



Monitoring the acute response in severe hypersensitivity reactions to drugs

Tetsuo Shiohara^{a,b}, Yoshiko Mizukawa^a, and Yumi Aoyama^c

Purpose of review

Severe adverse drug reactions (ADRs) including Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) are acute life-threatening conditions. There is the urgent need for reliable, noninvasive and standardized laboratory tests for identifying patients at higher risk of developing severe ADRs.

Recent findings

Although previous studies demonstrated the pathogenic role of TNF- α , IFN- γ , IL-10, perforin/granzyme B, Fas L and granulysin in the development of severe ADRs, there have been no biomarkers predicting progression to severe ADRs. We, therefore, measured serum levels of cytokines/chemokines as well as other biological markers in patients who presented with clinical symptoms suggestive of ADRs at their initial presentation. The results show that sFas L represents a useful early biomarker that can predict the subsequent progression to TEN, but not SJS, particularly when combined with the increase in IL-6 and IP-10. The increased levels of IL-6 and IP-10 are reliable biomarkers predictive of the progression to severe ADRs, such as SJS/TEN and DiHS/DRESS.

Summary

The use of a combination of several early biomarkers, although not sufficiently sensitive or specific on its own when used alone, could increase the diagnostic and prognostic utility for the prediction of severe ADRs.

Keywords

adverse drug reactions, biomarkers, cytokines, virus

INTRODUCTION

Although severe adverse drug reactions (ADRs) are uncommon, these reactions can occur in anyone who takes drugs: the list of drugs causing severe ADRs in susceptible individuals is constantly growing. Among severe ADRs, the most serious and potentially life-threatening ADRs are Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS). Because these severe ADRs initially present with a wide variety of clinical manifestations and the clinical course and pattern are unpredictable, it is quite difficult to label cutaneous lesions as severe ADRs in the early stage of disease before clinical signs compatible with severe ADRs develop. Indeed, the diagnosis of severe ADRs relies almost entirely on the presence of clinical symptoms and histopathological findings of the involved target organs, and there are no currently used laboratory tests that can predict

the risk of developing severe ADRs. Thus, there is the urgent need for reliable laboratory tests for identifying patients at higher risk of developing severe ADRs. Another diagnostic dilemma is determining whether eruptions occurring during viral or bacterial infections could be the result of medications or the infection itself. In this regard, Yoon *et al.* [1] reported that procalcitonin levels were preferentially increased in patients with bacterial infection, but not in those with delayed-type drug hypersensitivity. Thus, monitoring procalcitonin

^aDepartment of Dermatology, ^bDivision of Flow Cytometry, Kyorin University School of Medicine, Tokyo and ^cDepartment of Dermatology, Kawasaki Hospital, Kawasaki Medical School, Okayama, Japan

Correspondence to Tetsuo Shiohara, MD, PhD, Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan. Tel: +81 422 47 5511 Ext 3562; fax: +81 422 41 4741; e-mail: tpshio@ks.kyorin-u.ac.jp

Curr Opin Allergy Clin Immunol 2015, 15:294–299

DOI:10.1097/ACI.0000000000000180

KEY POINTS

- The increase in serum sFas L, IL-6 and IP-10 at the initial presentation represents a useful early biomarker that can predict the subsequent progression to TEN, but not SJS.
- The increased levels of IFN- γ , IL-7 and IL-10 at the initial presentation may be a useful predictor of SJS patients who will not progress to TEN.
- Identifying patients with a high risk of subsequently progressing to severe ADRs by the use of a combination of several early biomarkers is relevant for clinical decision-making.

levels can help clinicians distinguish ADRs from bacterial infections, although this biomarker has not been validated in a blind manner using samples from different institutions.

This review discusses profiling the most clinically relevant biomarkers of severe ADRs for discovering safe and effective treatment modality to reduce morbidity and mortality that might be more effective if introduced early.

