questionnaire survey was administered by mail. The questionnaire was sent to all the hospitals having dermatology specialists and that were providing a clinical training program for dermatology specialists certified by the Japanese Dermatological Association. Dermatology specialists were requested to provide the number of cases of DIHS seen in the hospital and those that developed FT1D from 2007–2009. The questionnaires were sent to 641 hospitals, and 441 replies were obtained. Because we received only one reply from a representative dermatology specialist in each hospital, no overlapping cases would be expected. Basically, DIHS was diagnosed by dermatology specialists, and FT1D was diagnosed by the physicians. The significance of the difference in the frequency of human leukocyte antigen (HLA) class I or II alleles between patients with FT1D with DIHS, and either those without DIHS or nondiabetic control subjects was determined by Fisher's exact probability test. A binomial test was used for assessing the significance of the difference in the frequency of FT1D between patients with DIHS and the general Japanese population.

Results

We found 15 reported cases (eight males, seven females) of FT1D associated with DIHS in Japan between 1985 and 2010. Their characteristics are summarized in Table 1. There were no differences in morbidity related to sex, in agreement with a previous nationwide survey of FT1D in Japan (2). The mean age at onset of FT1D with DIHS was 53.4 yr (males 50.6, females 55.9), which was older than that without DIHS reported in the nationwide survey (40.0 yr), even when FT1D cases with pregnancy (29.1 yr) were excluded (2). The mean levels of HbA1c and blood glucose at onset was 6.6% (National Glycohemoglobin Standardization Program value), and 617 mg/ml, respectively, similar to the values for FT1D in the nationwide survey. The duration from the onset of DIHS to the development of FT1D was 39.9 d on average.

No islet-related autoantibodies were detected in any of the cases analyzed. Of seven cases in which HLA class I were analyzed, the frequency of B62 was significantly higher than that for either FT1D without DIHS ($P < 0.001$) or nondiabetic controls ($P < 0.001$) (Table 1) (9). The frequency of A24 tended to be higher, but not significantly so. Of 10 cases in which HLA class II were analyzed, no difference was found in HLA class II compared with FT1D without DIHS.

The causative drugs were mexiletine and carbamazepine for three cases, diaminodiphenyl sulfone and allopurinol for two cases, and zonisamide, phenytoin, salazosulfapyridine, and minocycline, for one case each. In case 15, a causative drug was not identified, despite the typical symptoms of DIHS. The frequency of the use of an anticonvulsant, such as carbamazepine and phenytoin, appears to be lower (five of 15, 33.3%) than that observed in the previous study for DIHS in Japan (60 of 94; 63.9%) (10).

Of 13 cases in which anti-HHV-6 antibody was examined, reactivation of HHV-6 was detected in 11 cases, but not in two cases (cases 5 and 11). Cytomegalovirus (CMV) reactivation was detected in four cases, in which concom-

TABLE 1. Clinical characteristics of FT1D associated with DIHS

Case	Age (yr)	Sex	Causative drug	Onset of FT1D after DIHS (d)	Virus reactivation		HbA1c (at the time of onset of FT1D)[a]	GADAb (autoantibody)	HLA class I B	HLA class II DR	Underlying disease
					HHV-6	Other viruses					
1	70	F	Mexilletine	16	+	–	5.8	–	B62/B62	DRB1*04:03/*08:03	Arrhythmia
2	46	M	Mexilletine	43	+	ND	6.7	–	B62(5)/B48	DR2/DR4	Type 2 diabetes
3	61	F	Mexilletine	20	+	ND	7.0	–	B52/B62	DR2/DR4	Rheumatic fever, valve replacement
4	77	F	Carbamazepine	22	+	–	6.3	–	ND	DRB1 *15:01/*08:03	Post-therapeutic neuralgia
5	63	M	Carbamazepine	14	–	–	6.1	–	ND	ND	Alcoholism
6	19	F	Carbamazepine	35	+	–	7.5	–	B62/B62	DR9	Schizophrenia
7	31	F	DDS	21	+	ND	ND	ND	ND	ND	Livedo reticularis
8	60	F	DDS	24	ND	CMV coxsackie B3	6.2	ICA –	ND	DR4/DRW12(5)	Erythema nodosum
9	40	M	Allopurinol	45	ND	–	ND	ICA –	BW35/BW35	DR4/DRw8	Hyperuricemia
10	69	M	Allopurinol	23	+	CMV	ND	–	ND	ND	Atrial fibrillation, hyperuricemia
11	56	M	Phenytoin	199	–	CMV	7.2	–	ND	ND	Subarachnoid hemorrhage
12	61	M	Zonisamide	14	+	CMV	6.9	–	ND	ND	Cerebral hemorrhage, convulsion
13	19	M	Salazosulphapyridine	89	+	–	6.6	–	ND	DRB1*04:05/*16:02	Drug addiction
14	57	F	Minocycline or Cefdynyle	13	+	–	5.8	–	B44(12)/B61(40)	DRB1*04:05/*13:02	Hepatitis C
15	72	F	Unknown	20	+	–	6.7	–	B13/B62	DRB1*12:02/*14:06	Atrial fibrillation

