BJD
British Journal of Dermatology

Longitudinal analysis of antibody profiles against plakins in severe drug eruptions: emphasis on correlation with tissue damage in drug-induced hypersensitivity syndrome and drug reaction with eosinophilia and systemic symptoms

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Linked Comment: Chu. *Br J Dermatol* 2016; **175**:866–867

Summary

Background The evidence for severe drug eruption as a trigger for autoimmune disease has recently increased. No information is available on how tissue damage in severe drug eruptions can induce autoimmune responses.

Objectives To investigate whether the generation of autoantibodies (autoAbs) against plakin family proteins could be the cause or result of tissue damage in patients with severe drug eruptions and whether the generation of autoAbs could be prevented by systemic corticosteroids during the acute stage.

Methods We retrospectively analysed alterations of serum levels of autoAbs against plakin family proteins in patients with Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) during the acute stage and long after resolution over a period of more than 10 years.

Results AutoAbs against plakin family proteins were detected in patients with either SJS/TEN or DiHS/DRESS regardless of the epidermal damage in the acute stage, and were sustained even long after resolution in DiHS/DRESS, indicating that those autoAbs are neither the cause nor the consequence of epidermal damage, at least in DiHS/DRESS. Severe liver damage and noncorticosteroid therapy during the early and acute stages of DiHS/DRESS were associated with the subsequent generation of these autoAbs.

Conclusions These autoAbs are neither necessarily the cause nor the result of epidermal damage in DiHS/DRESS, because the presence of these autoAbs was not restricted to patients with SJS/TEN but was also observed in those with DiHS/DRESS, which is characterized by lack of epidermal damage. Severe liver damage and/or immune responses that could be prevented by corticosteroids in the acute stage of DiHS/DRESS are among the causal factors contributing to the generation of autoimmune responses.

What's already known about this topic?

- Evidence for severe drug eruption as a trigger for autoimmune diseases has increased.

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Accepted for publication
12 April 2016

Funding sources
This work was supported by a grant from the Ministry of Health, Labour and Welfare (Research for Intractable Diseases matching fund subsidy, H26-081) and the Ministry of Education, Culture, Sports, Science and Technology of the Japan Government.

Conflicts of interest
None declared.

DOI 10.1111/bjd.14677

- Sera from patients with Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) have been shown to contain autoantibodies (autoAbs) against epidermal proteins generated as a consequence of epidermal damage.
- No information is available about the existence of these autoAbs in drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS), characterized by lack of epidermal damage and gradual loss of regulatory T-cell function.

What does this study add?

- These autoAbs were more persistently and frequently detected in patients with DiHS/DRESS than in SJS/TEN.
- In DiHS/DRESS, liver damage during the early and acute phases, together with a regulatory T-cell dysfunction, would serve to generate autoAbs.

What is the translational message?

- Patients with severe drug eruptions benefit from corticosteroid therapy during the acute stage with regard to the subsequent development of autoimmune responses.
- Achievement of early resolution by corticosteroids is associated with a lower risk of subsequently developing autoimmune responses, particularly in patients with DiHS/DRESS.
- The need to relieve clinical symptoms by corticosteroids should be balanced with antimicrobial therapies aiming at reducing the risk of infectious diseases.

Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) represent the polar ends of a wide clinicopathological spectrum of severe drug eruptions. SJS/TEN is characterized by severe epidermal damage, while no epidermal damage is the hallmark of DiHS/DRESS.[1] Previous studies suggested that sera obtained from patients in the acute stage of SJS/TEN and erythema multiforme contain autoantibodies (autoAbs) against epidermal proteins, which could have been generated as a consequence of epidermal damage.[2] Moreover, although there is no information available about the existence of autoAbs against epidermal proteins in the sera of patients with DiHS/DRESS, an observational study conducted in our facilities over a period of 10 years found autoAbs or autoimmune diseases in approximately 10% of patients with DiHS/DRESS.[3] Thus, the evidence for severe drug eruption as a trigger for autoimmune disease has increased over the past 10 years based on recognition of the temporal association between severe drug eruptions and autoimmune diseases, and their pathogenetic association.[3–9]

An early clue to the pathogenetic link between preceding severe drug eruptions and the subsequent development of autoimmune responses was noted in our previous finding that SJS/TEN and DiHS/DRESS are characterized by defective regulatory T cell (Treg) responses during the acute and resolution stages, respectively.[10] Indeed, a series of studies addressed the role of defects in Treg number and function in human autoimmunity.[11–15] However, it is difficult to determine whether these defects are causative and/or a consequence of autoimmune pathology. Thus, there is a great need for longitudinal analyses to determine when and how autoimmune responses are generated, by using samples obtained at various time points including during the course of the disease and long after resolution.