CYTOKINES RELEVANT FOR THE DEVELOPMENT AND RESOLUTION OF ADVERSE DRUG REACTIONS

Because severe ADRs are considered to be T-cell-mediated [2,3], a number of previous studies investigated whether cytokines/chemokines released from activated T cells and other immune cells could play a key role in the development of these severe ADRs. Because cytokine/chemokine production and release are a complex and tightly controlled and sequentially orchestrated process, it is possible that cascades of cytokines/chemokines initially responsible for the development of inflammatory responses could later be not involved or alternatively involved in the resolution process, more likely is the combinatorial and sequential involvement of different sets of cytokines and soluble mediators, one or the other being more dominant at particular stages in the disease process.

Fixed drug eruption (FDE) is thought to be a simplified disease model for elucidating the mechanism(s) of how skin inflammation is induced by skin-resident T cells in severe ADRs [4,5]. A complicated multistep process from the initiation of inflammatory responses to eventual tissue damage observed in severe ADR lesions could be examined in sequence by following the evolution of individual FDE lesions after clinical challenge with the causative drug, because in this entity controlled

administration is safe and is still the most reliable method for establishing the causative drug in FDE [6]. The similarity between SJS/TEN and FDE, particularly the generalized form of FDE, in clinical features and histologic patterns has been proposed by several authors [7–9].

We, therefore, asked whether sequential cytokine measurements after clinical challenge in patients with the generalized form of FDE clinically and histologically mimicking SJS/TEN could provide the unique opportunity of following the evolution of drug-induced immune responses that may otherwise result in the development of SJS/TEN. Because our previous unpublished studies showed that cytokine/chemokine levels have returned to normal 1 month after clinical resolution, we analyzed the serum levels of cytokine/chemokines before and after challenge in patients with the generalized form of FDE who were challenged with the causative drug more than 1 month after clinical resolution. Such generalized form of FDE could be analyzed as a single disease process occurring early in the disease course of severe ADRs because mechanisms involved in drug-induced T-cell activation start as soon as the causative drug is administered. We expected that biomarkers involved in various stages of drug-induced immune responses will be identified that correlate with clinical or subclinical symptoms.

As shown in Table 1 [7], serum TNF- α and IL-8 levels were elevated dramatically at 3 h after clinical challenge, the earliest time point we examined, at which time slight erythema appeared as the initial symptoms [10]. The marked elevation of TNF- α and IL-8 levels was associated with an onset of a low fever, but not with abnormal laboratory findings, such as elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). The levels of TNF- α reached its maximum at 5.5 h and remained elevated at 5.5–9 h, whereas the IL-8 levels peaked at 3 h and decreased thereafter. The decrease in the IL-8 levels at 5.5 and 7 h was associated with the elevation in the IL-6 and IL-10 levels; circulating IL-10 was first detected at 5.5 h, peaked at 7 h and decreased thereafter. A gradual increase in the IFN- γ levels was observed at 3–9 h; but the IFN- γ levels after clinical challenge did not reach those observed on her first presentation (data not shown), probably because of systemic prednisone given at 9 and 12 h that may have prevented further production of IFN- γ . Of note was our observation that IL-10 levels were first detected at 5.5 h after challenge, at the time when circulating proinflammatory cytokine (TNF- α , IL-6 and IL-8) levels began to decrease, and the IL-10 levels at various time points showed a clear-cut inverse relationship with the corresponding TNF- α , IL-6 and IL-8 levels. In view of the action

Table 1. Serum cytokine levels before and after clinical challenge in a patient with generalized fixed drug eruption (modified from the data presented in reference [7])

	TNF- α (pg/ml)	IFN- γ (IU/ml)	IL-10 (pg/ml)	IL-8 (pg/ml)	IL-6 (pg/ml)	IL-4 (pg/ml)
Before challenge	21.5	<0.03	<1.4	7.0	<6.3	<4.9
3 h	130.0	0.03	<1.4	76.0	<6.3	7.0
5.5 h	135.0	1.00	170.0	33.0	85.8	<4.9
7 h	115.0	1.65	245.0	27.0	160.5	<4.9
9 h ^a	115.0	3.20	138.0	54.0	166.5	<4.9
24 h ^b	<5.5	1.10	<1.4	<3.0	<6.3	<4.9