For virus reactivation, a 4-fold or greater increase in IgG antibody titer was interpreted as significant (+). For autoantibodies, (–) represents negative for GADAb or ICA. DDS, Diaminodiphenyl sulfone; F, female; M, male; ND, not determined or not available; ICA, islet cell antibodies; GADAb, glutamic acid decarboxylase antibodies.

[a] HbA1c indicated by NGSP value.

itant HHV-6 reactivation was detected in cases 10 and 12, and coxsackie virus B3 reactivation in case 8 (5).

In a nationwide questionnaire survey concerning the frequency of FT1D with DIHS, we found 746 cases of DIHS between 2007 and 2009. Of 746 cases of DIHS diagnosed during these 3 yr, four cases developed FT1D (0.54%). In addition, four cases developed T1D other than FT1D.

Discussion

We analyzed 15 reported cases of FT1D associated with DIHS in Japan. No islet-related autoantibodies were detected in FT1D without DIHS, where most cases were reported to have negative antibodies (2). In Japanese subjects, HLA DR4 and DR9 are susceptible to FT1D as well as autoimmune T1D (3), whereas HLA DR2 confers strong protection against autoimmune T1D but weak protection against FT1D. In this study, the genetic contribution of HLA class II appeared to be similar in FT1D with and without DIHS. Although HLA B5801 and A3101were reported to be associated with DIHS with allopurinol (11) and carbamazepine (12), respectively, in Japanese, they were not found in the present study. There were no differences in the frequency of HLA B61 compared with nondiabetic controls, although HLA B61 was reported to be associated with FT1D in the nationwide survey in Japan (9). On the other hand, the frequency of HLA B62 was significantly increased in FT1D with DIHS. These findings suggest that some drugs that induce DIHS may bind to specific class I alleles, resulting in the direct activation of CD8 T cells. In a large number of activated T cells, a subset of T cells with simple T-cell receptor motifs may target an islet molecule as reported for the class I (13) or class II (14) major histocompatibility complex.

HHV-6 reactivation was observed in most of the cases. The mean duration from the onset of DIHS to the development of FT1D was 39.9 d and, in most cases, between 2 wk and 2 months. Because HHV-6 is commonly reactivated 2–4 wk after the onset of DIHS (4), FT1D appeared to develop just after HHV-6 reactivation. In fact, two cases of FT1D with DIHS were reported to be associated with HHV-6 reactivation (6, 15).

The reactivation of CMV and coxsackie virus B3 were also detected, in addition to HHV-6. It was reported that HHV-6 reactivation sometimes results in the sequential reactivation of CMV or Epstein-Barr virus (EBV) (4, 16). In fact, CMV activation was detected in two cases (cases 10 and 12) concomitant with HHV-6 activation. On the other hand, in the cases of FT1D without DIHS, antibody titers for viruses, such as coxsackie virus, rotavirus, EBV, and CMV, were elevated in nine of 55 FT1D patients (3). Furthermore, influenza B, herpes simplex, mumps, EBV, coxsackie, and hepatitis A virus were reported to be associated with FT1D. Despite the preceding DIHS, it appears that the viral infections may be involved in the pathogenesis of FT1D.