We therefore decided to evaluate thoroughly changes in the serum levels of autoAbs against epidermal proteins in patients with severe drug eruptions. We sought to extend previous observations on the existence of autoAbs in severe drug eruptions including DiHS/DRESS, and in the disease course beyond the acute stage, by describing how their profile could be altered over a long period of time after resolution. Long-term persistence of autoAbs is probably more important than their profile at the acute stage.

Materials and methods

Patients and clinical definitions

This study was approved by the institutional review board at Kyorin University School of Medicine and Okayama University School of Medicine, Dentistry and Pharmaceutical Sciences. Patients with severe drug eruptions who visited these university hospitals between 2002 and 2014 were analysed. The patients were divided into three groups according to the clinical presentation: SJS (n = 30, 19 male and 11 female), TEN (n = 9, two male and seven female) and DiHS/DRESS (n = 27, 12 male and 15 female), as previously described.[6] The diagnosis of SJS, TEN

and DiHS/DRESS was made based on their criteria: all patients in this study met the full criteria for SJS, TEN[16] and DiHS/DRESS[17] proposed by the Japanese Severe Cutaneous Adverse Reaction Group. The mean ages of the patients with SJS, TEN and DiHS/DRESS were 55·8 ± 18·6 years, 51·7 ± 16·1 years and 56·8 ± 17·2 years, respectively.

The culprit drug had been discontinued once the diagnosis was suspected. All patients with SJS and TEN received systemic corticosteroid therapy. Patients with DiHS/DRESS were classified into two groups according to their management: a systemic corticosteroid-treated group of 12 patients (four male and eight female, age 62·8 ± 15·4 years) and a noncorticosteroid-treated group of 15 patients who were given supportive care only without the use of immunosuppressive agents (eight male and seven female, age 52·1 ± 17·0 years). The initial oral corticosteroid dose for SJS, TEN and DiHS/DRESS was 0·6–1·0 mg kg^{-1} daily, after which the dose was gradually tapered.

Patient sera

After informed consent was obtained, serum samples were obtained from these patients at or near the time of the initial presentation before the start of therapy and thereafter on a biweekly or monthly basis during the course of the disease, even after clinical resolution. Blood samples obtained at the various time points were classified into four phases depending on the timing of sampling: the first 9 days after onset (early phase), 10–29 days after onset (acute phase), 30–99 days after onset (resolution phase) and ≥ 100 days after onset (late resolution phase). Analyses of samples were performed, which included 79 samples from 30 cases of SJS, 33 samples from nine cases of TEN, 74 samples from 27 cases of DiHS/DRESS and 27 samples from healthy volunteers.

Preparation of recombinant periplakin

The bacterial expression constructs of periplakin (PPL) were a kind gift from Dr Lars Komorowski. The recombinant PPL (N1–324) His-tagged protein is reported to be highly reactive to autoAbs in paraneoplastic pemphigus (PNP).[18] Recombinant PPL was prepared following a previously reported method.[18]

Indirect immunofluorescence of rat bladder epithelium

Indirect immunofluorescence (IIF) was conducted using cryostat sections of rat urinary bladder as previously described.[19] All sera from the patients and healthy volunteers were diluted 1 : 100. All samples were examined using confocal microscopy.

