



Immunological mechanisms of epidermal damage in toxic epidermal necrolysis

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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]

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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.

proposed that electrophilic drug metabolites can 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 tumor necrosis factor (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

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

Apoptotic 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.

detachment [12,13], although this effect 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 prevents 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 interferon (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 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 factor (NF)- κ B, 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- κ B activity is blocked [42]. If the activation of NF- κ B 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 Stevens-Johnson syndrome (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 [9²²]. CD80/CD86 and CD137L activate T cells through their receptors CD28 and CD137, respectively, with engagement of the TCR and a cognate peptide-major histocompatibility 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].

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

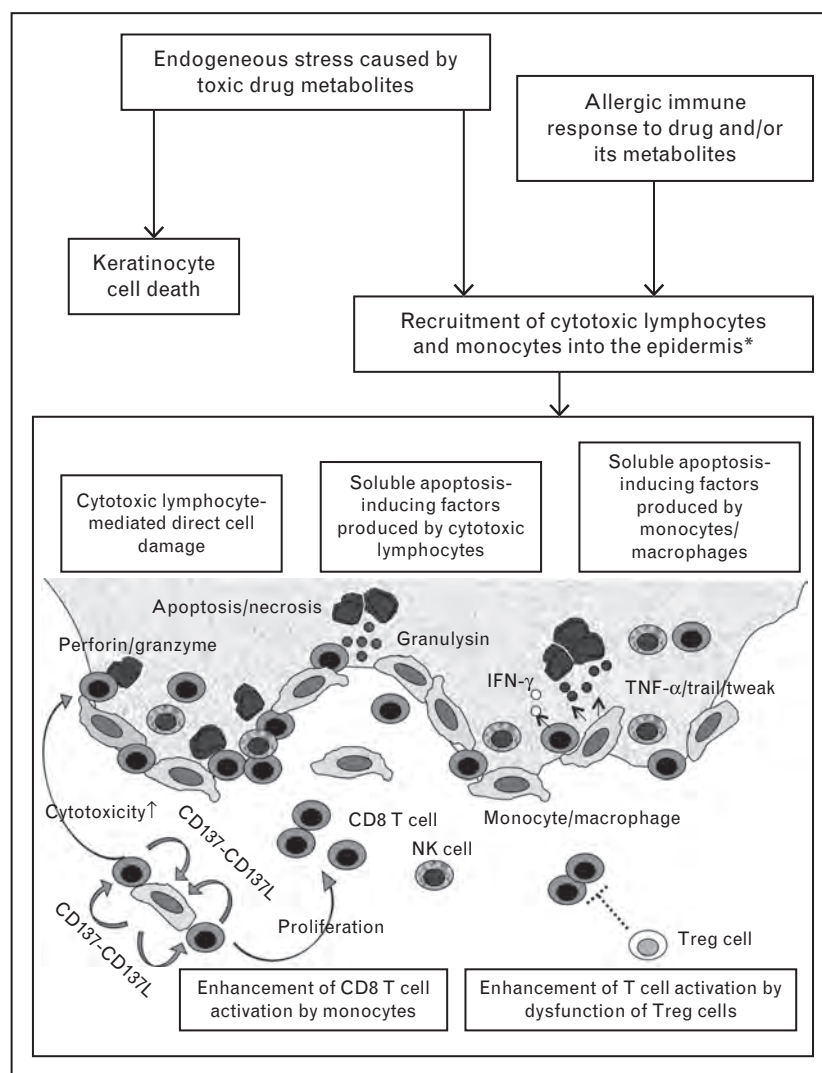


FIGURE 1. A conceivable pathomechanisms of epidermal cell death in toxic epidermal necrolysis. *It has remained unclear how cytotoxic lymphocytes and monocytes are recruited into the epidermis.

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 TNF- α and TRAIL by themselves cannot induce apoptosis in keratinocytes, the combination of cytokines or the existence of 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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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 441–442).

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