Whereas HHV-6 reactivation in DIHS is considered to require immunosuppression, skin inflammation may in-

duce immunosuppressive conditions (16). It was recently reported that plasmacytoid dendritic cells from the systemic circulation accumulate in skin, resulting in a reduced circulation. This could reduce antiviral responses, facilitating viral reactivation in areas other than the skin (4). Enteroviral infections and subsequent innate immunity, such as the expression of toll-like receptor-3 or laboratory of genetics and physiology 2 (LGP2), were observed in islets from autopsied patients with FT1D without DIHS (3, 17). Like viral infections of FT1D without DIHS, the accelerated innate immune response by viral reactivation may result in the rapid destruction of pancreatic β-cells in FT1D with DIHS.

In the questionnaire sent to the hospitals concerning the frequency of FT1D with DIHS, four of 746 cases (0.54%) developed FT1D between 2007 and 2009. However, caution is advised in the interpretation of the data. In the 15 reported cases, four cases were reported between 2007 and 2009. Of the four cases, two cases (cases 6 and 15) were overlapped with the cases from the questionnaire survey, and two cases (cases 5 and 10) were not included in the cases from the questionnaire survey. We also received only 441 replies of 641 hospitals in the nationwide survey. Therefore, the possibility that the frequency of FT1D with DIHS could be higher cannot be excluded.

In the Ehime study, a survey of T1D in Japan, nine cases of FT1D were observed in 4980 cases of diabetes (18). The frequency of FT1D in DIHS (0.54%) is much higher than that in the general Japanese population (0.010%) estimated from the Ehime study finding and diabetes morbidity in Japan ($P < 0.001$); therefore, FT1D should be kept in mind when DIHS develops.

On the other hand, four cases developed T1D other than FT1D. In addition to FT1D, autoimmune T1D associated with DIHS has been reported (19), and a case of autoimmune T1D with DIHS was found in the present study. It would be interesting to examine the difference between the DIHS cases that developed FT1D and autoimmune T1D.

In conclusion, the clinical features of 15 cases of FT1D with DIHS in Japanese subjects were investigated. HLA B62 was associated with FT1D with DIHS, suggesting that it may be involved in this pathogenesis. Further analyses of additional cases would be useful in clarifying the pathogenesis of FT1D associated with DIHS.

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REVIEW

Immunological mechanisms of epidermal damage in toxic epidermal necrolysis

Mikiko Tohyama and Koji Hashimoto

Purpose of review
The purpose of the present review is to introduce recent findings on the pathomechanisms of toxic epidermal necrolysis (TEN), which is characterized by widespread epidermal detachment due to keratinocyte apoptosis.

Recent findings
In the mechanism of epidermal damage, the roles of drug metabolites, cytotoxic lymphocytes, and apoptosis-inducing factors have been noted. In addition, recent studies have focused on monocytes/macrophages, which may participate in epidermal damage through the production of apoptosis-inducing factors and the expression of costimulatory factors with the ability to activate $CD8^+$ T cells.

Summary
Epidermal keratinocyte death is a hallmark of TEN. In a very high proportion of cases, drugs are responsible for TEN. It has been suggested that toxic drug metabolites produced by keratinocytes act like electrophilic agents to induce apoptosis and inflammation. Next, cytotoxic lymphocytes and monocytes function in the development of widespread epidermal damage through direct or indirect cytotoxic pathways. In addition, T-cell activation may be strengthened by the impairment of regulatory T-cell function and activated monocytes. The development of epidermal damage in TEN may require the coordinated action of these factors.

Keywords
apoptosis-inducing factor, cytotoxic lymphocyte, macrophage, monocyte, toxic epidermal necrolysis

INTRODUCTION

Toxic epidermal necrolysis (TEN) is a rare but life-threatening disease that is usually caused by medications [1▪]. TEN, which has an estimated incidence of around 1–2 per million persons per year, is characterized by widespread epidermal loss due to keratinocyte death. The eyes and mucosal membranes of the mouth and genitals are often affected.