Immunoblotting

Immunoblot analyses of extracts of HaCaT cells and recombinant PPL (N1–324) were performed. The separated proteins were transferred electrophoretically to a polyvinylidene difluoride membrane. The membrane strips were blocked with 5% skimmed milk in phosphate-buffered saline, and incubated with patient serum diluted at 1 : 1000, and then with horseradish peroxidase-conjugated antihuman IgG diluted at 1 : 10 000. The location of desmoplakin (DP) and PPL was determined with an anti-DP1+2 antibody and anti-PPL antibody (both Abcam, Cambridge, U.K.) diluted 1 : 1000. The enzyme reaction was visualized using the ECL Select Western Blotting Detection System and analysed using ImageQuant LAS 4000 (both GE Healthcare Japan, Tokyo, Japan).

Enzyme-linked immunosorbent assay

Envoplakin enzyme-linked immunosorbent assay (ELISA) (Euroimmun AG, Lübeck, Germany) was performed following the instruction manual.

Statistical analyses

Analysis for statistically significant differences was performed with Student's t-test, Wilcoxon test, Welch's t-test and Fisher's exact test. Results were considered significant with $P < 0·05$.

Results

Detection of autoantibodies against epidermal proteins in patients with severe drug eruptions

As shown in Figure 1, the sera of many patients contained multiple autoAbs against epidermal proteins, including 250-kDa, 210-kDa and 190-kDa polypeptides that comigrated with DP1, DP2 and PPL. Thus, these sera contained various autoAbs

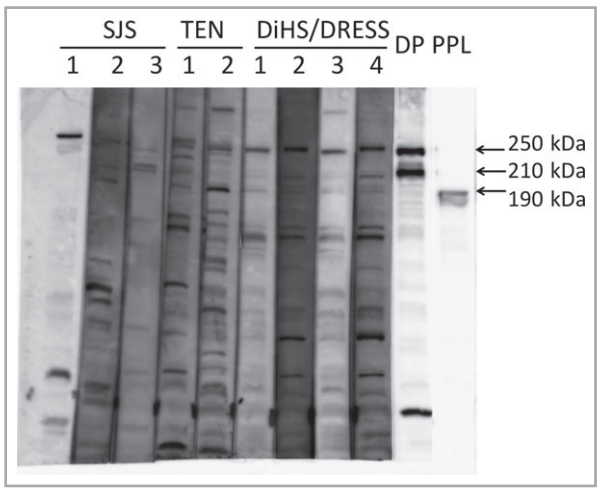

Fig 1. (a) Profiles of autoantibodies (autoAbs) in representative sera from patients with Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS). Representative result of Western blotting using HaCaT cell lysate with SJS, TEN and DiHS/DRESS sera. Some patient sera reacted with the 250-kDa, 210-kDa and 190-kDa antigens and other antigens. For controls, labelling was performed using specific autoAbs against desmoplakin (DP)1 and DP2 and periplakin (PPL) in the right two lanes.

that bind to characteristic subsets of epidermal proteins observed in PNP.[20]

The presence of autoAbs against epidermal proteins in serum samples from the 66 cases was examined by IIF using rat bladder epithelium, because this test was shown to be highly specific for the diagnosis of PNP.[20] Positive staining was observed in 13 cases (20%) regardless of the clinical phenotype, but the staining pattern observed in these severe drug eruptions was different from that in PNP, in that most of the positive cases showed a positive cytoplasmic or nuclear staining pattern and no positive intercellular staining (Fig. 2 and Table 1). No positive staining was observed with control samples including sera from healthy volunteers (data not shown).

Because autoAbs bound to antigens of 190 kDa in size may have reacted against epidermal proteins other than PPL, we next examined whether patients' sera contained autoAbs against recombinant fragments of PPL. Among the recombinant fragments, we used fragment PPL N1–324, as it represents the domain that is frequently recognized by autoAbs in patients with PNP.[18] A negative control was included, using serum from a healthy control. AutoAb binding to the PPL N1–324 fragment was detected in patients' sera at different intensities, whereas binding was not detected in sera from healthy controls (data not shown). Importantly, the reactivity of sera was persistently detected in patients with DiHS/DRESS, while it gradually decreased with time and eventually disappeared at later times in patients with SJS and TEN (Fig. 3).

