RIP3 as a diagnostic and severity marker for Stevens-Johnson syndrome and toxic epidermal necrolysis

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Clinical Implications

- Receptor-interacting kinase-3 may serve as a biomarker for the severity of Stevens-Johnson syndrome, and toxic epidermal necrolysis, and may aid in choosing an early and appropriate treatment.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening diseases.[1] Medication and infections, including those caused by *Mycoplasma pneumoniae* and cytomegalovirus, are common causes.[1] Both diseases are characterized by skin detachment, differentiating them from other generalized skin eruptions such as maculopapular exanthema and erythema multiforme (EM). However, clinical manifestations of SJS/TEN are occasionally undistinguishable from those of early EM because blisters or mucous lesions are mild. Finding a severity and diagnostic biomarker for SJS/TEN is an urgent need.

Levels of serum Fas ligand and granulysin increase SJS/TEN; however, they have not been correlated with disease severity.[2,3] Su et al[4] reported that IL-15 levels correlate with the severity and mortality.[4] However, their correlation with clinical parameters was not analyzed.

SJS/TEN is characterized by the death of keratinocytes. Although keratinocytes die through apoptosis, we and others have shown that necroptosis, or programmed necrosis, also contributes to keratinocyte death.[5,6] Necroptosis is mediated by receptor-interacting kinase 3 (RIP3) and mixed lineage kinase domain-like pseudokinase phosphorylation.[5] In necroptotic cells, RIP3 expression is increased and it is released extracellularly.[7] Herein, we investigated whether necroptotic keratinocytes release RIP3 extracellularly and whether serum RIP3 levels correlate with the severity of SJS/TEN.

First, we confirmed that necroptotic keratinocytes released RIP3 extracellularly, specifically in HaCaT cells treated with necroptosis inducers (see Figure E1, *A-E*, in this article's Online Repository at www.jaci-inpractice.org). Supernatant of HaCaT cells stimulated with T/S/Z had increased RIP3 levels (see Figure E1, *F*). This indicated that necroptotic keratinocytes release RIP3 extracellularly.

Next, we analyzed whether the necroptosis pathway is activated in lesional keratinocytes of SJS/TEN, EM, and maculopapular exanthema. Abundant RIP3 and phosphorylated mixed lineage kinase domain-like–expressing keratinocytes were detected in the epidermis of SJS/TEN skin lesions, whereas only small numbers of RIP3 and phosphorylated mixed lineage kinase domain-like–positive cells were observed in EM skin lesions (Figure 1). Our results suggest that the necroptosis pathway is activated not only in SJS/TEN but also in EM, albeit to a different degree.

We hypothesized that the degree of necroptosis could be predicted by measuring serum levels of RIP3 released by necroptotic keratinocytes, suggesting serum RIP3 as a diagnostic and severity biomarker for SJS/TEN. The patients' clinical attributes are presented in Table E1 in this article's Online Repository at www.jaci-inpractice.org. In the first 10 days following onset, the SJS/TEN group (799.2 ± 732.6) had significantly higher serum RIP3 levels than the other groups (EM major: 178.3 ± 135.2, $P < .001$; EM minor: 91.5 ± 57.6, $P < .001$; maculopapular exanthema: 65.8 ± 76.7, $P < .001$; drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms: 25.3 ± 48.6, $P = .002$; healthy controls: 13.9 ± 31.1, $P < .001$) (Figure 2, *A*). In the SJS/TEN group, the patients with TEN (n = 7) had higher RIP3 levels than did patients with SJS (n = 15) ($P = .007$) (Figure 2, *B*). Although autoimmune blistering disorders such as bullous pemphigoid and pemphigus vulgaris are occasionally difficult to differentiate from early SJS/TEN, serum RIP3 levels were significantly higher in the SJS/TEN group than in the bullous pemphigoid and pemphigus vulgaris groups (see Figure E2, *A* in this article's Online Repository at www.jaci-inpractice.org).

The EM major group's serum RIP3 levels were significantly higher than those of the control and drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms groups. Consistent with these results, the skin detachment areas of patients were positively correlated with serum RIP3 levels (Figure 2, *C*). There was no significant difference between the medication-caused and infection-caused groups (see Figure E3, *A*, in this article's Online Repository at www.jaci-inpractice.org).

We also analyzed the association between serum RIP3 levels and mucosal involvement, organ dysfunction, vital signs, data from laboratory examinations, and histopathologic frequency of dead cells. The worst level for each clinical parameter during medical examination was used for evaluation. In histopathologic examination, the group with more than 10 dead keratinocytes revealed significantly high serum RIP3 levels (Figure 2, *D*). In SJS/TEN, mucosal involvement or organ dysfunction is related to severity and mortality.[1] Consistent with these data, positive correlations were observed between serum RIP3 levels and body temperature, number of sites with mucosal involvement, and number of organs with dysfunction (Figure 2, *E-G*). C-reactive protein levels showed a positive correlation with serum RIP3 levels, but not with white blood cell counts (see Figure 2, *H*, and Figure E3, *B*). These results suggest the utility of RIP3 as a severity and diagnostic biomarker for SJS/TEN.