Various, possibly interrelated factors have been identified as being critical for epidermal damage. This review discusses current knowledge regarding the epidermal damage in TEN and summarizes recent findings.

THE MECHANISMS OF KERATINOCYTE DEATH

Severe epidermal damage due to keratinocyte death is a hallmark of TEN. Keratinocyte death in the early phase of TEN is the result of apoptosis, rather than necrosis [2]. Therefore, apoptosis-inducing factors may be central players in the pathogenesis of TEN (Table 1). However, the pathogenesis of epidermal damage has not been completely elucidated, and therapy for TEN is not always sufficient to prevent the progression of epidermal loss. Several therapeutic agents and procedures have been tested. Understanding the mechanisms of epidermal damage will facilitate the development of effective treatments.

Apoptosis-inducing factors in toxic epidermal necrolysis

Apoptotic-cell death can be triggered through intrinsic and extrinsic pathways. Paquet *et al.* [2,3] proposed that electrophilic drug metabolites can

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KEY POINTS

- Severe epidermal damage due to keratinocytes cell death is a hallmark of TEN.
- Endogenous stress by toxic drug metabolites, cytotoxic effect of lymphocyte and monocyte, and enhancement of T-cell activation may be involved in the epidermal cell death of TEN.
- It is conceivable that these factors may be intertwined with each other, and not a single specific factor causes epidermal cell death in TEN. Identification of principal pathways involved in epidermal detachment would be important in evaluating the mechanism of action and effectiveness of immunomodulatory therapies.

cause apoptosis in keratinocytes through mitochondrial damage. Mitochondrial damage increases the production of reactive oxygen species (ROS), which can induce the production of proinflammatory cytokines such as TNF-α and directly damage cellular components. A marker of oxidative stress, glutathione-S-transferase pi, is expressed in suprabasal keratinocytes in both lesional and uninvolved skin in TEN [4]. This may explain the frequent observation of full-thickness epidermal damage with reduced infiltration of lymphocytes in TEN.

Secondly, extrinsic factors such as the death receptor pathway and other cytotoxic proteins may cause keratinocyte apoptosis in TEN in an immunogenic fashion. The infiltration of cytotoxic lymphocytes such as $CD8^+$ T cells and natural killer (NK) cells has been observed in the lesional skin of patients with TEN. $CD8^+$ T cells and NK cells express death receptor ligands and possess cytotoxic granules [5]. Fas ligand (FasL), a death receptor ligand, is expressed on cytotoxic lymphocytes. The interaction of FasL with Fas on target cells induces apoptosis in those cells through cell-to-cell contact. The release of cytotoxic chemicals from granules in the lymphocytes, including perforin, granzymes, and granulysin, is also important in the cytotoxic effects of these cells. Granulysin may cause more widespread cell damage than the perforin–granzyme pathway. In addition, the numerous monocytes/macrophages that infiltrate the lesional skin in TEN patients may participate in cell death through the production of death receptor ligands such as TNF-α, TNF-related apoptosis-inducing ligand (TRAIL), and TNF-related weak apoptosis inducer (TWEAK) [6,7▪]. Cytotoxic lymphocytes and monocytes/macrophage infiltrate the epidermis and dermoepidermal junction [6,7▪,8,9▪▪]. As dermal damage is minimal in TEN, the recruitment of these cells into the epidermis has important implications for the development of epidermal damage.