^aBefore administration of systemic corticosteroids.^b16 h after administration of systemic corticosteroids.

of IL-10 on immune responses [10–13], elevated levels of serum IL-10 during the development of ADRs could reflect an appropriate response that serves to protect tissues from destructive autoimmune attack by activated tissue-resident T cells [4,5] and the relative balance between IL-10 and proinflammatory cytokines in a disease tissue would influence whether the inflammation progresses to the life-threatening outcome (severe ADRs) or is suppressed (FDE). If so, the levels of IL-10 may have prognostic value in the setting of ADRs. Indeed, our unpublished observation indicates that serum IL-10 levels were never elevated despite dramatic increases in proinflammatory cytokine levels in a patient with TEN who resulted in the fatal outcome. Contrary to this finding, however, recent studies have demonstrated that increased serum IL-10 levels can also be detected in patients with TEN [14]. In this regard, we found that IL-10 was not elevated in association with progression to TEN, as described later. Another important factor is the timing of IL-10 production, which can determine the outcome of drug-induced immune responses. Thus, our data provide one potential explanation for why drug-induced immune responses in the generalized form of FDE resolve spontaneously upon discontinuation of the causal drug despite the clinical and histological resemblance to TEN: whether IL-10 can be produced at a relatively early time point after drug introduction may determine whether the patient has self-limited FDE or progress into TEN. It is apparent, however, that measurements of single cytokine are not sufficiently sensitive on its own for identifying patients at higher risk of severe ADRs.

COMBINATION OF MULTIPLE BIOMARKERS FOR PREDICTION OF DEVELOPING SEVERE ADVERSE DRUG REACTIONS

Although numerous previous studies [15–24] demonstrated the pathogenic role of soluble mediators including cytokines/chemokines secreted by

different immune cells including TNF- α , IFN- γ , IL-10, perforin/granzyme B, Fas L and granulysin, there are few biomarkers or prognostic tests available to predict disease progression that can be used in clinical routine for diagnosis and to monitor patients with ADRs. In this regard, the simultaneous use of these biomarkers may increase specificity and sensitivity as a diagnostic tool. We, therefore, determined serum levels of various cytokines/chemokines in patients with various types of ADRs at their initial presentation before the appearance of typical clinical symptoms. We sought to elucidate a characteristic cytokine profile associated with each clinical phenotype and possibly linked to the pathogenesis, because thus far no studies have addressed cytokine/chemokine profiles predictive of progression to severe ADRs in patients with ADRs at their initial presentation before treatment.

Because we sought to determine which combination of cytokines/chemokines could identify patients who eventually progressed to severe ADRs, serum samples were obtained from patients who were initially presented with clinical symptoms suggestive of ADRs or viral infections and were eventually diagnosed as the following six categories: SJS, TEN, DiHS/DRESS, generalized FDE, maculopapular ADR and viral exanthema: excluded were patients who had received systemic corticosteroids before the initial presentation. Diagnosis of each phenotype was made at later times based on their criteria.

As shown in Fig. 1, the IL-6 and IP-10 levels were significantly higher in SJS/TEN and DiHS patients, in particular those in TEN were the highest: they may reflect the severity of the disease (Mizukawa Y *et al.* article submitted). The IL-16 levels were significantly increased in FDE, SJS and DiHS/DRESS patients, but not in TEN patients. These results clearly indicate that increased levels of IL-6 and IP-10 in sera are reliable biomarkers predictive of the progression to severe ADRs such as SJS/TEN and probably DiHS/DRESS. Because increased IL-6 and interferon gamma-induced protein 10 (IP-10) levels

