Uncovering Histologic Criteria With Prognostic Significance in Toxic Epidermal Necrolysis

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Objective: To identify histologic criteria and prognostic significance in patients with toxic epidermal necrolysis (TEN), a frequently lethal disease that usually represents an adverse drug reaction.


Setting: North American tertiary care, university-based burn unit.

Patients: Thirty-seven patients treated for TEN between 1994 and 2004 who had sloughing of 30% or more of their total body surface area and who underwent skin punch biopsies immediately following admission.

Main Outcome Measure: The degree of dermal mononuclear (DM) inflammation was graded (sparse, moderate, or extensive) at least 2 high-power fields (HPF) away from the perimeter of epidermal detachment, and the mean number of DM cells/5 HPF was quantified for each patient. Clinical records were reviewed and the following data extracted: age, history of cancer, percentage of total body surface area slough, heart rate, and serum glucose, bicarbonate, and serum urea nitrogen values on admission. Severity scores for TEN (SCORTEN) were calculated, and clinical outcome was recorded as survived or died during hospitalization.

Results: Extent of inflammation was assessed by categorizing the mean±SD DM cell counts as follows: sparse, 161±36 cells/HPF (n=15); moderate, 273±76 cells/HPF (n=15); and extensive, 392±124 cells/HPF (n=7). There was good concordance between observer ratings (P<.001). While 73% of patients (n=11) with sparse inflammation survived, only 47% (n=7) with moderate and 29% (n=2) with extensive inflammation survived. The accuracy in predicting patient outcome was 65% using grade of inflammation, 68% with mean cell count, and 71% with SCORTEN.

Conclusions: There is a histologic spectrum with TEN that ranges from sparse to extensive DM inflammation, and degree of inflammation predicts clinical outcome approximately as well as SCORTEN. Future clinical trials should consider the possibility that various patient subsets exist within the TEN population, and a role for immunocytes needs to be critically reevaluated in this devastating disease.

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Despite significant advancement in technological methods for investigating immune-mediated diseases, our understanding of the pathogenesis of toxic epidermal necrolysis (TEN), first described in 1956,1 remains relatively unclear. Current classification schemes recognize TEN as a life-threatening, desquamating skin disorder that occurs on a clinical spectrum with the less severe disorders erythema multiforme (EM) and Stevens-Johnson syndrome (SJS). While studies have identified both infectious and drug-related causes for EM and SJS, TEN is virtually always associated with an adverse drug reaction. A clinical diagnosis is made with regard to pertinent history of an inciting agent and physical examination findings, including the extent of epidermal detachment and the appearance and location of lesions.2,3 Epidermal sloughing of more than 30% of the total body surface area (TBSA) occurs with many cases of TEN, while sloughing of less than 30% TBSA can be found in cases that clinically overlap with EM and SJS. Owing to the rapid onset and severity of the disease, patients with TEN are treated in specialized burn cen-

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Pathologic examination of perilesional skin can be used to support or exclude a clinical diagnosis of TEN. Characteristic histologic features include extensive keratinocyte destruction via apoptosis with separation of the epidermis from the dermis at the dermoepidermal junction. A paucicellular, dermal mononuclear (DM) infiltrate has been commonly described. For comparison, EM and SJS often demonstrate less keratinocyte destruction in a background of extensive DM inflammation. Lymphocytes cross the dermoepidermal junction with moderate infiltration of the epidermis. Most EM cases are associated with a herpesvirus infection in which the cytotoxic mechanism involves a delayed-type hypersensitivity reaction where the target antigen is presented on the surface of vulnerable keratinocytes. This explains the presence of extensive lymphocytic infiltration of the epidermis in these cases. A smaller percentage of EM cases are found to be drug related, and the mechanism of pathogenesis in these cases may be similar to that of TEN.