We also analyzed changes in serum RIP3 levels during the course of disease progression. In all SJS/TEN cases, patients received some form of therapy: oral corticosteroid, steroid pulse therapy, or intravenous immunoglobulin therapy. In the recovery phase, serum RIP3 levels were decreased after treatment in all cases (TEN: n = 3, SJS: n = 5) (Figure 2, *I*). Next, we compared

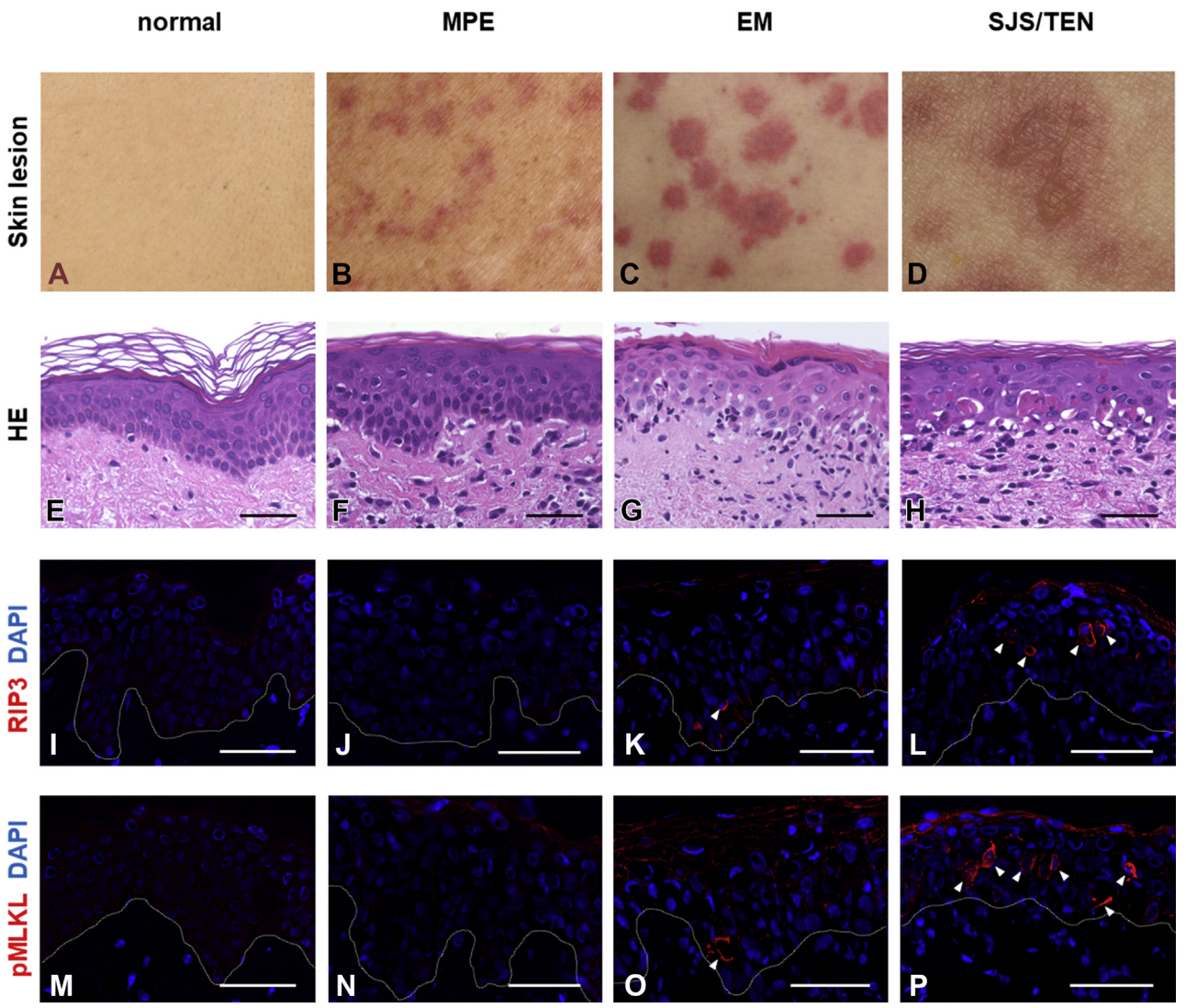

FIGURE 1. Skin lesions and epidermal RIP3 expression. (**A-D**) Skin lesions in controls and patients with MPE, EM, and SJS, respectively. (**E-H**) Hematoxylin-eosin staining. (**I-L**) Immunofluorescence staining for RIP3 (red). (**M-P**) Immunofluorescence staining for pMLKL (red). (*I-P*) Merged images with nuclear staining (blue). Scale bars = 50 μm. Arrowheads indicate RIP3- or pMLKL-positive cells. *DAPI*, 4′,6-diamidino-2-phenylindole; *MPE*, maculopapular exanthema; *pMLKL*, phosphorylated mixed lineage kinase domain-like.

serum RIP3 levels from before treatment and at the worst time in 2 of the TEN cases. In both cases, the level of serum RIP3 peaked when the skin detachment area was maximal, then decreased in the recovery phase (Figure 2, *J*). We show the details for one of the TEN cases in this article's Online Repository at www.jaci-inpractice.org (Figure E4, *A*).

We further tested serum RIP3 level as a diagnostic and severity marker for SJS/TEN. Cells undergoing necroptosis release RIP3 extracellularly,[7] which we confirmed in this study for necroptotic keratinocytes. Necroptosis is associated with acute kidney injury[8] and systemic inflammatory response syndrome.[9] Plasma RIP3 level has been identified as a useful prognostic biomarker for acute kidney injury and sepsis.[8,9] We found that serum RIP3 levels in the acute phase were significantly higher in the SJS/TEN group than in the other groups. Moreover, skin detachment areas and necrotic changes in histopathologic examination revealed a positive correlation with serum RIP3 levels. It is suggested that elevation of serum RIP3 in patients with SJS/TEN is associated with the amount of necrotic change in the epidermis. Serum RIP3 levels were higher in EM cases than in healthy controls, and epidermal necroptosis in EM skin lesions was detected. Although it is controversial as to whether EM belongs to the same spectrum of disease as SJS/TEN that differs only in severity, our results suggest that it does.

