A Review of the Pathogenesis of Toxic Epidermal Necrolysis

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Toxic epidermal necrolysis (TEN) is a rare skin condition, most often drug-induced, known for its skin detachment and high mortality. In general, acute TEN is considered a T-cell mediated, type IV hypersensitivity disorder. It mostly results from a cumulative effect of risks from the drug structure, drug metabolism, HLA alleles and T cell clonotypes. However, the precise mechanism of TEN is still unknown. Apoptosis or necroptosis causes keratinocytes to lose their shape and adhesion, and necrosis predominates within a few days. Total epidermal necrosis separates the epidermis from the dermis. TEN is regarded as an immune reaction with predominantly CD8+ T lymphocytes, monocytes/macrophages, and natural killer cells. Impaired regulatory T-cells, T helper 17 cells, cytotoxic granules such as perforin-granzyme and granulysin, tumor necrosis factor α, annexin, microRNA-18a-5p, and drug metabolites are all thought to be involved. From what is known, it can be assumed their mechanism is complex, and there is still much to be investigated. New findings will contribute to the identification of effective active methods of intervention. (J Nippon Med Sch 2016; 83: 216–222)

Key words: Fas-Fas ligand, apoptosis, pathogenesis, tumor necrosis factor α, toxic epidermal necrolysis

Introduction

Toxic epidermal necrolysis (TEN) is a rare skin condition, most often drug-induced, known for its skin detachment and high mortality (Fig. 1). The incidence rate is nearly 1 per million per year and the average mortality rate is as high as 25–50%. Following a flu-like prodrome, erythematous or purpuric macules appear on the skin. These macules coalesce to become flaccid blisters that slough off as areas of necrotic epidermis. Drugs are the most common cause, with allopurinol, sulfonamides, and carbamazepine especially noted. In general, acute TEN is considered a T-cell mediated, type IV hypersensitivity disorder. It mostly results from a cumulative effect of risks from the drug structure, drug metabolism, HLA alleles and T cell clonotypes. However, the precise mechanism of TEN is still unknown. Apoptosis or necroptosis causes keratinocytes to lose their shape and adhesion, and necrosis predominates within a few days. Total epidermal necrosis separates the epidermis from the dermis. TEN is regarded as an immune reaction with predominantly CD8+ T lymphocytes, monocytes/macrophages, and natural killer cells. Impaired regulatory T-cells (T-reg cells), T helper 17 cells, cytotoxic granules such as perforin-granzyme and granulysin, tumor necrosis factor α, annexin, microRNA-18a-5p, and drug metabolites are all thought to be involved. The proposed mechanisms of apoptosis can be divided into 2 groups: the extrinsic and intrinsic pathways. The extrinsic pathway involves electrophilic toxic drug metabolites produced by keratinocytes. Drug metabolites damage the mitochondria and produce reactive oxygen species, leading to production of TNFα, which damages the cell further. The extrinsic pathway includes Fas-Fas ligand (FasL) interactions, soluble FasL (sFasL), perforin-granzyme, granulysin, TNFα, and miR-18a-5p, with cytotoxic lymphocyte and monocyte/macrophage involvement. Annexin induces cell death through necroptosis, and this can be considered an extrinsic pathway. The CD137L-CD137 system of monocytes augments damaging effects of CD8+ T cells, and CD94/NKG2C+ cytotoxic T cells binding to keratinocytes expressing HLA-E induce cell death (Table 1).