Insights gained from effective treatment

Blocking T-cell activation with corticosteroids may be somewhat beneficial in preventing the progression of epidermal loss, although systemic corticosteroid treatment during the later phase is not only ineffective, but also increases the risk of infection [10,11]. Some reports indicate that cyclosporine, a potent inhibitor of T-cell activation, is also effective in preventing the progression of epidermal detachment [12,13], although this effect

Table 1. Apoptosis-inducing factors implicated in epidermal cell death of toxic epidermal necrolysis AQ7

Apototic pathway	Apoptosis-inducing factor	Cellular source
Intrinsic pathway		
	Electrophilic drug metabolite	Keratinocyte
	Reactive oxygen species (ROS)	Keratinocyte
Extrinsic pathway		
	Death receptor ligand	
	Fas ligand	CD8 T-cell, NK cell
	Soluble Fas ligand	T-cell in peripheral blood
	TNF-α	Monocyte/macrophage, T-cell, keratinocyte
	TRAIL	Monocyte/macrophage, T-cell, keratinocyte
	TWEAK	Monocyte/macrophage
	Cytotoxic protein	
	Perforin, granzyme	CD8 T-cell, NK cell
	Granulysin	CD8 T-cell, NK cell

NK, natural killer; ROS, Reactive oxygen species; TRAIL, TNF-related apoptosis-inducing ligand; TWEAK, TNF-related weak apoptosis inducer.

remains controversial [14]. These findings indicate that T-cell activation is important in the development of epidermal damage in TEN. However, systemic corticosteroids are often insufficient to arrest the progression of epidermal damage in TEN; thus, additional therapies are required. The effectiveness of plasmapheresis was demonstrated previously, although it is of limited value [10,11]. The beneficial effect of plasmapheresis may be due to the reduction of proinflammatory cytokines such as TNF-α [15▪]. High-dose intravenous immunoglobulin (IVIG) may also improve mortality in TEN [10,11]. It is thought that anti-Fas antibody, other inhibitory antibodies, or inhibition of lymphocyte activation prevent epidermal keratinocyte apoptosis [16,17]. In addition, IVIG has numerous actions, including the blockade of Fc receptors, inhibition of complement deposition, enhancement of regulatory T (Treg) cells, modulation of adhesion molecules and cell receptors, and activation of regulatory macrophages [18]. Notably, the blockade of TNF-α immediately arrests epidermal detachment in TEN. Biological therapy with anti-TNF-α monoclonal antibodies (infliximab) or a soluble fusion protein that binds TNF-α (etanercept) is effective in the treatment of TEN [19,20]. Soluble and additional factors are presumed to play important roles in the development of epidermal damage in TEN.

CYTOTOXIC LYMPHOCYTE-MEDIATED DIRECT CELL DAMAGE

The blister fluid of TEN patients contains many lymphocytes, most of which are $CD8^+$ T cells [21–23]. NK cells are also more frequently detected in lesional skin in TEN compared with other drug rashes that show milder epidermal damage [24▪▪]. $CD8^+$ T cells and NK cells are able to kill target cells directly by the perforin–granzyme pathway and Fas–FasL system, both of which depend on cell–cell contact. When cytotoxic T cells recognize their target, degranulation is triggered. Perforin polymerizes on the target cell membrane and forms a pore through which granzymes enter to induce apoptosis [5]. Lymphocytes collected from the blister fluid of TEN patients show high granule expression of perforin and granzyme B [25], and exert cytotoxic effects on keratinocytes treated with IFN-γ [26]. Previous reports have demonstrated that cytotoxic lymphocytes express FasL on their membranes, and that FasL binds Fas on target cells to induce apoptosis in various tissues. However, the involvement of the Fas–FasL system of cytotoxic lymphocytes in TEN is controversial. Lymphocytes from TEN blister fluid do not express high levels of FasL [23].

SOLUBLE APOPTOSIS-INDUCING FACTORS PRODUCED BY LYMPHOCYTES

Lymphocytes produce soluble cytotoxic protein and cause cell death without direct contact.

Soluble Fas ligand

Soluble FasL (sFasL) triggers apoptosis through its interaction with cell-surface Fas in keratinocytes *in vitro*. Serum sFasL has been evaluated as a candidate molecule in epidermal damage in TEN [27]. Murata *et al.* [28] showed that serum sFasL levels increased AQ2 during the earliest phase of TEN. We confirmed the early rise of sFasL in serum collected within 5 days after the onset of TEN [29]. However, the rise in serum sFasL levels was observed not only in TEN, but also in other types of drug-induced rashes [29–31]. Furthermore, sFasL levels were correlated with the severity of hepatic damage: high serum sFasL concentrations were observed in patients with liver damage, regardless of the type of drug rash [31].