We also investigated whether serum RIP3 levels correlate with disease activity. Although sFasL and granulysin levels have been reported to decrease immediately after initiation of treatment, even in patients with no improvement in skin symptoms,[2,3] serum RIP3 levels correlated with disease activity even after the initiation of immunosuppressive therapy.

In conclusion, the measurement of RIP3 at an early stage of disease may help us to determine diagnosis and severity of SJS/TEN. Our study suggests a diagnostic role for RIP3 that needs to be evaluated in larger studies.

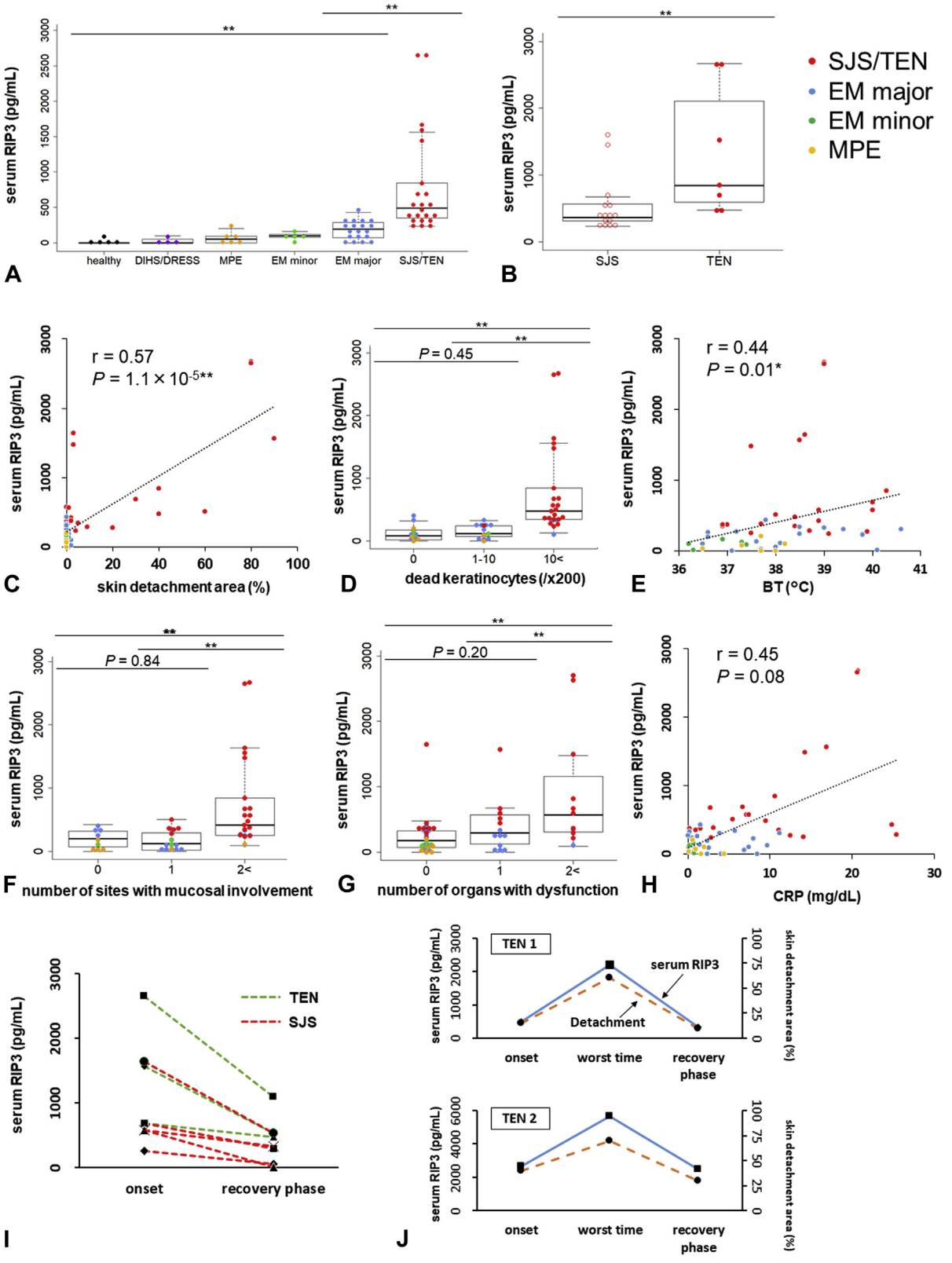

FIGURE 2. Association between serum RIP3 levels and clinical and laboratory parameters. (**A**) Serum RIP3 levels by disease type. (**B**) Serum RIP3 levels in SJS and TEN groups. (**C**) Skin detachment areas. (**D-H**) Correlations between serum RIP3 levels and (*D*) keratinocyte death, (*E*) body temperature, (*F*) sites with mucosal involvement, (*G*) organs with dysfunction, and (*H*) CRP levels. (**I**) Serum RIP3 levels at initial examination and the course of disease. (**J**) Changes in serum RIP3 levels and skin detachment areas of patients with TEN. *BT*, Body temperature; *CRP*, C-reactive protein. For all panels, $*P < .05$ and $**P < .01$.