Drug Antigen Presentation

An allergic immune response in which an antigenic drug-host tissue complex is made is thought to start the
reaction leading to TEN. There are currently 4 theories as to how drugs stimulate T cells to induce TEN: 1) The hapten/pro-hapten theory, 2) pharmacological interaction of drugs with immune receptors (p-i) theory, 3) altered peptide theory, and 4) altered T cell receptor (TCR) repertoire theory. In the hapten/pro-hapten theory, the drug itself is not antigenic. A chemically inert drug becomes reactive after undergoing metabolism to form a hapten and stimulates an immune response. Neoantigens are formed by binding to carrier proteins recognized by T cells. These are presented to T cells through antigen-presenting cells (APCs). This is because common skin reaction-inducing drugs tend to be small molecules. Drug hypersensitivity to a hapten-peptide complex is less likely to be HLA-restricted because the protein has multiple binding sites. A variety of drug-bound peptides is available for loading onto different types of HLA alleles. In the p-i theory, drugs bind to T cell receptors and/or major histocompatibility complex molecules to trigger specific T cells. The drug does not bind to proteins as in the hapten/pro-hapten theory. A hapten is not made and not involved. This theory was proposed when it was found that even fixed APCs were able to activate specific T cell clones although they cannot process antigens. In the altered peptide model, the drug binds directly to the pocket of a specific HLA but does not bind to a closely related HLA molecule. HLA and the drug form a complex before the HLA molecules are loaded with peptides.

![Patient of toxic epidermal necrolysis with epidermal sloughing showing bare dermis. There is widespread involvement of trunk and lower extremities.](image)

**Table 1** Summary of apoptosis or necroptosis-inducing factors of TEN

<table>
<thead>
<tr>
<th>Apoptotic pathway</th>
<th>Apoptosis-inducing factors</th>
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<tr>
<td>Intrinsic pathway</td>
<td>Electrophilic drug metabolites</td>
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<td>Reactive oxygen species</td>
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<td>Extrinsic pathway</td>
<td>Receptor related</td>
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<td>Fas ligand</td>
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<td>Soluble Fas ligand</td>
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<td>CD94/NKG2C+</td>
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<td>Cytokines/Chemokines</td>
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<td>TRAIL</td>
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<td></td>
<td>TWEAK</td>
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<td></td>
<td>Interleukins (IL-2, 5, 6, 10, 12, 13, 15, 18)</td>
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<td></td>
<td>α defensin</td>
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<td>Cytotoxic proteins</td>
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<td>Granulysin</td>
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<td>Perforin-granzyme</td>
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<td>miR-18a-5p</td>
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<td>Necroptotic pathway</td>
<td>Annexin A1 etc</td>
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This table has been modified from Tohyama et al6.

TEN, toxic epidermal necrolysis; TNFα, Tumor necrosis factor α; IFNγ, interferon γ; TRAIL, TNF-related apoptosis inducing ligand; TWEAK, TNF-related weak apoptosis inducer; IL, interleukin; miR, microRNA
inside the cell. As a result, self peptides that bind to the HLA-drug complex for display to TCR are altered from the self-peptides that bind to the original HLA. Thus different T cell are triggered. In the altered TCR repertoire, the same thing happens as in the altered peptide repertoire, but this time the drug binds to a specific TCR, and changes the structure of the TCR. The HLA-drug-TCR may activate the immune reaction leading to TEN. It can be thought that the p-i and altered peptide repertoire theories favor specific HLA phenotypes. Also in the TCR repertoire theory, the favoring of specific HLA phenotypes is likely. A drug serving as an allergen binds directly to specific HLA molecules and/or TCRs without prior processing by antigen-presenting cells. In the p-i theory, the interactions of certain drugs with immune receptors cause a drug hypersensitivity reaction. Abacavir and carbamazepine are thought to cause TEN through the altered peptide model. A direct interaction between carbamazepine and HLA-B*1502 may induce TEN. Genetic susceptibility may be explained by the possibility that specific drug-related HLA alleles increases the likelihood of TEN happening.