	TEN	SJS	DiHS/DRESS	FDE	MP	Virus
IL-6	↑↑	↑↑	↗	~	~	↗
IL-8	↑↑	↑	↗	~	~	↑
G-CSF	↑↑	↑	↗	~	~	↗
IL-15	↑↑	↑↑	↑	~	~	↑
Granulysin	↑↑	↑↑	↑↑	~	~	~
sFas L	↑↑	~	↗	~	~	~
IP-10	↑↑	↑	↑	↗	↗	↗
TNF- α	↑	↑	↑↑	↗	~	↑
IL-10	↗	↑	↑↑	~	~	↑
IL-5	~	↑	↑↑	~	↑	~
IL-1 α	↗	↗	↗	↑↑	↑	↑
IL-2	~	↑	↗	↑↑	~	↑
IL-17	↗	↑	~	~	↑	↑↑
IFN- γ	~	↑	↗	↗	↗	↑↑
IL-4	↑	~	↗	~	~	↑↑
IL-16	↑	↑↑	↑	↑↑	N.D.	~
IL-13	~	↑	↑↑	~	~	↑

FIGURE 1. Increased cytokine/chemokines in sera of patients with adverse drug reactions and viral exanthem at their initial presentation before typical clinical symptoms develop. Each symbol represents the magnitude of alterations of cytokine/chemokine levels in patients when compared with the mean cytokine/chemokine levels in healthy controls. Double 'increase' or 'decrease' symbol indicates a profound increase or decrease. Single 'increase' or 'decrease' symbol indicates an increase or decrease. Wavy lines represent no significant alterations. Slanting 'increase' represents a slight increase. N.D., not done.

apparently preceded the appearance of the full-blown pictures by days, these cytokines could serve as both a diagnostic and a predictive tool in monitoring patients with ADRs. These findings suggest that both IL-6 and IP-10 might be involved in the early event required for the development of severe ADRs but may not be unique to each clinical phenotype as late events; although SJS/TEN and DiHS/DRESS represent opposite ends of severe ADRs, they may share important common pathophysiologic processes. Although additional studies are needed to determine whether IL-6 and IP-10 act synergistically or independently, serum IL-6 and IP-10 levels at the initial presentation could be promising surrogate biomarkers for predicting disease progression to severe ADRs. Similar tendency was also observed in the IL-8 and granulocyte-colony stimulating factor (G-CSF) levels: increased

levels of IL-6, IL-8, G-CSF and IP-10 may reflect the severity of the disease, indicating an injurious outcome.

Importantly, serum sFas L levels at the initial presentation were the highest in TEN patients who subsequently progressed to TEN, but not to SJS. Although sFas L [18–22] and granulysin [23,24] have been implicated in the pathological process of SJS/TEN, these findings suggest that sFas L represents a useful early biomarker that can predict the subsequent progression to TEN, but not SJS, particularly when combined with the increase in serum IL-6 and IP-10 levels (Fig. 2). Because TEN has been placed in the same disease category with SJS, we were surprised to find a highly significant difference in the sFas L level between SJS and TEN at the earliest stage. Thus, serum sFas L levels at the initial presentation can be used for effectively distinguishing patients at the risk of subsequently developing TEN from patients with SJS.

Surprisingly, the IL-17 level was significantly increased in patients with viral exanthema but not in these severe ADRs and rather decreased in DiHS/DRESS patients, although there was no statistically significant difference among these ADRs groups. Although IL-17 has been implicated in the pathogenesis of inflammatory and autoimmune diseases [25,26], we did not detect a correlation with the serum level and the disease phenotype of ADRs. This was an unexpected finding because it has been reported that IL-17 expression was increased in TEN and its level correlated with clinical severity of the disease [27]. This could be explained by the fact that the first measurement of cytokine levels in our cohort was performed at the earliest time point, 3–7 days after onset, whereas previous studies measured samples that were taken at later time points (usually 2 weeks after onset), even after starting therapy. In view of our finding that the IL-17 levels rather increased after starting treatment with corticosteroids, increased IL-17 levels that had been previously reported in TEN may have resulted from a possible delay in taking serum samples during the disease course. Alternatively, it is tempting to speculate that increased serum IL-17 levels during the course of the disease may serve to prevent progression to severe ADRs and may be bifunctional depending on other cytokine levels.