Granulysin

In 2009, Chung *et al.* [32] detected high concentrations of granulysin in TEN blister fluid. Granulysin release was detected from cytotoxic T cells and NK cells, and few granulysin-expressing cells were observed in other skin diseases and controls [32]. Granulysin, which permits cell-mediated cytotoxicity without direct cell-to-cell contact [33], is responsible for the poor cell infiltration of TEN. Granulysin is a cationic protein that binds to the cell surface based on charge interactions without a specific receptor. The resulting cell membrane disruption induces mitochondrial damage, leading to cell death. Serum granulysin levels were increased in TEN patients 2–4 days before the appearance of mucous membrane disorders [34,35]. However, Schlapbach *et al.* [24▪▪] reported that the appearance of granulysin-expressing cells was not specific to TEN. Granulysin-expressing cells were detected in lesional skin in various types of drug rashes. Although the number of granulysin-expressing cells was higher in TEN than in a maculopapular-type drug rash, granzyme B was expressed at comparable levels to granulysin. The authors concluded that granulysin is one of multiple cytotoxic molecules used by cytotoxic T cells and NK cells.

SOLUBLE APOPTOSIS-INDUCING FACTORS PRODUCED BY MONOCYTES/MACROPHAGES

Monocytes/macrophages are able to produce apoptosis-inducing factors and may participate in epidermal damage.

TNF-α

Soluble TNF-α is present at high levels in blister fluid and serum in TEN patients [23,36]. By immunohistochemical analysis, Paquet *et al.* [6] showed that monocytes/macrophages, the most numerous cells in the epidermis in TEN, express TNF-α. Recently, we found that monocytes/macrophages also express CD16 [9▪▪]. $CD16^+$ monocytes are a minor fraction of peripheral blood monocytes, accounting for less than 10% of cells [37]. Compared with ordinary monocytes, $CD16^+$ monocytes produce higher levels of proinflammatory cytokines such as TNF-α following stimulation, but not IL-10 [38,39].

TNF-α can activate TNF receptor 1 (TNF-R1), which in turn activates Fas-associated death domain proteins and downstream caspases, resulting in apoptosis. However, TNF-R1 also activates nuclear fctor (NF)-κB, which has an antiapoptotic effect. Therefore, TNF-α alone cannot induce apoptosis in keratinocytes. On the contrary, the combination of TNF-α and IFN-γ may induce apoptosis in keratinocytes. It was previously demonstrated that significant apoptosis, mainly in the basal cell layer of the epidermis, was induced when normal skin was cultured in medium containing TNF-α and IFN-γ [40,41]. However, it remains controversial whether the combination of TNF-α and IFN-γ induces apoptosis.

TNF-related apoptosis-inducing ligand and TNF-related weak apoptosis inducer

TRAIL and TWEAK are death receptor ligands. Recently, de Araujo *et al.* [7▪] reported that TRAIL and TWEAK were present in TEN blister fluid at high concentrations. According to their findings, $CD1a^+$ or $CD14^+$ cells from blister fluid produced these molecules, and TRAIL was additively produced by $CD8^+$ T cells. It is known that keratinocytes also produce TRAIL following stimulation with IFN-γ [42]. Although TRAIL can induce keratinocyte death, the induction of apoptosis is observed only in transformed keratinocytes [42,43]. However, TRAIL can induce apoptosis in normal keratinocytes when NF-κB activity is blocked [42]. If the activation of NF-κB signaling, which is part of the TNF-α survival pathway, is disrupted by factors such as electrophilic drug metabolites or ROS [44], TRAIL and/or TNF-α may induce apoptosis. TWEAK is known as a weak inducer of apoptosis in keratinocytes. A recent report demonstrated that TWEAK and TNF-α induce apoptosis cooperatively in normal keratinocytes [45]. TWEAK may also contribute to epidermal damage in TEN.