**CD8+ T Lymphocytes**

CD8+ T lymphocytes are crucial to the pathogenesis of TEN, and infiltration of CD8+ T lymphocytes with monocytes/macrophages was observed in a study by Le Cleach et al., who analyzed cells from the blister fluid at the site of dermal-epidermal detachment of 4 TEN patients. Early in the fluid, CD8+ T lymphocytes predominated, while monocyte/macrophages increased as the lesion developed. It is hypothesized that the dominance of CD8+ lymphocytes in early lesion development is related to their role in keratinocyte apoptosis, as opposed to monocytes/macrophages. In a recent study, Yang et al. found that CD8+ T cells and NK cells were predominant in the blister fluid of Stevens-Johnson syndrome (SJS)/TEN patients but their percentage gradually decreased, while that of CD14+ cells increased. The change in the blister cells may have caused the change of cytokines. In maculopapular rashes, it has been reported that CD4+ T lymphocytes are the most common and drug-specific T cells.

**T-reg Cells**

It has been proposed that an enhancement of T-cell activation occurs via a decrease in T-reg cell function and upregulation of T-cell activation by monocytes. CD8+ T-cells themselves are not specific to TEN, and are seen in other drug reactions; therefore, the function of T-reg cells in augmenting the activation of CD8+ cells may be a determining factor in TEN. Enhanced activation of CD8+ T-cells may induce severe epidermal damage. The mechanism via which T-reg cell function becomes impaired remains unclear, but a loss of CD8+ T-cell inhibition is observed. T-reg cells collected from the peripheral blood of TEN patients do not inhibit T-cells. The number of T-reg cells in TEN patients does not appear to differ from that of normal patients, but their function is impaired in the acute phase of TEN.

**Th17 Cells**

Th17 cells are a subset of CD4+ T cells found to be in a high percentage of SJS/TEN patients compared to normal patients and those with maculopapular rash from 2–6 days after onset. Th17 cells produce IL-17 and IL-22. There are more IL-17-producing CD4+ T cells in SJS/TEN patients than in erythema multiforme patients and healthy persons. When the disease improves, the number of Th17 cells become lower. Among Th17 cells, there is a high proportion of cells in the CLA+CCR4+ subset, i.e. cells with skin-homing properties. Skin-homing Th17 cells may regulate recruitment of neutrophils and other inflammatory leukocytes, affecting inflammation and skin damage. Th17 cells may alter their phenotype and become T-reg cells. In SJS/TEN patients, Th17 cells decrease while T-reg cells increase. In addition, neutropenia, which causes higher mortality in TEN patients, may be caused by the effect of Th17 cells on neutrophils.

**Fas-FasL Interaction and Perforin-Granzyme**

Cytotoxic T-cells, specifically CD8+ T-cells and NK cells, exhibit the membrane-bound death receptor ligand FasL, and contain cytotoxic granules. Lymphocytes are responsible for cell death, either directly or indirectly. CD8+ T-cells and NK cells kill target cells directly via the perforin-granzyme pathway, or with the Fas-FasL system, both of which require cell contact. Perforin is a protein that attaches itself within the cell membrane of the target cell and gathers together with other perforin molecules to form a pore. Through this pore, granzymes enter. Granzymes are serine proteinases that cleave caspasess and induce apoptosis. Cytotoxic T-cells exhibit membrane-bound FasL, which is responsible for binding Fas on target cells. Fas is a cell surface receptor and, when interaction occurs between Fas and FasL, induction of apoptosis is observed via a cascade of intracellular events, culminating in the activation of caspasess, the effectors of apoptosis. In this Fas signaling pathway, the Fas-associated death domain (FADD) binds to a Fas-Fas complex. Pro-caspase-8 is recruited, followed by autoactivation of procaspase 8 to caspase 8, which initiates a caspase cascade.