The presence of autoAbs against envoplakin (210 kDa) was also examined by a commercially available ELISA kit. AutoAbs against envoplakin were not detected in any of the serum

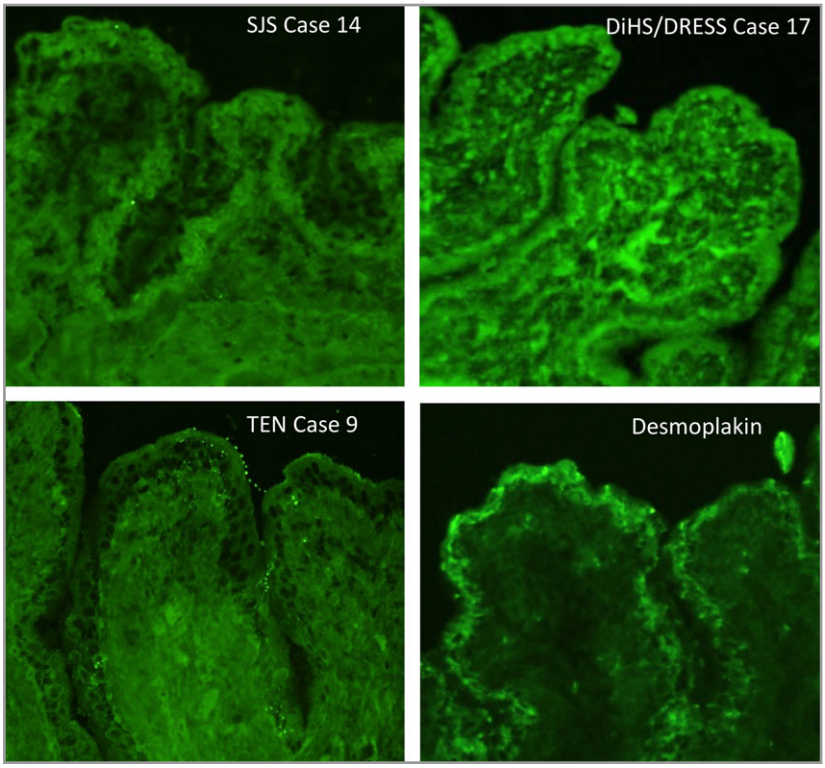

Fig 2. Representative result of indirect immunofluorescence of rat bladder epithelium using Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) sera. Note that an intercellular staining pattern was not observed with any serum, and only a cytoplasmic or nuclear staining pattern was seen.

Table 1 Summary of Western blotting and indirect immunofluorescence (IIF) results of patients with Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) and healthy controls

Antigen	SJS (n = 30)	TEN (n = 9)	DiHS/DRESS (n = 27)	Healthy controls (n = 27)
250 kDa	24 (80)	7 (78)	15 (56)	0
210 kDa	6 (20)	4 (44)	9 (33)	0
190 kDa	13 (43)	7 (78)	16 (59)	3 (11)
Recombinant periplakin N1–324	7 (23)	5 (56)	13 (48)	1 (4)
IIF on rat bladder epithelium	6 (20): 5 cytoplasmic, 1 nuclear	4 (44): 4 cytoplasmic	3 (11): 1 cytoplasmic, 2 nuclear	0

Numbers (%) of positive results are shown. A case was considered positive if an autoantibody was detected at least once during the clinical course. Serum samples were obtained at various time points after onset from each case over a 2–10-year period.