Unlike other Th1-type cytokines, the IL-2 level appeared to be the opposite of what would be expected if increased production were simply a Th1-type response: the IL-2 level was the highest in FDE, a milder form of ADRs, whereas the increase was not observed in other ADRs. If this finding can be validated, high IL-2 levels could be interpreted as increased IL-2 levels being early biomarkers

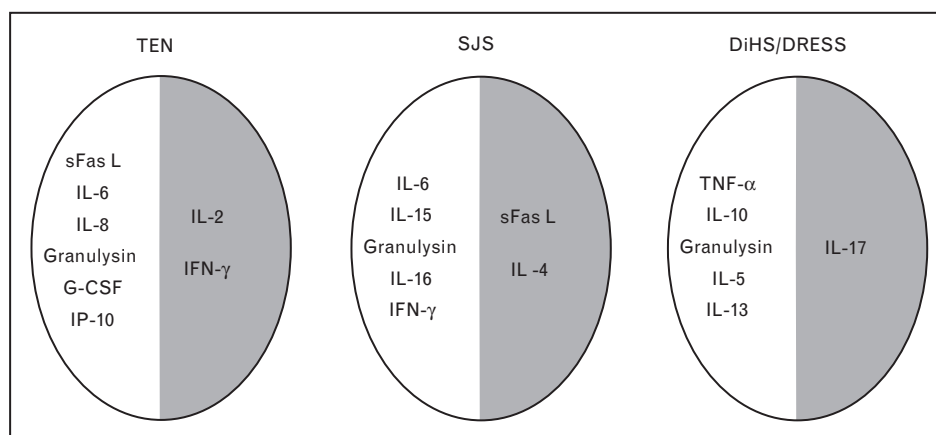


FIGURE 2. Combinations of multiple early biomarkers useful for predicting progression to severe ADRs. A light area indicates increased cytokines/chemokines in each type of severe ADRs. A dark area indicates decreased cytokines/chemokines. ADRs, adverse drug reactions.

predictive of beneficial outcomes: the IL-2 levels, particularly when combined with increased IL-16 levels, may predict better prognosis. To determine the physiologically relevant source of IL-16, we have recently analyzed skin lesions of severe ADRs, SJS/TEN, by using immunohistochemistry with anti-IL-16 and antitryptase antibodies. The results show that expression of IL-16 was colocalized with tryptase-positive mast cells (Mizukawa Y *et al.* article submitted). Because accumulation of IL-16-positive mast cells beneath the epidermis was preferentially observed in the vicinity of Foxp3⁺ regulatory T cells (Tregs) in FDE lesions, but not in SJS/TEN lesions, a likely interpretation of these observations, in consideration of the data presented here, is that mast cells residing in the FDE lesions represent a major source of IL-16 in the setting of ADRs and that mast cells contribute to the resolution of ADR lesions by recruiting Tregs through the release of IL-16. This emphasizes the importance of Tregs as a negative regulator of TEN [28²²,29²³,30]. Interestingly, the IL-16 levels were significantly increased not only in FDE and SJS, but also in DiHS/DRESS patients, but not in TEN patients.

Contrary to our initial expectation, increased IL-10 levels were observed in SJS and DiHS/DRESS, but not in TEN. Because IL-10 generally acts as an immunosuppression during inflammatory diseases [11,12], the serum IL-10 level was initially thought to be associated with milder forms of ADRs. However, the increase was associated with the development of severe ADRs. Nevertheless, one surprising finding was that serum IL-10 levels in SJS were much higher than those in TEN despite clinical similarities between the two diseases. Our data could be interpreted as an indication that increased IL-10 levels associated with increased levels of IL-2, IL-5 and IFN- γ at the initial presentation may be a useful

predictor of SJS patients who will not progress to TEN: alternatively, relatively low levels of IL-10 associated with increased IL-6 and IP-10 levels could be interpreted as suggesting the subsequent progression to TEN.