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This study was supported by Japan Agency for Medical Research and Development (AMED) (grant no. 18ek0109317h0001 to R.A.) and Grant-in-Aid for Scientific Research (KAKENHI) (grant no. 18H04047 to R.A. and grant no. 18K16020 to N.H.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication September 4, 2019; revised January 2, 2020; accepted for publication January 3, 2020.

Available online January 15, 2020.

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2213-2198

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https://doi.org/10.1016/j.jaip.2020.01.006

REFERENCES

1. Pereira FA, Mudgil AV, Rosmarin DM. Toxic epidermal necrolysis. J Am Acad Dermatol 2007;56:181-200.
2. Abe R, Yoshioka N, Murata J, Fujita Y, Shimizu H. Granulysin as a marker for early diagnosis of the Stevens-Johnson syndrome. Ann Intern Med 2009;151:514-5.
3. Murata J, Abe R, Shimizu H. Increased soluble Fas ligand levels in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis preceding skin detachment. J Allergy Clin Immunol 2008;122:992-1000.
4. Su SC, Mockenhaupt M, Wolkenstein P, Dunant A, Le Gouvello S, Chen CB, et al. Interleukin-15 is associated with severity and mortality in Stevens-Johnson syndrome/toxic epidermal necrolysis. J Invest Dermatol 2017;137:1065-73.
5. Saito N, Qiao H, Yanagi T, Shinkuma S, Nishimura K, Suto A, et al. An annexin A1-FPR1 interaction contributes to necroptosis of keratinocytes in severe cutaneous adverse drug reactions. Sci Transl Med 2014;245:245ra95.
6. Kim SK, Kim WJ, Yoon JH, Ji JH, Morgan MJ, Cho H, et al. Upregulated RIP3 expression potentiates MLKL phosphorylation-mediated programmed necrosis in toxic epidermal necrolysis. J Invest Dermatol 2015;135:2021-30.
7. Qing DY, Conegliano D, Shashaty MG, Seo J, Reilly JP, Worthen GS, et al. Red blood cells induce necroptosis of lung endothelial cells and increase susceptibility to lung inflammation. Am J Respir Crit Care Med 2014;190:1243-54.
8. Shashaty MG, Reilly JP, Sims CA, Holena DN, Qing D, Forker CM, et al. Plasma levels of receptor interacting protein kinase-3 (RIP3), an essential mediator of necroptosis, are associated with acute kidney injury in critically ill trauma patients. Shock 2016;46:139-43.
9. Wang B, Li J, Gao HM, Xing YH, Lin Z, Li HJ, et al. Necroptosis regulated proteins expression is an early prognostic biomarker in patient with sepsis: a prospective observational study. Oncotarget 2017;8:84066-73.

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METHODS

Cell culture and induction of necroptosis

HaCaT cells were purchased from COSMO BIO (Tokyo, Japan) and cultured in Dulbecco modified Eagle medium (Gibco, Waltham, Mass), supplemented with 10% FBS (Gibco), 100 unit/mL penicillin, and 100 μg/mL streptomycin (Sigma, St Louis, Mo), in an incubator at 37°C under 5% CO_2. Cells were treated with 40 ng/mL TNF-α (T) (Sigma), 100 μM Smac-mimetics (S) (BioVision, Milpitas, Cass), and 40 μM z-VAD-fmk (Z) (R&D Systems, Minneapolis, Minn) to induce necroptosis.

RNA isolation and quantitative real-time PCR

Total RNA was isolated from HaCaT cells cultured with necroptosis inducers for 24 hours, using the RNeasy Mini Kit (Qiagen, Hilden, Germany). The RNA was transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, Calif) according to the manufacturer's instructions. Quantitative real-time PCR was performed using the THUNDERBIRD SYBR qPCR Mix (TOYOBO, Osaka, Japan). The sequences of primers used in the study were as follows: *RIP3* forward primer, 5′-CCTTCCAGGAATGCCTACCA-3′; *RIP3*-reverse primer, 5′-CGTGGAGACAGCAGCATTCA-3′; *β-actin* forward primer, 5′-AGAAAATCTGGCACCACACC-3′; *β-actin* reverse primer, 5′-GGGGTGTTGAAGGTCTCAAA-3′. Total RNA concentration in each sample was normalized to β-actin mRNA levels. The expression of *RIP3* is presented as the ratio of the *RIP3* mRNA to β-actin mRNA. Data were analyzed by the delta-delta Ct method.

Western blot analysis

HaCaT cells were lysed in a buffer containing 0.5% Triton X-100 with protease and phosphatase inhibitor cocktails. Insoluble material was removed by centrifugation. Lysates were boiled in sample buffer for 10 minutes and electrophoresed on a 4% to 12% SDS-polyacrylamide gel (Invitrogen). The resolved proteins were transferred to polyvinylidene fluoride or nitrocellulose membranes. The membranes were blocked with 5% nonfat milk in Tris-buffered saline containing Tween-20 (TBS-T) for 30 minutes and then incubated overnight at 4°C with the appropriate primary antibody prepared in the blocking solution. Antibodies against phosphorylated mixed lineage kinase domain-like (1:1000, Abcam, Cambridge, UK), mixed lineage kinase domain-like (1:1000, Abcam), phosphorylated RIP3 (1:1000, Cell Signaling Technology, Danvers, Mass), and RIP3 (1:1000, Abcam) were used. After a minimum of 10-hour incubation, the membranes were washed thrice with TBS-T for 5 minutes and incubated in secondary antibodies for 1 hour at room temperature. The membranes were then washed with TBS-T for 5 minutes and imaged using LuminoGraph I (ATTO, Tokyo, Japan).