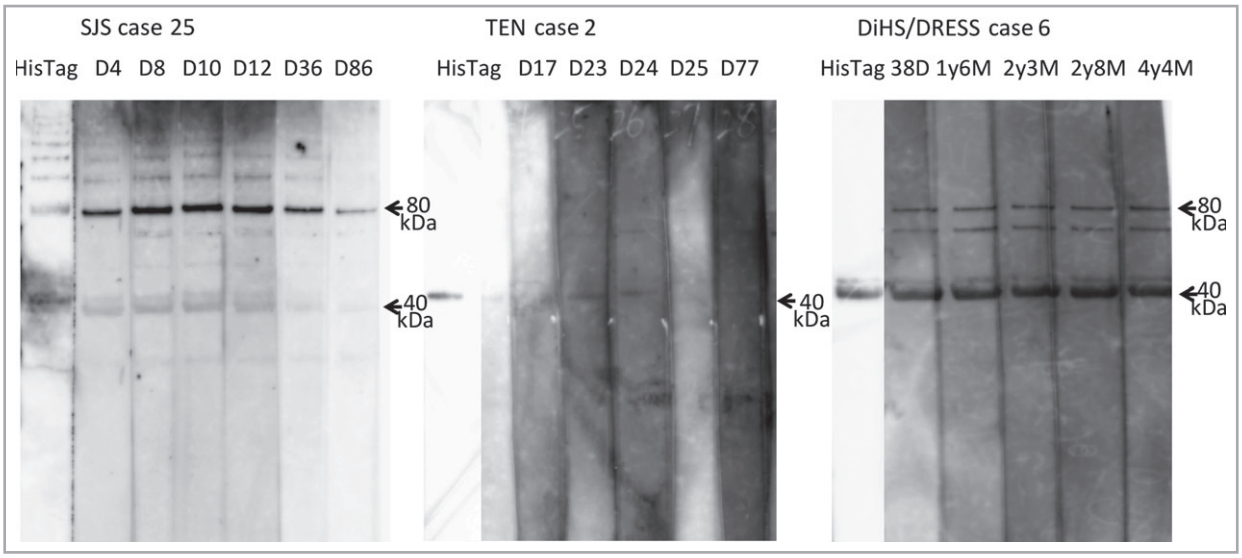

Fig 3. Representative results of immunoblotting in cases of Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) showed alterations of autoantibody (autoAb) levels against recombinant periplakin (PPL) N1–324 during the disease course. AutoAbs against recombinant PPL N1–324 were initially detected on day 4 and then decreased on day 36 in SJS (case 25) and on day 25 in TEN (case 2). In contrast, autoAbs against recombinant PPL N1–321 were clearly detected during the entire observation period for more than 4 years in DiHS/DRESS in case 6. Arrows indicate the position of recombinant PPL N1–324.

samples examined (data not shown). The detailed results of Western blotting and IIF for each clinical phenotype are shown in Table 1. AutoAbs against PPL (190 kDa and PPL N1–324) were more frequently detected in sera from patients with TEN and DiHS/DRESS, although there was no significant difference between the phenotype and the antigens detected. These results indicate that autoAbs against epidermal proteins including PPL can be generated during and after the development of severe drug eruptions. The low frequency of autoAbs against the conformational epitope detected with IIF was consistent with the findings in a previous study.[18] This suggests that the epitopes of autoAbs against epidermal proteins detected in patients with severe drug eruptions are different from those in patients with PNP.

Persistent detection of autoantibodies in patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms

Sequential analyses of the autoAbs against PPL N1–324 at different time points in a representative patient with DiHS/DRESS showed that autoAbs appeared 38 days after onset, and the levels increased continuously with time (Fig. 3). We therefore compared the detection rate of various autoAbs against 250-kDa, 210-kDa and 190-kDa polypeptides or PPL N1–324, depending on the clinical phenotype and the clinical phases examined. Because of the relatively small number of SJS/TEN samples available for analysis, data from cases of SJS and TEN were combined for further analyses. As shown in Table 2, the autoAb detection rates were highest in sera from patients with DiHS/DRESS that had been obtained at the late resolution phase ($\geq$ 100 days after onset, $P < 0{\cdot}001$). Overall, patients' antigen profiles at each time point appeared to correlate with the clinical phenotype; these autoAbs tended to persist much longer in patients with DiHS/DRESS than in those with SJS/TEN.

Correlation between liver dysfunction and the subsequent generation of autoantibodies

As shown in Table 3, the only significant difference between the autoAb-positive and -negative groups was the mean serum level of alanine aminotransferase (ALT) during the early and acute phases of disease. Although the mean serum levels of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were also higher in the autoAb-positive group than in the negative group, the differences were not statistically significant (AST, $P = 0{\cdot}14$; ALP, $P = 0{\cdot}16$). In addition, severe liver dysfunction, defined as ALT > 200 IU L^{-1}, was not observed during the early and acute phases in patients who were negative for autoAbs against epidermal proteins (Fig. 4). These results indicate that severe liver damage during the early and acute phases of DiHS/DRESS may be one of the causal factors contributing to the generation of these autoAbs. However, no relevant link could be established between other internal organ involvement, such as the kidneys, and the generation of autoAbs. A link between the severity of clinical illness as evidenced by the area of erythema and the generation of autoAbs was not established.

