Phosphorylated MLKL and cleaved caspase8 are useful for early diagnosis of Stevens-Johnson syndrome and toxic epidermal necrolysis.

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Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but extremely severe and life-threatening cutaneous adverse drug reactions. It is difficult to distinguish erythema multiforme (EM) and SJS/TEN in early stage. Histopathology shows keratinocyte death in SJS/TEN and EM in various frequency. Although keratinocytes have been suggested to die as apoptosis, our recent study revealed necroptosis, programmed necrosis, also contribute to keratinocyte death in SJS/TEN. Monocytes secrete Annexin A1 in stimulation of causative drugs. The released annexin A1 binds to FPR1 and mediates the RIP1/RIP3 complex. MLKL is phosphorylated by RIP3 and initiate necroptosis¹. Since necroptosis is considered to be specific to SJS/TEN, we hypothesized that analyzing the form of keratinocyte death is useful for early diagnosis of SJS/TEN.

Materials and Methods

The skin samples were obtained from non-bullous erythematus lesion of the patient with acute phase drug eruption. We included 4 patients diagnosed as SJS and 10 patients diagnosed as EM. We performed immunofluorescence staining. Anti-phosphorylated MLKL (pMLKL) staining was used to detect necroptosis, and anti-cleaved caspase8 (cCas8) staining was used to detect apoptosis. The number of death keratinocyte in H-E staining and pMLKL and cCas8 positive cell in immunofluorescence staining were counted.

Result

No	Age	Sex	cause	skin rush area (*)	Musosal involvement	Other symptoms	Diagnosis
S1	28	M	acetaminophen	2+	Conjunctival injection Erosion of lip and oral mucosa	liver injury	SJS
S2	23	F	drug for common cold	4+	Congestion, corneal erosion Erosion of oral mucosa	liver injury	SJS
S3	65	F	tegretol	±	Erosion of lip and oral mucosa	—	SJS
S4	67	F	loxoprofen	3+	Conjunctival injection Erosion of lip and oral mucosa	liver injury	SJS
E1	75	M	acetaminophen or tranexamic acid	3+	Conjunctival injection Erosion of oral mucosa	—	EM
E2	67	F	salazosulfapyridine	4+	—	—	EM

*skin rush area 0-20%: ±, 20-40%: 1+, 40-60%: 2+, 60-80%: 3+ 80-100%: 4+

In case of EM, 2 of 10 cases were shown. One was only pMLKL positive and the other was both pMLKL and cCas8 were negative.

Frequency of cell death in epidermis

	HE (%)	pMLKL (%)	cCas8 (%)
SJS	13.43 ± 5.39	3.78 ± 2.70	17.80 ± 4.94
EM	1.02 ± 1.38	1.47 ± 2.56	1.09 ± 2.15

In 4 all cases diagnosed as SJS, both of pMLKL and cCas8 cell were positive. In contrast, it was only 1 case that both pMLKL and cCas8 were positive and either pMLKL or cCas8 was positive in 4 cases in EM. The number of pMLKL(+) plus cCas8(+) cell were statistically higher than the number of death keratinocyte detected by HE staining.

Conclusion

Both of necroptosis were apoptosis are occurred in keratinocyte death in SJS. Although, in EM, both of necroptosis and apoptosis were shown, the frequency of cell death was lower than SJS/TEN. It is suggested that the frequency of cell death is important for differentiation between SJS and EM. It is expected that pMLKL and cCas8 are useful for early diagnosis of SJS.