Cell viability assay

Cell viability was evaluated using the RealTime-Glo MT cell Viability Assay kit (Promega, Madison, Wis). HaCaT cells were treated in 96-well plates with TNF-α, Smac-mimetics, and z-VAD-fmk (T/S/Z), then incubated at 37°C in a 5% CO_2 incubator. Luminescence in each well was measured at 3-hour intervals for 24 hours.

Patient selection

Patients diagnosed with SJS/ TEN (n = 22), EM minor (n = 5), EM major (n = 19), or maculopapular exanthema (MPE) (n = 6) were enrolled for the study. In patients presenting with a skin rash that was indistinguishable from MPE, EM, or SJS/TEN at the time of blood collection (SJS/TEN: n = 2, EM minor: n = 1, EM major: n = 5, MPE: n = 2), final diagnoses were made on the basis of skin manifestations and histopathologic findings. All patients with TEN had been clearly diagnosed as suffering from TEN when blood for the RIP3 assay was drawn. The patients for whom any medication had been initiated during the period 1 month before the enrollment and who did not have any episode of infection were classified into the "medication-caused" group, whereas those who did not receive any medication during this period but had had a clear episode of infection were classified into the "infection-caused" group. Patients for whom the cause of disease was not clear were classified into the "unknown" group. The patients in the infection-caused group were diagnosed with either herpes simplex virus (EM minor: n = 1, EM major: n = 1) or *Mycoplasma pneumoniae* (SJS/TEN: n = 4) infection. Patients with drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms (n = 4), bullous pemphigoid (BP) (n = 8), and pemphigus vulgaris (PV) (n = 6) were enrolled as disease controls. Drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms is a severe cutaneous adverse drug reaction characterized by herpesviruses reactivation. In the pathomechanisms of drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms, the association of necroptosis has not been evident.[E1] Controls (5 healthy adults) had no history of drug hypersensitivity. The details of the study were fully explained to each patient or his or her guardian, after which written informed consent was obtained. The study protocol was approved by the Ethics Committees of Niigata University, Showa University, and Yamanashi University. The study was conducted in accordance with the Declaration of Helsinki.

Laboratory tests and evaluation of clinical scores

White blood cell counts, C-reactive protein levels, body temperatures, number of mucosal involvement sites, number of injured organs, and histopathologic parameters of the patients were evaluated. The worst level for each clinical parameter was used for determining the correlation with the serum RIP3 level. We performed skin biopsies on all patients except 1 patient with EM minor. In histopathologic analysis, we assigned the patients to 3 groups depending on the number of dead keratinocytes observed in a field of view of hematoxylin and eosin−stained samples at ×200 magnification; the 3 groups had 0, 0 to 10, and more than 10 dead cells, respectively.

Measurement of serum RIP3 levels by ELISA

Serum RIP3 levels were determined at the first visit before the initiation of treatment and after clinical recovery using an ELISA kit, according to the manufacturer's instructions (Cusabio, Wuhan, China).[E2]

