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INVESTIGATIVE REPORT

Diagnostic Role of Soluble Fas Ligand Secretion by Peripheral Blood Mononuclear Cells from Patients with Previous Drug-induced Blistering Disease: A Pilot Study

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Toxic epidermal necrolysis and Stevens-Johnson syndrome are severe blistering diseases generally considered to be hypersensitivity reaction to drugs. The aim of this study is to explore the diagnostic role of soluble Fas ligand secretion by peripheral blood mononuclear cells (PBMCs) in patients with previous drug-induced blistering diseases with or without stimulation with the offending drug. The results revealed that PBMCs from the patient group showed significant soluble Fas ligand secretion after stimulation with 100 μg/ml culprit drug ($p<0.05$). No significant change was seen in the control groups ($p>0.05$). Taken as a group, PBMCs from the patient group secreted more soluble Fas ligand than the normal control group regardless of drug stimulation. These results indicate that individuals whose PBMCs are high secretors of soluble Fas ligand are prone to severe toxic epidermal necrolysis/Stevens-Johnson syndrome. The possibility of using soluble Fas ligand secretion as a tool for retrospective determination of culprit drugs in patients with previous drug-induced blistering disease awaits further investigation. ***Key words: Stevens-Johnson syndrome; toxic epidermal nercolysis; peripheral blood mononuclear cell; soluble Fas ligand.***

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Toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS) are severe blistering diseases generally considered to be hypersensitivity reactions to drugs. The most commonly encountered offending agents include antibiotics, anticonvulsants, non-steroidal anti-inflammatory agents and allopurinol (1, 2). A thorough medical history is the basis of a correct diagnosis. Nevertheless, identification of the causative drug(s), which is essential for future prevention, cannot always be made from the available clinical data. The drug challenge test remains the most specific and sensitive method for determining culprit drugs, but the risk of severe reaction associated with drug re-challenge prevents both physicians and patients from participating in this.

T lymphocytes have been shown to participate in the pathogenesis of cutaneous drug eruptions. The phenotype of the involved T cells and the cytokine secretion profile of these T cells may correlate with the type of immune response that evolves after drug exposure (3). At present, the lymphocyte transformation test (LTT) is the most commonly used *in vitro* test for detecting drug sensitization regardless of the effector mechanisms and the clinical phenotype of the reaction (4, 5). However, since LTT involves working with radioactive substances and has its limitations, routine use of this method is considered impractical (4, 5). A recent review by Merk described the current concepts for LTT and the modification of LTT readouts using cytokine secretions from the peripheral blood mononuclear cells (PMBC) (6).

Several reports have shown that high-dose intravenous immunoglobulin therapy may be effective in blocking the progression of SJS, although some have argued otherwise (7, 8). TEN and SJS are still considered as a potentially fatal diseases, from which it is difficult to guarantee complete recovery (9, 10). Identification of the offending drug is essential to prevent patients from future exposure. Viard et al. (11) has previously shown that up-regulation of keratinocyte Fas ligand expression may trigger keratinocyte destruction during disease progression of TEN. It has been reported recently that soluble Fas ligand (sFasL) produced by PBMCs in patients with TEN and SJS also play a crucial role in the pathogenesis of the disease process (12). Moreover, in the same report, it was shown that sFasL secretion by PBMCs from a patient with active disease was up-regulated after stimulating with the causal drug. Therefore, we aimed to explore the sFasL secretion profile of PBMCs from patients with previous TEN/SJS with or without stimulation with the offending drug and hoped to determine its potential clinical application.

MATERIALS AND METHODS

Patient selection

Since this is the first report investigating the use of sFasL on patients with TEN/SJS after active disease, we recruited

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our patient group from those who had been admitted to our hospital due to TEN/SJS in the past 3 years. The culprit drug was confirmed by the stringent drug-allergy history-category A according to the criteria previously described by Nyfeler & Pichler (5). Because carbamazepine (CBZ) was the most frequently encountered culprit drug for causing TEN/SJS in our hospital, the patients with previous TEN/SJS due to this agent were recruited. Four patients fulfilled our inclusion criteria and agreed to participate in the study (Table I). Two control groups were also included in this study. A healthy, age- and sex-matched control without known allergy to drugs was matched to every patient. The second control group, the CBZ control group, consisted of two individuals, a 55-year-old man and a 63-year-old woman, taking CBZ for more than 4 months without clinical signs of adverse drug reaction.