The view that DM cell inflammation is characteristically sparse in TEN concurs with the theory that widespread keratinocyte cytotoxic effects are caused by cytokines and soluble Fas ligand rather than by cell-mediated death. Very little attention has been paid to the observation that not all TEN biopsy specimens show paucicellular infiltrate. In the largest series comparing the histologic characteristics of EM, SJS, and TEN, an intermediate or extensive infiltration of the dermis was found in 5 (22%) of 23 patients with TEN. The relevance of this finding has not been addressed; most histologic descriptions of TEN continue to focus on the classic paucicellular variant. In addition, there has been no attempt to correlate histologic findings with clinical outcome. Currently, the only validated method for predicting severity of illness in TEN is the assessment of specific clinical findings known as SCORTEN. Herein, we show not only that histologic assessment has a role in predicting survival among patients with TEN but also that our current model of pathogenesis may require further modification in a subset of patients.

**METHODS**

Following approval by the Loyola University institutional review board, a search of the pathology database was performed to identify all patients from January 1994 to January 2004 with a skin biopsy diagnosis of EM, SJS, or TEN. The electronic medical record database was used to confirm which of these patients had received treatment for their condition at the Loyola University Burn Center.

All of these patients were diagnosed and treated by physicians and nurses in a consistent fashion. Patients were admitted directly to the burn intensive care unit where they underwent a complete history and physical examination per protocol. Punch biopsy specimens of perilesional skin were taken and histopathologic examination was used to confirm the diagnosis of TEN. Wounds were cleansed and debrided on admission. Wound diagrams were recorded on admission and as often as significant changes were noted in the area of skin loss throughout the hospital stay. Silver nitrate dressings were applied in the early period of the study; thereafter, nanocrystal-line silver dressings (Acticoat; Smith & Nephew, Largo, Fla) were applied on admission and changed every 3 days. Wounds were treated with routine dressing, debrided as necessary, and dressings changed throughout hospitalization by using a standardized protocol.

All patients were resuscitated as needed to maintain adequate organ perfusion. All antibiotics were discontinued in the absence of a documented infection. The inciting agent was discontinued, and steroids were administered only if patients had been treated for other chronic conditions. Patients who had been recently started on steroid treatment underwent a rapid taper as indicated. Nutritional support was initiated on admission either by nasogastric feedings or oral nutritional supplements. In those patients who experienced tube-feeding intolerance, parenteral nutrition was used.

For the purpose of strictly defining a TEN study population, only patients with sloughing of 30% TBSA or more were included. It was also required that at least 1 biopsy specimen per patient show an area of epidermal attachment with adjacent epidermal detachment, indicating that it had been obtained from the perimeter of a lesion. Biopsies were not performed where the entire epidermis was denuded or there was no evidence of keratinocyte apoptosis.

Two investigators blinded to clinical data reviewed hematoxylin-eosin–stained sections. Slides were graded according to the extent of DM inflammation (sparse, moderate, or extensive) underlying attached epidermis at least 2 high-power fields (HPF) away from the perimeter of detachment (Figure 1). Mean cell counts of DM cells were obtained by calculating the average number of DM cells/5 HPF.

Immunophenotypic characterization of the DM cells was accomplished by selecting 3 representative specimens for each of the 3 inflammatory grades and immunostaining each using a highly sensitive avidin-biotin technique following the manufacturer’s instructions ( Vectastain; Vector Laboratories; Burlingame, Calif), as previously described. Briefly, tissue sections were deparaffinized followed by antigen retrieval (10 mM citrate buffer, pH 6.0; microwaving at 500 W for 15 minutes) and then addition of primary antibody to detect the following antigens: CD3 (polyclonal rabbit; 1:100 dilution; DakoCyto-mation, Carpinteria, Calif), CD4 (monoclonal mouse 1F6; prediluted by Ventana Medical Systems, Tucson, Ariz), CD8 (monoclonal mouse 1A5; prediluted by Ventana Medical Systems), TIA-1 (monoclonal mouse; 1:100 dilution; Beckman Coulter Inc, Fullerton, Calif), factor XIIIa (polyclonal rabbit; 1:1000 dilution; Calbiochem, San Diego, Calif), and CD1a (monoclonal mouse JPM30; prediluted by Ventana Medical Systems). Positive staining was accomplished using the chromogen 3-amino-4-ethylcarbazole, generating a positive red reaction product that was then counterstained with hematoxylin-eosin. Cells expressing each of the selected antigens were counted in 5 HPF. The SCORTEN values were calculated by giving 1 point each for age older than 40 years, heart rate on admission greater than 120 bpm, TBSA sloughing greater than 10%, history of malignancy, serum urea nitrogen level greater than 28 mg/dL (~7.14 mmol/L), bicarbonate value lower than 2.38 mEq/L, and glucose level greater than 252 mg/dL (~14 mmol/L). Patient outcome was recorded as survived or died during hospitalization.