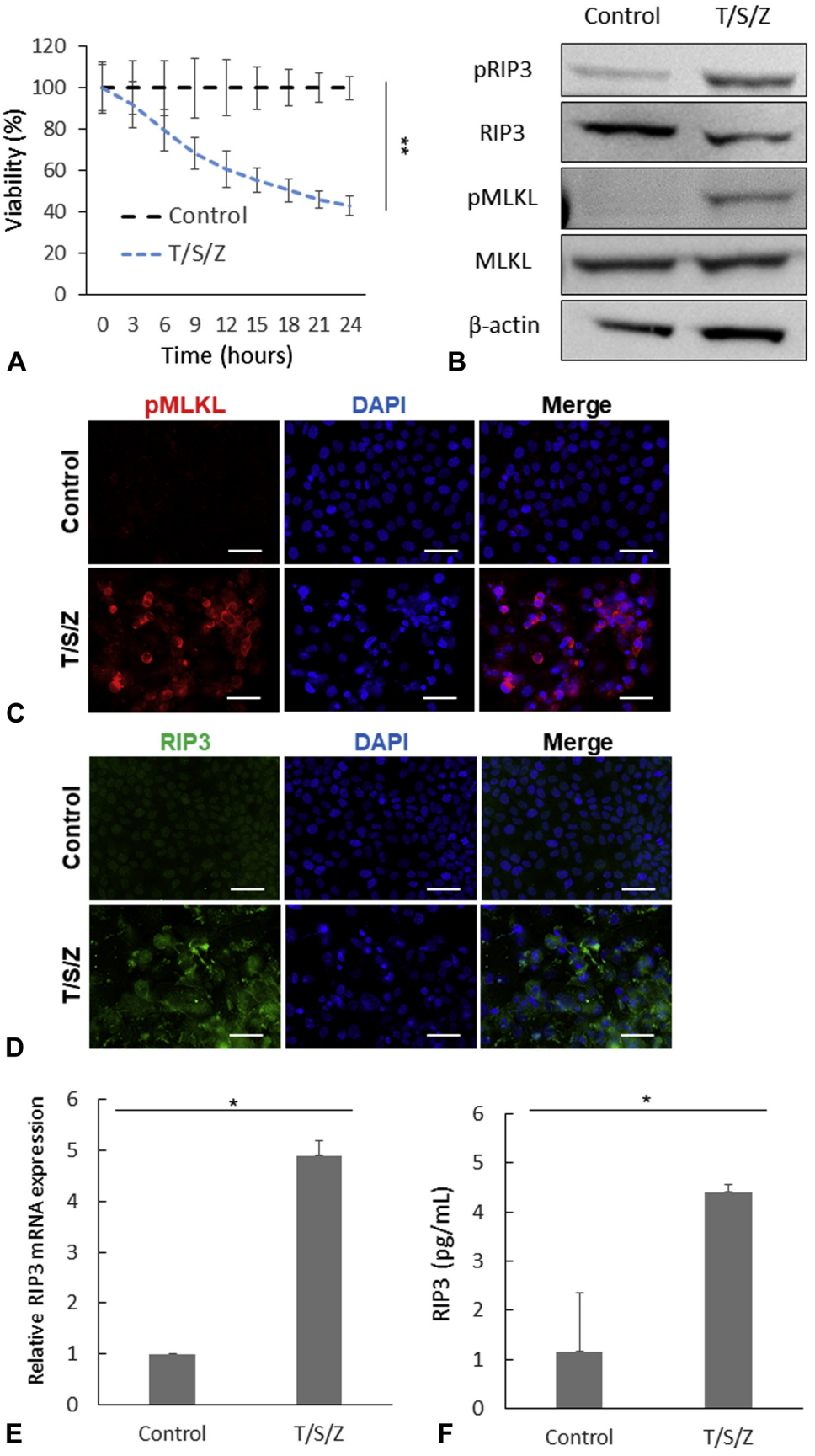
Immunofluorescence

Skin samples were obtained from nonbullous erythematous lesions of patients with SJS/TEN, EM, and MPE. Cryostat sections or HaCaT cells were fixed with 4% paraformaldehyde for 10 minutes and blocked with 10% goat serum for 30 minutes

at room temperature. Immunolabeling was performed with polyclonal anti-RIP3 (1:100, Novus Biologicals, Littleton, Colo), anti−phosphorylated RIP3 (1:100, Cell Signaling Technology), or anti−phosphorylated mixed lineage kinase domain-like (1:100, Signalway Antibody, College Park, Md) antibody overnight at 4°C, followed by incubation with secondary antibody for 30 minutes at room temperature in the dark. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (1:100, Vector Laboratories, Burlingame, Calif). Images were acquired using a Keyence BZ-X710 all-in-one fluorescence microscope (Keyence, Osaka, Japan). For immunofluorescence staining of frozen sections, we changed the green color of RIP3 to red using the BZ-X image converter (Keyence).

Statistical analyses

Statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for the R environment (The R Foundation for Statistical Computing, Vienna, Austria).[E3] Independent group comparisons between serum RIP3 levels and clinical parameters were determined using the Mann-Whitney *U* test. Correlations with clinical data were assessed by Spearman rank correlation test. The Wilcoxon signed-rank test was used to compare pairs of related samples. Statistical significance was assumed at $P < .05$.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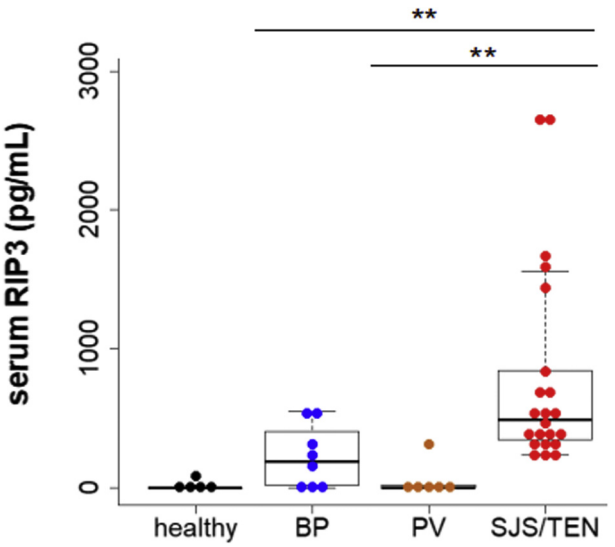

FIGURE E2. Comparison of serum RIP3 levels between the SJS/TEN group and autoimmune blistering disorders groups. Serum RIP3 levels compared between SJS/TEN and autoimmune blistering disorders. The serum RIP3 levels were significantly higher in the SJS/TEN group than in the BP ($n = 8$) and PV ($n = 6$) groups (BP: $P = .006$; PV: $P < .001$). $^{**}P < .01$. Notably, serum RIP3 levels of 2 patients in SJS/TEN group whose diagnosis was unclear because there was no observable skin detachment at the time of blood collection were higher (420.3 pg/mL, 247.6 pg/mL) than those of patients with BP or PV who had blisters or erosion (BP: 222.3 ± 220.7, PV: 53.3 ± 119.8). In addition, no significant differences were observed between the levels in BP or PV group and those of healthy controls (BP: $P = .05$; PV: $P < .73$).