Table 2 Summary of the detection rate of autoantibodies in serum samples obtained in the different clinical phases of Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS)

	SJS/TEN (n = 39)			DiHS/DRESS (n = 27)			
Sampling date (days)	Total samples (cases)	Positive samples (cases)	% Positive samples (cases)	Total samples (cases)	Positive samples (cases)	% Positive samples (cases)	P-value[a]
1–9 (early phase)	47 (32)	6 (4)	13 (12)	12 (11)	5 (4)	42 (36)	NS
10–29 (acute phase)	30 (18)	9 (7)	30 (39)	14 (12)	4 (3)	29 (25)	NS
30–99 (resolution phase)	19 (16)	4 (4)	21 (25)	17 (12)	6 (5)	35 (42)	NS
≥ 100 (late resolution phase)	16 (10)	1 (1)	6 (10)	31 (17)	22 (10)	71 (59)	< 0·001

NS, not significant. Total and positive numbers of samples and numbers of cases are shown. Serum samples were obtained from each case over a 2–10-year period. In most cases, multiple samples were obtained at various time points: intervals from onset to days of sampling are shown as 'sampling date'. A case or sample was considered to be positive if at least one of the autoantibodies against any plakin family protein was positive. [a]Determined using Fisher's exact test.

Table 3 Differences in clinical features including laboratory data during the early and acute phases of drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) between the autoantibody-positive group who developed antibodies against 250-kDa, 210-kDa and 190-kDa polypeptides or recombinant periplakin N1–324 and the autoantibody-negative group.

	Antiplakin antibodies	
	Positive (n = 20)	Negative (n = 5)
ALT (IU L^{-1})[a,b]	356·2	88·2
AST (IU L^{-1})[a]	252·6	101·6
ALP (IU L^{-1})[a]	669·5	364·4
Blood urea nitrogen > 20·0 mg dL^{-1}, n (%)	5 (25)	2 (40)
Creatinine > 1·07 mg dL^{-1}, n (%)	3 (15)	2 (40)
Area of erythema (% body surface area), mean ± SD	81 ± 3·8	80 ± 7·1
Detection of autoantibody, n (%)[c]	9 (45)	2 (40)
Autoimmune disease, n (%)	4 (20)	1 (20)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. [a]Mean serum levels. [b]P < 0·01 was considered statistically significant, Welch's t-test. [c]Autoantibodies include antinuclear antibodies, extractable nuclear antigens and antithyroid antibodies.

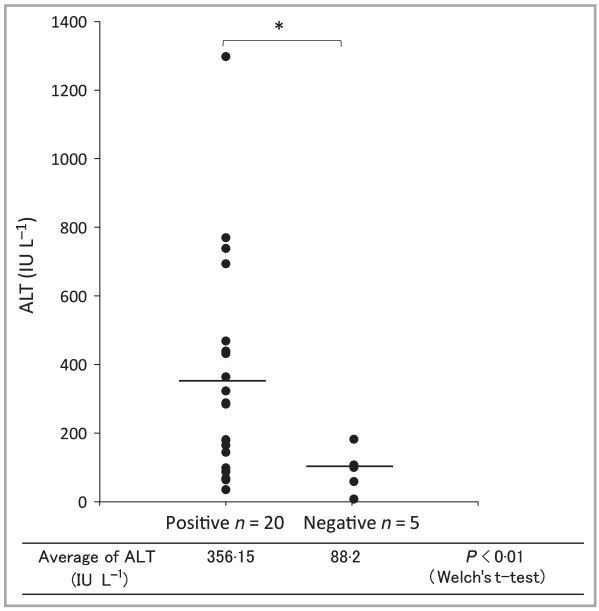

Fig 4. Differences in serum alanine aminotransferase (ALT) levels in the early and acute phases of drug-induced hypersensitivity syndrome (DiHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) between the autoantibody-positive group (antibodies against either 250-kDa, 210-kDa and 190-kDa antigens or recombinant periplakin N1–324) and the autoantibody-negative group. The serum ALT levels shown represent the highest seen during the early and acute phases of DiHS/DRESS. A statistically significant difference was determined using Welch's t-test.