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crease cascade. This results in intracellular degradation. This entire process, and the subsequent apoptosis, can begin in a matter of minutes, and only takes a few hours to complete when the death signal is strong. One could assume that, in widespread epidermal necrosis, the amount of FasL would increase dramatically. However, this assumption is somewhat controversial, as the lymphocytes in TEN blisters do not express high levels of FasL, in contrast to the substantial perforin/granzyme in phagocytes. In TEN blisters do not express high levels of FasL, in contrast to the substantial perforin/granzyme increases observed. Indeed, in a study by Nassif et al., the cytotoxicity of TEN blister lymphocytes was attenuated when perforin/granzyme lysis was inhibited. Additionally, there was no observed change in cytotoxicity with the addition of an anti-Fas monoclonal antibody. Keratinocytes also express FasL, and thus the keratinocyte suicide theory has been proposed. In this theory, keratinocytes self-destruct via autocrine or paracrine secretion, causing widespread epidermal necrolysis. Indeed, increased keratinocyte FasL expression and conservation of keratinocyte Fas expression have been identified in vivo. Keratinocyte FasL does not typically exhibit a cytotoxic effect, but may become cytotoxic in TEN. Viard et al. noted that frozen skin sections from TEN patients, when combined with Fas sensitive cells, exhibited 3-4 times more cell death than skin sections from healthy controls and patients with maculopapular rashes.

**sFasL and Granulysin**

Soluble cytotoxic proteins produced by lymphocytes are responsible for indirect cell death without cell contact. This indirect mechanism may account for the relatively sparse cell infiltration in TEN, despite widespread epidermal damage. The soluble cytotoxic proteins include sFasL and granulysin. sFasL is typically produced by keratinocytes or peripheral blood mononuclear cells, and is responsible for triggering apoptosis through interaction with membrane-bound Fas in keratinocytes, inducing the same reactions as the Fas-FasL interaction. This has been identified in vitro, with TEN patient serum inducing apoptosis in cultured keratinocytes. An increase in serum sFasL has been observed in the early stages of TEN, with the levels of sFasL being highest before the appearance of mucocutaneous lesions, and decreasing approximately 5 days after the appearance of the lesions, suggesting a role in the pathogenesis of TEN. However, the degree of involvement of sFasL is unknown as it is not specific to TEN and is observed in other drug reactions. Furthermore, the cytotoxicity of sFasL is 1,000 times less than that of membrane-bound FasL. Granulysin is a 9-kD cationic protein that binds to the cell surface based on charge interactions without a specific receptor. The precursor is a 15-kD granulysin protein. Both proteins are cytotoxic, but the 9-kD protein is secreted by cytotoxic cells in a granule, whereas the 15-kD protein is secreted by exocytosis. Granulysin is secreted by CD8+ T-cells, NK, and NKT cells. NKT cells are T-cells with properties of both CD8+ T-cells and NK cells. Granulysin is involved in apoptosis, but also has antitumor, antimicrobial, chemotactic, and proinflammatory properties. The negative charge on the molecule disrupts the positively charged cell membrane when binding, and this charge relationship damages the mitochondria, resulting in cell death. High serum levels of granulysin have been detected 2-4 days before TEN. In addition, granulysin levels are higher than perforin, granzyme, or sFasL levels in the blister fluid from TEN patients. Injecting granulysin into mouse skin results in induction of skin lesions similar to those in TEN, whereas depletion of granulysin mitigates cytotoxic effects. Serum granulysin levels directly relate to skin lesion severity, unlike FasL. However, as noted with FasL, granulysin is not specific to TEN, and is also observed in other drug reactions.

**Annexin A1 and Formyl Peptide Receptor 1 (FPR1)**

Annexin A1 binding to FPR1 can trigger keratinocyte death, suggesting it may be involved in the pathogenesis of SJS/TEN. It is a direct mechanism with cell contact. Annexin is an immune regulatory protein secreted from immune cells, including monocytes, and has many functions, including membrane aggregation, inflammation, phagocytosis and proliferation. FPR1 is a type of G protein-coupled receptor involved in tissue damage. Secreted annexin A1 binds with FPR1 on the surface of skin cells and necroptosis, a programmed form of cell death, occurs. When necroptosis was prevented in a mouse model of SJS/TEN, SJS/TEN like responses did not occur. Necroptosis is thought to be another kind of cell death in addition to apoptosis.