FIGURE E1. RIP3 release from necroptotic keratinocytes. (**A**) Cell viability of HaCaT cells treated with TNF-α (40 ng/mL), Smac-mimetics (100 μM), and Z-VAD (40 μM) for 24 hours. Cell viability was measured for 3 hours for 24 hours. The viability of T/S/Z-treated HaCaT cells was significantly decreased compared with that of nontreated control cells. $^{**}P < .01$. (**B**) Western blot detection of RIP3, pRIP3, MLKL, and pMLKL in HaCaT cells treated with TNF-α (40 ng/mL), Smac-mimetics (100 mM), and Z-VAD (40 mM) for 24 hours. (**C** and **D**) Immunofluorescent staining of HaCaT cells treated with TNF-α (40 ng/mL), Smac-mimetics (100 μM), and Z-VAD (40 μM) for 24 hours. Cells were stained with pMLKL (red) and pRIP3 (green). Scale bar = 50 μm. (*B-D*) In T/S/Z-treated HaCaT cells, phosphorylation of RIP3 and MLKL was detected. (**E**) Expression of RIP3 mRNA. Values are normalized to nontreated controls. The mRNA expression of *RIP3* was increased in T/S/Z-treated HaCaT cells over that in nontreated control cells. $^{*}P < .05$. (**F**) Concentrations of RIP3 in the supernatants of HaCaT cells treated with TNF-α (40 ng/mL), Smac-mimetics (100 μM), and Z-VAD (40 μM) for 48 hours. *DAPI*, 4′,6-Diamidino-2-phenylindole; *MLKL*, mixed lineage kinase domain-like; p*MLKL*, phosphorylated mixed lineage kinase domain-like; *pRIP3*, phosphorylated RIP3. $^{*}P < .05$.

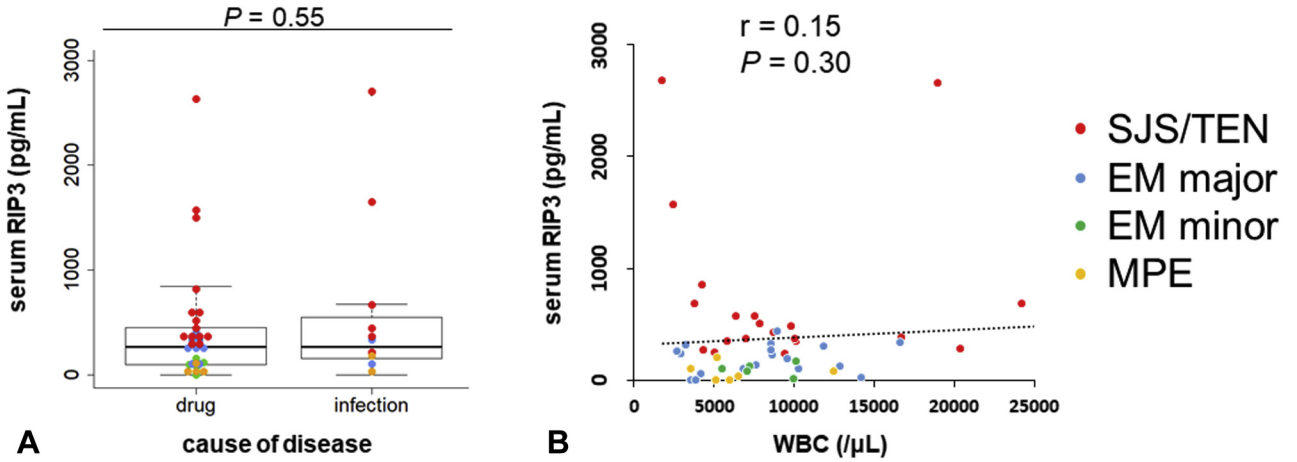

FIGURE E3. Associations between serum RIP3 levels and clinical and laboratory parameters. (**A**) Serum RIP3 levels in cases of medication-caused and infection-caused disease. There was no significant difference between the groups. Boxes represent medians with interquartile ranges. (**B**) Correlation between serum RIP3 levels and WBC counts. Statistical analysis by Spearman rank correlation test. *WBC*, White blood cell.