Effects of systemic corticosteroids on the subsequent generation of autoantibodies

Because 12 of 27 patients with DiHS/DRESS were treated with systemic corticosteroids during the early, acute and resolution phases, while the remaining patients were not given systemic corticosteroids, patients with DiHS/DRESS were divided into two groups: corticosteroid-treated and noncorticosteroid-treated groups. These two groups were age matched (62·8 ± 15·4 years and 52·1 ± 17·0 years, respectively), and no significant difference was seen in disease severity at the initial presentation (e.g. fever, body surface area of involvement and degree of liver dysfunction).

As shown in Table 4, the detection rates of autoAbs against 210-kDa and 190-kDa polypeptides were significantly higher in the noncorticosteroid-treated group than in the corticosteroid-treated group (P < 0·01). The number and frequency of patients in whom autoAbs against the 250-kDa polypeptide,

Table 4 Differences in the detection rates of autoantibodies during the observation period between noncorticosteroid and corticosteroid treatment groups in drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms

Antigens	Noncorticosteroid (n = 15)	Corticosteroid (n = 12)	P-value[a]
250 kDa	11 (73)	4 (33)	NS
210 kDa	9 (60)	0	< 0·01
190 kDa	12 (80)	3 (25)	< 0·01
Periplakin N1–324	8 (53)	5 (42)	NS
Both 190 kDa and periplakin N1–324	8 (53)	3 (25)	NS

Values are n (%) positive. NS, not significant. [a]Statistically significant difference was determined using Fisher's exact test.

PPL N1–324 and 190-kDa polypeptide plus PPL N1–324 were generated were also higher in the noncorticosteroid-treated group (Table 4). The results indicate that immune responses that are preventable with systemic corticosteroids could trigger the generation of these autoAbs. Based on our results, we conclude that severe drug eruptions could be a trigger of autoimmunity in a clinical phenotype-specific manner, and that these patients might benefit from corticosteroid therapy during the acute stage with regard to the subsequent development of autoimmune responses.

Discussion

In this study, we show that circulating autoAbs to various epidermal proteins including DP1 and DP2 (250 kDa and 210 kDa), envoplakin (210 kDa) and PPL (190 kDa) were present in the sera of patients with DiHS/DRESS at the same frequency as in sera of patients with SJS/TEN. Because the clinical findings of SJS/TEN show striking similarities to those of PNP, and autoAbs against plakin family proteins such as DP1, DP2, envoplakin and PPL have been consistently detected in PNP,[18–20] it has been suggested that these autoAbs play a pathogenic role in acantholysis in PNP.[21] In contrast, several previous studies demonstrated that autoAbs against DPs 1 and 2 and PPL were also detected in patients with erythema multiforme major[21–23] and SJS/TEN,[2] respectively, and they suggested that these autoAbs might be associated with epidermal damage. In this regard, our results clearly indicate that these autoAbs were present not only in patients with SJS/TEN but also in patients with DiHS/DRESS, particularly beyond the time frame of the acute inflammatory response in the latter. These contradictory results could be reconciled by assuming that the generation of autoAbs may be a direct consequence of severe epidermal damage in SJS/TEN but not in DiHS/DRESS.

In view of our findings that autoAb levels appeared to increase gradually with time in some patients with DiHS/DRESS, tissue damage occurring during the early or acute phase could act as a trigger for the subsequent development of autoimmunity: severe drug eruptions are likely to be a trigger of autoimmunity, at least in a proportion of patients. Consistently with this possibility, we reported that antinuclear antibodies were not detected during the acute stage of DiHS/DRESS, but their level gradually elevated and was 1 : 5120 at 4 years after resolution in a patient with DiHS/DRESS who eventually developed sclerodermoid graft-versus-host disease-like lesions.[24] In addition, a recent case report has shown that DiHS/DRESS preceded the autoimmune blistering disease by up to 2 years.[25] Indeed, there will always be some amount of collateral tissue damage other than epidermal damage during the course of severe drug eruptions.