ENHANCEMENT OF T-CELL ACTIVATION IN TOXIC EPIDERMAL NECROLYSIS

$CD8^+$ T-cell expansion is not specific to TEN; it is also observed in drug-induced hypersensitivity syndrome [46,47]. In addition, the number of T cells infiltrating the lesional skin in TEN patients is not significantly different from that for other types of drug-induced rashes. Therefore, the trafficking of inflammatory cells into the epidermis, which is to some extent unique to TEN, may be important in understanding the mechanism of epidermal damage. However, the trafficking mechanism is an issue to be resolved in the future. On the contrary, several findings suggest that T-cell activation is enhanced in TEN. The enhancement of $CD8^+$ T-cell activation may induce more severe epidermal damage.

Decrease in regulatory T cells

In an animal model of TEN, Azukizawa *et al.* [48▪] demonstrated that $CD4^+CD25^+$ cells prevented skin damage. Therefore, a decrease in Treg cell numbers may strengthen T-cell activation. Takahashi *et al.* [49] analysed the $CD4^+CD25^+FoxP3^+$ Treg cells in peripheral blood from 11 TEN patients. Although the frequency of Treg cells in acute TEN was not different from that in normal patients, they found that Treg-cell function was profoundly impaired in TEN. Treg cells collected from TEN patients at an acute stage lost their inhibitory effects on T cells stimulated with culprit drugs to produce inflammatory cytokines and proliferate. These findings suggest that the impairment of Treg-cell function during the acute stage of TEN is related to severe epidermal damage.

IVIG and anti-TNF-α antibodies may be effective in reversing Treg-cell dysfunction in TEN. Indeed, it was reported that IVIG and anti-TNF-α antibodies enhanced the number and/or function of Treg cells [50–53].

Involvement of alarmins

Alarmins, endogenous molecules released from damaged cells or immune cells, recruit inflammatory cells and activate innate immune system like exogenous pathogen-associated molecular patterns [54]. Overexpression of alarmins such as S100A proteins and α-defensins was found in peripheral blood mononuclear cells from SJS/TEN patients during the acute phase [55,56▪]. In addition, high-mobility group box 1 (HMGB1) alarmin protein was detected at high concentrations in the serum of SJS/TEN patients [57▪]. HMGB1 may be released from damaged keratinocytes [57▪]. These findings suggest

that drugs may trigger an expression of alarmins leading to highly activated immune system and damaged cells result in further release of alarmins in TEN.

T-cell activation by monocytes

We recently found that the monocytes infiltrating lesional skin in TEN patients expressed costimulatory factors such as CD80, CD86, and CD137L [9▪▪]. CD80/CD86 and CD137L activate T cells through their receptors CD28 and CD137, respectively, with engagement of the TCR and a cognate peptide-MHC complex [58]. Activated T cells express CD137, a receptor for CD137L, and CD137L-CD137 signaling specifically expands $CD8^+$ T cells [58]. CD137 signaling could avoid activation-induced cell death, sustain the proliferation of $CD8^+$ T cells [59,60], and directly augment the cytotoxic function of $CD8^+$ T cells [61]. Costimulation with CD80/CD86 and CD137L has been reported to enhance the proliferation and cytotoxicity of $CD8^+$ T cells as a result of CD137 signaling [62]. AQ4

The CD137L–CD137 system also transduces signals in monocytes expressing CD137L by a process referred to as reverse signal transduction [63]. Dendritic cells generated by CD137L reverse signaling induced more potent T-cell responses than classical dendritic cells, as evidenced by increased T-cell proliferation, increased secretion of IL-12 and IFN-γ, and decreased secretion of IL-10 [64]. Taken together, these data suggest that the CD137L–CD137 system, operating between monocytes and $CD8^+$ T cells in the skin, may play an important role in the development of SJS/TEN.