As systemic corticosteroids are recommended for the treatment of many ADRs, and could have a significant impact on the levels of these biomarkers' levels, it is important to determine to what extent these markers could be influenced by the systemic corticosteroids and the treatment outcomes. However, although our preliminary findings indicate that many of these biomarkers were profoundly decreased in ADR patients after starting systemic corticosteroids, it would be premature to conclude which biomarkers could be prospectively used for predicting patients' clinical response to systemic corticosteroids before starting treatment with oral prednisolone. Despite it being well recognized that skin biopsies are also of value for diagnosing severe ADRs at the earliest stage of the disease, measurements of these biomarkers, rather than biopsy samples, are more practical, noninvasive and easily repeatable approaches for identifying patients at risk of progressing to severe ADRs and those who may respond to treatment. This is because a biopsy sample only represents a 'snapshot' during the dynamic process and time course of the ADR as it progresses. An important limitation of the present study was the relatively small number of samples from patients with severe ADRs that were available for our analysis, especially, because samples from patients who had received systemic corticosteroids before their initial presentation were excluded from this study and those from patients who had been complicated by apparent bacterial and viral infections at the initial presentation were also excluded. Thus, this limitation needs to be

taken into account when drawing conclusions from the data presented.

CONCLUSION

The use of a combination of several early biomarkers, although not sufficiently sensitive or specific on its own when used alone, could increase the diagnostic and prognostic utility for the prediction of severe ADRs before typical clinical symptoms develop. These biomarkers also provide information on the clinical course of the disease to distinguish patients destined to have beneficial outcomes from those who will have poor outcomes. Identifying patients with a high risk of subsequently progressing to severe ADRs by such a noninvasive method is relevant for clinical decision-making, as these patients may benefit from timely and selective therapeutic interventions. These biomarkers may represent novel therapeutic targets that could be inhibited by future anticytokine treatment. The findings presented here provide the theoretical basis for the use of various biologicals for control of the evolution of severe ADRs, for which no specific therapy has received wide acceptance as the best evidence-based therapy in patients with severe ADRs to date. This search should be more widely validated to assess the exact sensitivity and specificity of these measurements in a larger, independent, prospective, multicenter study. This should include samples from patients with other inflammatory diseases other than ADRs, because of the rarity of severe ADRs.

Acknowledgements

None.