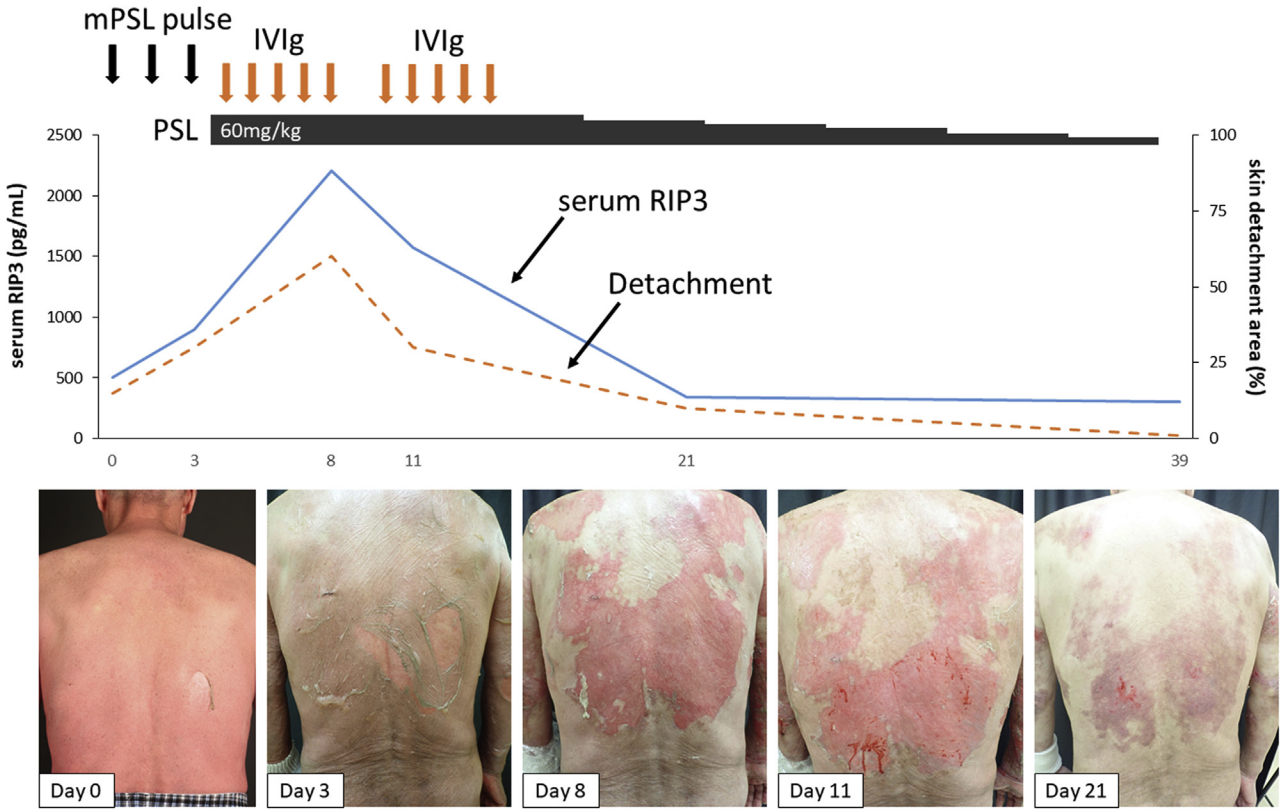

FIGURE E4. Clinical course of a patient with TEN. Changes in serum RIP3 levels and detachment areas with representative photos. The patient was a 67-year-old man. Vildagliptin and metformin hydrochloride combined was initiated for diabetes mellitus, and 2 weeks later, erythema and erosions appeared on the entire body. The skin detachment area at the first analysis was 15%. Mucosal symptoms were observed in the genital area and oral mucosa and the patient had mild kidney dysfunction. The severity-of-illness score for toxic epidermal necrolysis (SCORTEN) was 3 and the predicted mortality rate was 33%. The serum RIP3 level was 505.6 pg/mL at day 0. Although steroid pulse therapy was initiated, skin detachment spread further and serum RIP3 level increased to 896.3 pg/mL. Because the reactivity to steroid pulse therapy was poor, intravenous immunoglobulin (IVIg) therapy (400 mg/kg/d for 5 days) was performed immediately after the steroid pulse therapy and treatment with oral corticosteroid (1 mg/kg/d) was started. However, the skin detachment area spread to 60% of the body and the serum RIP3 level increased to 2205 pg/mL at the time the IVIg therapy was finished. Because the patient developed catheter infection, a second course of IVIg therapy was initiated, and disease activity gradually decreased. After recovery, the serum RIP3 level decreased to 341.1 pg/mL. Thirty-nine days after the initiation of treatment, the patient was completely healed and oral corticosteroid was discontinued. The serum RIP3 level decreased to 300.0 pg/mL. *mPSL*, methylpredonisolone; *PSL*, prednisolone.

TABLE E1. Patient attributes and outcomes

Variable	SJS/TEN	EM major	EM minor	MPE	DIHS/DRESS
n	22	19	5	6	4
Sex, male/female	12/10	8/11	2/3	2/4	2/2
Age (y), median (range)	61 (7-89)	65 (16-82)	65 (16-82)	57 (35-84)	66 (35-73)
Cause, n (%)					
Drug	14 (64)	11 (58)	5 (100)	4 (67)	4 (100)
Infection	6 (27)	4 (21)	0 (0)	2 (33)	0 (0)
Unknown	2 (9)	4 (21)	0 (0)	0 (0)	0 (0)
BT >38°C	15 (68)	9 (38)	0 (0)	2 (33)	2 (50)
Mucosal involvement, n (%)					
Eyes	15 (68)	7 (37)	1 (20)	2 (33)	1 (25)
Lips/mouth	22 (100)	13 (68)	4 (80)	1 (17)	0 (0)
Genital area/urinary tract	12 (55)	1 (5)	0 (0)	0 (0)	0 (0)
Organ dysfunction, n (%)					
Kidney	9 (41)	3 (16)	0 (0)	0 (0)	0 (0)
Liver	12 (55)	8 (42)	0 (0)	0 (0)	4 (100)
Lung	2 (9)	0 (0)	0 (0)	0 (0)	0 (0)
Gastrointestinal tract	3 (14)	1 (5)	0 (0)	0 (0)	0 (0)
Laboratory examination					
WBC count (/μL), median (range)	7850 (1790-24,200)	8610 (2730-16,630)	7230 (5530-10,130)	5600 (3540-12,460)	7850 (2710-10,290)
CRP (mg/dL), median (range)	9.54 (0.22-25.4)	2.6 (0.09-11.1)	0.7 (0.1-0.88)	1.31 (0.1-4.46)	1.78 (1.43-2.91)

BT, Body temperature; *CRP*, C-reactive protein; *DIHS*, drug-induced hypersensitivity syndrome; *DRESS*, drug rash with eosinophilia and systemic symptoms; *WBC*, white blood cell.

REFERENCES

E1. Shiohara T, Mizukawa Y. Drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS): an update in 2019. Allergol Int 2019;68:301-8.

E2. Qing DY, Conegliano D, Shashaty MG, Seo J, Reilly JP, Worthen GS, et al. Red blood cells induce necroptosis of lung endothelial cells and increase susceptibility to lung inflammation. Am J Respir Crit Care Med 2014;190:1243-54.

E3. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452-8.