Optimal data analysis was used to calculate and compare the accuracy in predicting patient outcome for inflammatory grade, mean cell count, SCORTEN, and percentage of TBSA sloughed through the statistical derivation of cut points for each method. Values below each cut point were used to predict survival, while values above each cut point were used to predict death. Accuracy was expressed as a percentage of correct predictions, and validation was performed using the leave-1-out method.
A total of 67 patients were diagnosed and treated for TEN or SJS during the 10-year period. Biopsy material was unobtainable (outside consult slides or patient did not undergo biopsy) for 9 cases. Of the remaining 58 patients, 19 were excluded for less than 30% TBSA sloughing, 1 for absence of keratinocyte death, and 1 for completely denuded epidermis on biopsy, resulting in a final study population of 37 patients. Hospital mortality was 46% among the study population and 34% overall.

Based on the routine histologic assessment of the degree of inflammation in perilesional skin (Figure 1), patients with TEN were assigned to 1 of 3 groups (histologic grades 1, 2, or 3, representing sparse, moderate, or extensive DM inflammation, respectively). Regardless of the method used (inflammatory grade or mean cell count), hospital mortality was found to increase with increasing DM inflammation (Table 1). Good concordance was achieved between observers in rating the extent of inflammation \( (P<.001) \). While 73% of patients with sparse inflammation survived \( (n=11) \), only 47% \( (n=7) \) with moderate and 29% \( (n=2) \) with extensive inflammation survived. The mean ± SD cell count for sparse inflammation was 161 ± 36 cells/HPF \( (n=15) \); moderate inflammation, 273 ± 76 cells/HPF \( (n=15) \); and extensive inflammation, 392 ± 124 cells/HPF \( (n=7) \), where mean cell counts ranged from 93 to 535 cells/HPF. Using the cut point of 215 cells/HPF, mortality was 24% for counts below this value \( (n=17) \) and 65% for those equal to or above it \( (n=20) \). The SCORTEN values ranged from 1 to 6 (mean ± SD, 2.8 ± 1.4). Accuracy in predicting clinical outcome was 65% for histologic grade of inflammation, 68% for mean cell count of DM cells, and 71% for SCORTEN (Table 2). The mean numbers of days from the onset of symptoms to the skin biopsy procedure were 9.4, 9.9, and 10.2 for histologic grades 1, 2, and 3, respectively. Thus, there was no significant difference among the groups with regard to the timing of tissue procurement. Eosinophils were only sporadically identified in tissue samples regardless of the histologic grade of inflammation.

As was seen by immunohistochemical staining of 5 representative cases in each of the 3 different histologic grades, the predominant mononuclear cell was the T cell with an admixture of both CD4 and CD8+ lymphocytes (Figure 2). Greater than 90% of the CD3+ T cells were present in the papillary dermis, with rare T cells extending into the reticular dermis. Occasional specimens had focal intraepidermal CD3+ T cells, but this was not a consistent finding in any of the groups, regardless of histologic grade. Using a marker for cytotoxic T cells (TIA-1), only occasional TIA-1+ T cells were identified in the biopsy specimens, regardless of the histologic grade of inflammation. The predominant dendritic cell was the factor XIIa+ dermal dendrocyte, with only rare CD1a+ Langerhans cells.