There is a constant risk of autoreactive responses damaging host tissue if adequate suppressive controls are missing. A gradual loss of Treg function has been reported to occur after resolution of DiHS/DRESS, although they are expanded during the acute stage.[10] Even in SJS/TEN, Treg function is severely impaired during the acute stage but not in the resolution stage.[10] Such time-dependent, defective Treg responses observed in these severe drug eruptions provide an explanation for why autoimmune responses can be generated at different time points in the two types of severe drug eruptions, in the acute phase of the SJS/TEN and after clinical resolution of DiHS/DRESS.

Because recent studies have demonstrated that target antigens such as PPL, which were thought to be specific for the epidermis, are also expressed in the liver,[26] generation of autoAbs to PPL could reflect a large amount of collateral tissue damage, probably occurring in the liver during the course of DiHS/DRESS and even after resolution. In support of this possibility, the proportion of sera showing positive reactivity to plakin family proteins was significantly higher in patients with DiHS/DRESS who exhibited severe liver dysfunction (ALT > 200 IU L^{-1}) than in those who showed moderate liver dysfunction during the early or acute phase. Furthermore, the mean serum levels of ALT during the early and acute phases of DiHS/DRESS were significantly higher in patients with autoAbs than in those without. The currently favoured mechanism is that defective Treg responses occurring either during the early or acute phase of the disease (SJS/TEN) or after clinical resolution (DiHS/DRESS) drive chronic and debilitating inflammatory responses that can cause severe collateral tissue damage and generate excessive or inappropriately directed immune response to self-components.

We infer from our results that at least two factors, severe liver or epidermal damage and defective Treg function, would be needed for the subsequent generation of autoAbs against epidermal proteins in patients with DiHS/DRESS. Indeed, our recent unpublished observations indicate that in DiHS/DRESS, Treg functions became defective during the acute and early resolution phases (days 11–36), immediately after the peak of severe liver damage, while in SJS/TEN, Treg functions were defective only during the acute phase when severe epidermal damage was at its peak. Thus, both factors need to be present sequentially but not concomitantly to achieve optimal generation of autoAbs against epidermal proteins in DiHS/DRESS. In other words, the timing of defective Treg function is a crucial factor in the generation of autoAbs against epidermal proteins.

Because there was no significant difference in levels of other autoAbs, such as antinuclear antibodies, antiextractable nuclear antigens and antithyroid antibodies, between the antiplakin antibody-positive and -negative groups (Table 3), severe liver damage during the early and acute phases is not crucial for the generation of antinuclear antibodies, antiextractable nuclear antigens and antithyroid antibodies. These data illustrate the need for more longitudinal studies examining the temporal relationship between defective Treg function and severe tissue damage in order to understand the sequence of immune events that take place after the onset of severe drug eruptions.

A key question is whether preventing inflammation, particularly by systemic corticosteroids, can prevent generation of autoimmunity. Although further large-scale controlled trials are needed to resolve this issue, our results clearly demonstrate that immune responses responsible for the development of autoAbs could be prevented in part by treatment with corticosteroids during the early and acute phases of DiHS/DRESS. Our data further illustrate the importance of achieving early resolution as a therapeutic goal in severe drug eruptions. Although systemic corticosteroids are clearly effective for alleviating a variety of clinical symptoms observed during the acute stage of severe drug eruptions, particularly in DiHS/DRESS, their effect on the long-term outcomes is far from certain. During the course of DiHS/DRESS, systemic corticosteroids have shown promising results in terms of ameliorating vigorous immune responses to either drugs or viruses. Because a gradual loss of Treg function occurring after resolution of DiHS/DRESS[10] could also increase the risk, systemic corticosteroids administered during the early and acute phases may be able to prevent a gradual loss of Treg function by restoring impaired Treg activity.

In conclusion, self-inflicted immune responses occurring during the early and acute phases of severe drug eruptions could be a trigger for the development of autoAbs. Longitudinal analyses of autoAbs offer the possibility for screening patients at high risk of autoimmune disease. In the wider context, the scenario in severe drug eruptions may be applicable to many other inflammatory conditions as important events that trigger autoimmune responses.

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