IVIG inhibits monocyte/macrophage activation [16]. It was previously shown that the number of $CD16^+$ monocytes and their production of TNF-α

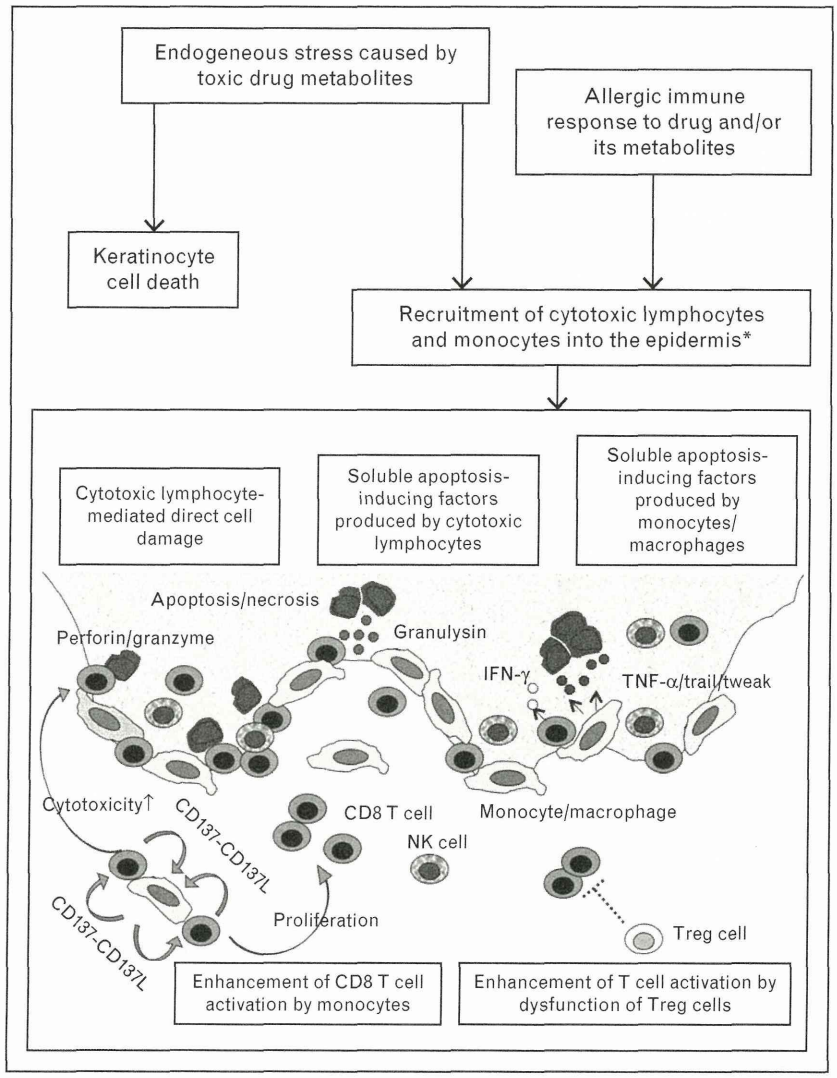

FIGURE 1. A conceivable pathomechanisms of epidermal cell death in toxic epidermal necrolysis. *It has remained unclear how cytotoxic lymphocytes and monocytes are recruited into the epidermis.

were rapidly reduced by IVIG [65]. In addition, anti-TNF-α antibodies inhibited T-cell activation in a monocyte-dependent manner [66].

CONCLUSION

Epidermal death in TEN may be induced by multiple factors, rather than one specific factor (Fig. 1). Both cytotoxic lymphocytes and multiple soluble apoptosis-inducing factors produced by lymphocytes and monocytes may play important roles in epidermal damage in TEN. Although ~~factors such as~~ TNF-α and TRAIL cannot induce apoptosis in keratinocytes, ~~combinations~~ of cytokines ~~such as IFN-γ and~~ endogenous stressors such as toxic drug metabolites may facilitate the apoptotic process. In addition, the strengthening of T-cell activation by the impairment of Treg-cell function and by activated monocytes/macrophages has become an important issue in TEN. Understanding the pathogenesis of epidermal cell death will help clarify the mechanisms governing treatment efficacy, and will assist in the development of improved treatment strategies.

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Conflicts of interest

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There are no conflicts of interest.

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- ■■ of outstanding interest

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