Two additional statistical evaluations were performed using this subset of patients with TEN. First, we assessed the correlation between histologic grade and percentage of TBSA sloughing. The mean TBSA sloughing for histologic grades 1, 2, and 3 were 53.0%, 63.0%, and 79.9%, respectively. A 1-way analysis of variance revealed a significant linear relationship \( (P=.005) \). Additional computations \( (\eta^2) \) indicated that 21% of the variance in TBSA sloughing is explained by histologic grade classification. This corresponds to a correlation coefficient of 0.46, which is in the moderate range of the strength of association between histologic grade and percentage of TBSA sloughing. Thus, it appears that patients with TEN whose biopsy specimens are characterized by a prominent cell inflammatory infiltrate experience more extensive skin involvement as reflected by the percentage of TBSA sloughing.

Second, we performed a statistical analysis exploring a potential correlation between the percentage of TBSA sloughing and mortality. Indeed, in this subset of patients, our current results indicate a significant correlation between TBSA sloughing and outcome \( (P=.03) \).
There is considerable controversy regarding both clinical and pathologic criteria for defining disease categories within the EM-SJS-TEN spectrum. This may partially explain why further characterization of the histopathologic characteristics is required. It has been suggested that drug-related cases of EM share a common pathogenic mechanism with TEN. From a histologic perspective, it has been stated, “a more prominent dermal infiltrate is seen in those cases [of TEN] which overlap with EM.” As previously stated, EM is typically associated with a more extensive dermal infiltrate, which may reflect cell-mediated hypersensitivity to antigen deposition within the epidermis. This disease process in general has a self-limiting course. If extensive dermal inflammation in TEN truly represents histologic overlap with EM, then one would expect these patients to follow a milder clinical course than those with paucicellular TEN. However, our findings demonstrate the opposite: where 7 (19%) of 37 patients had the same degree of inflammation typically associated with SJS and EM, increased inflammation correlated with a worse prognosis after the exclusion of patients within the area of clinical overlap (10%-30% TBSA slough).

Furthermore, in this subset of patients with TEN who had at least 30% TBSA slough, we found that the degree of DM as assessed by histologic grade 1, 2, or 3 correlated with the percentage of TBSA slough. Further studies are warranted on larger samples to validate this association and to delineate mechanistic links between the degree of inflammation and the extent of TBSA slough in patients with TEN. While our results indicate that the percentage of TBSA slough can be correlated with the mortality of patients with TEN, one report supports this finding while another fails to correlate TBSA slough with survival. Thus it remains unclear whether percentage of TBSA slough can consistently predict outcome as an independent prognostic factor in patients with TEN.

Our data suggest that pathologic examination can provide useful prognostic information through the grading of DM inflammation. This can be objectively achieved by counting DM cells per HPF, where a mean cell count of greater than 215 predicts a worse prognosis (65% vs 24% mortality) in patients with 30% or more TBSA sloughing. This basic histologic assessment was found to be approximately as accurate as the current gold standard, SCORTEN, in predicting patient outcome. In addition to predicting survival status, another potential benefit to stratifying patients by degree of dermal inflammation lies in the application of separate treatment strategies to various histologic subsets. While the role of immunotherapy (eg, intravenous immunoglobulins, cyclosporin A, and corticosteroids) remains to be defined, the expression of different biological pathways in patients with similar clinical findings may help explain some of the in-

| Table 1. Data Summary Showing an Inverse Relationship Between Survival and Grade of Dermal Inflammation in TEN Cut Point |
|-----------------------------------------------|------------------|-----------------|-----------------|-----------------|
| Dermal Inflammation Characteristic | No. of Patients | Survival, % | No. of Cells/HPF | SCORTEN |
| Grade | | | | |
| 1 | 15 | 73 | 161 | 2.5 |
| 2 | 15 | 47 | 272 | 2.6 |
| 3 | 7 | 29 | 392 | 4.2 |
| No. of cells/HPF | | | | |
| <215 | 17 | 76 | NA | NA |
| ≥215 | 20 | 35 | NA | NA |
| Total | 37 | 54 | 250 | 2.8 |

Abbreviations: HPF, high-power field; NA, not applicable; SCORTEN, severity of illness index for TEN; TEN, toxic epidermal necrolysis.