Financial support and sponsorship

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (to T.S.) and the Health and Labour Sciences Research Grants (Research on Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan (to T.S.).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Yoon SY, Baek SH, Kim TB, *et al.* Serum procalcitonin as a biomarker differentiating delayed-type drug hypersensitivity from systemic bacterial infection. *J Allergy Clin Immunol* 2013; 132:981–983.
2. Castrejón JL, Berry N, Naisbitt DJ, *et al.* Stimulation of human T cells with sulfonamides and sulfonamide metabolites. *J Allergy Clin Immunol* 2010; 125:411–418.
3. Depta JP, Altnauer F, Pichler WJ, *et al.* Drug interaction with T-cell receptors: T-cell receptor density determines degree of cross-reactivity. *J Allergy Clin Immunol* 2004; 113:519–527.
4. Shiohara T. Fixed drug eruption: pathogenesis and diagnostic tests. *Curr Opin Allergy Clin Immunol* 2009; 9:316–321.
5. Shiohara T, Mizukawa Y. Fixed drug eruption: the dark side of activation of intraepidermal CD8⁺ T cells uniquely specialized to mediate protective immunity. *Chem Immunol Allergy* 2012; 97:106–121.
6. Breathnach SM. Management of drug eruptions: part II. Diagnosis and treatment. *Australas J Dermatol* 1995; 36:187–191.
7. Kauppinen K. Fixed drug eruptions and oral challenge. *Cleve Clin J Med* 1991; 58:64–65.
8. Brahimi N, Routier E, Descamps V, *et al.* A three-year analysis of fixed drug eruptions in hospital setting in France. *Eur J Dermatol* 2010; 20:461–464.
9. Lipowicz S, Sekula P, Mockenhaupt M, *et al.* Prognosis of generalized bullous fixed drug eruption: comparison with Stevens-Johnson syndrome and toxic epidermal necrolysis. *Br J Dermatol* 2013; 168:726–732.
10. Mizukawa Y, Shiohara T. Nonpigmenting fixed drug eruption as a possible abortive variant to toxic epidermal necrolysis: immunohistochemical and serum cytokine analyses. *Clin Exp Dermatol* 2010; 35:493–497.
11. Mosser DM, Zhang X. Interleukin-10 new perspectives on an old cytokine. *Immunol Rev* 2008; 226:205–218.
12. Cope A, Le Friec G, Kemper C, *et al.* The Th1 life cycle: molecular control of IFN- γ to IL-10 switching. *Trends Immunol* 2011; 32:278–286.
13. Rutz S, Ouyang W. Regulation of interleukin-10 and interleukin-22 expression in T helper cells. *Curr Opin Immunol* 2011; 23:605–612.
14. Correia O, Delgado L, Barbosa IL, *et al.* Increased interleukin 10, tumor necrosis factor alpha, and interleukin 6 levels in blister fluid of toxic epidermal necrolysis. *J Am Acad Dermatol* 2002; 47:58–62.
15. Wolkenstein P, Latarjet J, Revuz J, *et al.* Randomised comparison of thalidomide versus placebo in toxic epidermal necrolysis. *Lancet* 1998; 352:1586–1589.
16. Polak ME, Belgi G, Ardern-Jones MR, *et al.* In vitro diagnostic assays are effective during the acute phase of delayed-type drug hypersensitivity reactions. *Br J Dermatol* 2013; 168:539–549.
17. Caproni M, Antiga E, Fabbri P, *et al.* Elevated circulating CD40 ligand in patients with erythema multiforme and Stevens-Johnson syndrome/toxic epidermal necrolysis spectrum. *Br J Dermatol* 2006; 154:1006–1007.
18. Viard I, Wehrli P, French LE, *et al.* Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 1998; 282:490–493.
19. Abe R, Shimizu T, Shimizu H, *et al.* Toxic epidermal necrolysis and Stevens-Johnson syndrome are induced by soluble Fas ligand. *Am J Pathol* 2003; 162:1515–1520.
20. Stur K, Karhofer FM, Stingl G. Soluble FAS ligand: a discriminating feature between drug-induced skin eruptions and viral exanthemas. *J Invest Dermatol* 2007; 127:802–807.
21. 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.
22. Posadas SJ, Padial A, Blanca M, *et al.* Delayed reactions to drugs show levels of perforin, granzyme B, and Fas-L to be related to disease severity. *J Allergy Clin Immunol* 2002; 109:155–161.
23. Chung WH, Hung SI, Chen YT, *et al.* Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Nat Med* 2008; 14:1343–1350.
24. Saito N, Abe R, Shimizu H, *et al.* Prolonged elevation of serum granulysin in drug-induced hypersensitivity syndrome. *Br J Dermatol* 2012; 167:452–453.
25. Ghoreschi K, Laurence A, O'Shea JJ, *et al.* T helper 17 cell heterogeneity and pathogenicity in autoimmune disease. *Trends Immunol* 2011; 32:395–401.
26. Yosef N, Shalek AK, Regev A, *et al.* Dynamic regulatory network controlling TH17 cell differentiation. *Nature* 2013; 496:461–468.
27. Teraki Y, Kawabe M, Izaki S. Possible role of TH17 cells in the pathogenesis of Stevens-Johnson syndrome and toxic epidermal necrolysis. *J Allergy Clin Immunol* 2013; 131:907–909.
28. Shiohara T, Takahashi R, Ushigome Y, *et al.* Regulatory T cells in severe drug eruptions. *Curr Immunol Rev* 2014; 10:41–50.
- This review summarizes the function of Treg cells in patients with severe drug eruptions and the functional defect developing at the different stages.
29. Yang C, Mosam A, Saavedra A, *et al.* HIV infection predisposes skin to toxic epidermal necrolysis via depletion of skin-directed CD4⁺ T cells. *J Am Acad Dermatol* 2014; 70:1096–1102.
- This study demonstrates the importance of CD4⁺CD25⁺Foxp3⁺ Treg cells in mediating protection against T-cell-mediated epidermal injury.
30. Dang EV, Barbi J, Pan F, *et al.* Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* 2011; 146:772–784.