**miR-18a-5p**

miR-18a-5p has been observed to be increased in the skin of patients with TEN. miRs are small noncoding RNAs only 22 nucleotides long on average, and are thought to be the most abundant class of regulators. Immune response, cell development, cell differentiation, organogenesis, growth control, and apoptosis are known to be related. The transfection of miR-18a-5p into keratinocytes results in an increase of apoptotic cells, and caspase-9 activity. Because the expression of an anti-intrinsic apoptotic protein, B-cell lymphoma/leukemia-2 like protein 10 (BCL2L10) is decreased by miR-18a-5p,
apoptosis is more likely to occur. The level of miR-18a-5p in the serum correlates with areas of erythema or erosion, suggesting its involvement in the pathogenesis of TEN.

TNFα, Interferon γ (IFNγ) and Other Cytokines/Chemokines

Monocytes/macrophages produce soluble apoptotic proteins. These may be involved in the epidermal damage associated with TEN. Importantly, monocytes/macrophages are the most numerous cell type in the epidermis during TEN, and are responsible for expressing the cytokine TNFα. TNFα activates TNF receptor 1 (TNF-R1), which induces apoptosis in the same way as the Fas signaling pathway. However, in addition to the conventional Fas pathway, TNF-R1 activates nuclear factor-κB (NF-κB), which has an antiapoptotic effect. It remains unknown which of these effects is dominant and, subsequently, the degree to which TNFα contributes to epidermal damage. Some cytokines are important in the apoptotic pathogenesis of TEN, as they are responsible for initiating and amplifying apoptosis by inducing the simultaneous production of other cytokines. Drug-specific CD8+ T-cells produce IFNγ, which recruits monocytes/macrophages and dendritic cells, which in turn produce other proinflammatory cytokines such as TNF-related apoptosis-inducing ligand (TRAIL) and TNF-related weak apoptosis inducer (TWEAK). CD1a+ and CD14+ T-cells are involved in the production of these cytokines. TRAIL is also produced by CD8+ T-cells. TNFα and IFNγ increase inducible nitric oxide synthase and FasL expression, and are involved in FasL-mediated cytotoxic apoptosis in keratinocytes. Other cytokines/chemokines involved in the pathogenesis of TEN are interleukins (IL-2, 5, 6, 10, 12, 13, 15, 18), CCR3, CXCR3, CXC4, CXCR10 and CCL27. The elevation of these cytokines/chemokines in skin lesions and plasma blister fluids has been reported and might affect the trafficking, proliferation, regulation or activation of T cells and other leukocytes involved in TEN. Intracellular flow cytometry of mononuclear cells revealed that defensin was expressed in NK cells and T cells from patients with cutaneous drug reactions, and might be involved as well.

CD137L-CD137

Monocytes infiltrating lesions express costimulatory factors such as CD80/86 and CD137L. These activate T-cells through their receptors CD28 and CD137, along with binding of TCR and the cognate peptide major histocompatibility complex. Specifically, the CD137L-CD137 system augments the damaging effect of CD8+ T-cells by facilitating avoidance of cell death and, subsequently, continuous proliferation. Additionally, this system signals to dendritic cells via reverse signal transduction, which results in increased potency of T-cell responses with production of IL-12, increased IFNγ, and decreased IL-10.

CD94/NKG2C+

In SJS/TEN patients, CD94/NKG2C+ peripheral blood T and NK cells are increased during the acute phase of SJS/TEN. HLA-E-specific activating receptor CD94/NKG2C+ can trigger TCR-independent cytotoxicity in cytotoxic T lymphocytes. The keratinocytes from affected skin of SJS/TEN patients express HLA-E, and the binding of those CD94/NKG2C+ cytotoxic lymphocytes may cause their death.

Conclusion

The pathogenesis of TEN has not been established, and there are several proposed mechanisms. The mechanism is complex, and there is still much to be investigated. New findings will contribute to the identification of effective active methods of intervention.

Conflict of Interest: None declared.

References

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