* A quantitative cut point of 215 cells/HPF was derived for predicting survival vs death.

| Table 2. Accuracy of Methods for Predicting Patient Survival in TEN* |
|-----------------------------------------------|------------------|-----------------|-----------------|-----------------|
| Survival Prediction Method | Accuracy, % |
| Inflammatory grade | 65 |
| No. of cells/HPF | 68 |
| SCORTEN | 71 |

Abbreviations: HPF, high-power field; SCORTEN, severity of illness index for TEN; TEN, toxic epidermal necrolysis.

* Note the approximate equivalence between histologic evaluation and SCORTEN.

Figure 2. Quantitative immunophenotypic analysis of immunocytes in biopsy specimens from patients with toxic epidermal necrolysis, inflammation grades 1, 2, and 3. Using a panel of antibodies to immunostain mononuclear cell subsets, we found that the predominant inflammatory cell was the CD3+ T cell, including both CD4+ and CD8+ T cells, admixed with factor XIIIa+ dermal dendrocytes (F13A). T cells expressing a cytotoxic marker (ie, TIA-1) and Langerhans cells expressing CD1a were less common. Error bars represent standard deviations.
consistencies in patient response to therapy. To better understand the basis for immunotherapy in TEN, we need to clarify the role of immunopathogenic and immunoregulatory cells in the disease process.

Early immunophenotypic studies in TEN demonstrated a depletion of circulating CD4+ helper T lymphocytes in the blood with deposition in cutaneous perivascular spaces. A correlation was drawn between TEN and other cytotoxic skin disorders; however, it was suggested that epidermal damage in TEN might be due in part to nonantibody circulating lymphokines as an explanation for the “paucity” of dermal inflammation. Paquet and Pierard showed that TEN is characterized immunopathologically by an increased ratio of dermal dendrocytes to dermal lymphocytes, in contrast to the opposite pattern seen in EM where lymphocytes predominate. A correlation was also found between the number of tumor necrosis factor α–producing macrophages and dermal dendrocytes in TEN, suggesting that the release of tumor necrosis factor α may serve as a potential pathway leading directly or indirectly to the cytotoxic damage of keratinocytes. More recently, other apoptotic mechanisms have received attention, including activation of Fas (CD95) by soluble Fas ligand.

In the present series of tissue samples, we observed that the predominant mononuclear cell was the T lymphocyte, accompanied by factor XIIIa+ dermal dendrocytes (Figure 2). Among the T-cell population, both CD4+ and CD8+ T cells were present, including a subset of T cells expressing TIA-1. The relative frequency of individual subsets of immunocytes was not consistently different among the 3 histologic grades.

The presence of extensive dermal inflammation in TEN would suggest the possibility of additional unknown pathogenic mechanisms or variable expression of a common pathway. Future investigations will be required to sort this out. However, since the disease is relatively uncommon, difficulty lies in collecting a sufficient number of cases where both diagnosis and treatment have been performed in a consistent fashion. Most of the immunopathologic data come from a limited number of cases, not accounting for wide variation in histologic characteristics. While immunohistochemical analysis provides an opportunity for studying archived paraffin-embedded tissue, future investigations should include the use of proteomic and molecular technologies, where blood and tissue need to be stored in the fresh and frozen state. By addressing these issues, an increased understanding of the pathogenesis of TEN should ultimately lead to better therapeutic methods for circumventing the rapid and destructive nature of this life-threatening disease.

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REFERENCES