Isolation of mononuclear cells and experimental conditions

PBMCs were isolated from heparinized venous blood after centrifugation at 900×g for 30 min over a Ficoll-Paque cushion (Amersham Biosciences, Uppsala, Sweden; specific gravity 1.077) as previously described (13). Ten ml of heparinzed blood collected from the patient and control groups were layered onto 3 ml of Ficoll-Paque. The tubes were then centrifuged at 900×g for 30 min at 18°C. The upper layer was drawn off using a clean pipette. Subsequently, the layer containing mononuclear cells (including monocytes and lymphocytes) was aspirated into a clean tube. The mononuclear cells were washed twice using phosphate-buffered saline (PBS). The pellets were then suspended with RPMI 1640 supplemented with 10% of foetal calf serum (Gibco, Grand Island, New York, USA) at a concentration of 1×10^6 cells/ml. Freshly isolated mononuclear cells (1×10^6 cells/ml) were stimulated with carbamazepine (CBZ) (0, 10, or 100 μg/ml) or sulindac, a commonly used non-steroidal anti-inflammatory agent (0, 10, or 100 μg/ml) (Sigma, St Louis, MO, USA), according to the experimental protocol, for 24 h. The concentrations of CBZ chosen were modelled after Abe et al. (12) who have used similar concentrations in their study. The concentration used (10 μg/ml) was comparable to the therapeutic plasma concentration of CBZ (4–12 μg/ml). The supernatants were collected and analysed with a commercially available sFasL ELISA kit (R&D Systems, Minneapolis, MN, USA) for determination of sFasL levels.

Statistical analysis

The statistical analysis was performed with SPSS system for Windows version 10.0 (SPSS Inc., Chicago, IL, USA). The significance of the differences within the groups after various treatments (CBZ/sulindac) was tested with non-parametric Friedman's test. Dunn's test was used for *post hoc* analysis if there were statistically significant changes within the group after treatment. The comparison of sFasL levels between the normal control group and the other studied groups (i.e. control group vs. patient group) were assessed using the Mann–Whitney U test. A p value <0.05 was considered significant.

RESULTS

The Friedman's test showed that the levels of sFasL secretion by the PBMC after 0, 10, and 100 μg/ml CBZ treatment are significantly different among the patient group ($p<0.05$). Moreover, the *post hoc* Dunn's test indicated significant difference between PBMCs stimulated with 100 μg/ml CBZ and those without CBZ stimulation ($p<0.05$) (Fig. 1a). To ascertain that the elevation of sFasL is a drug-specific response, PBMCs of the patient group were also stimulated with sulindac, a commonly used non-steroidal anti-inflammatory agent. Contrasting CBZ treatment, equivalent sulindac stimulation did not result in significant alteration of sFasL secretion in the patient group ($p>0.05$) (Fig. 1b). No significant differences in the PBMC secretion of sFasL were seen in the control groups after CBZ stimulation ($p>0.05$) (Fig. 1a). It should be pointed out that stimulating the PBMCs with 10 μg/ml of a causal drug may not always result in up-regulation of sFasL secretion as demonstrated in patient 4 (Table I).

Taken as a group, the PBMCs of the patient group secreted more sFasL than the normal control group regardless of CBZ stimulation (Fig. 1a). No correlation was found between the severity of body surface area involvement during active disease and the basal level of sFasL secreted by PBMCs (Table I). In addition, no correlation was found between the IgE level during active disease and the sFasL secretion by PBMCs retrospectively (Table I).

DISCUSSION

This is the first study demonstrating increased sFasL secretion by PBMCs from patients with previous TEN/SJS. As shown in our results, PBMCs from the patient group showed higher basal sFasL secretion compared with the control groups. In addition, subsequent stimulation with the causal drug induced a statistically significant increase in sFasL secretion in the patient group. Abe et al. (12) have reported that the serum from

Table I. *Clinical information for patients with carbamazepine-induced TEN/SJS in previous 1–3 years and secretion of soluble Fas ligand (sFasL) by peripheral blood mononuclear cells (PBMCs) after treatment with carbamazepine in vitro*

Pat. no.	Sex/Age (years)	Disease onset (years)	BSA (%)	IgE	Basal sFasL secretion	sFasL secretion (10 μg/ml)[a]	sFasL secretion (100 μg/ml)[a]
1	M/53	3	19	36.2	33.38	42.02	57.41
2	F/23	2	50	627	37.25	58.84	65.00
3	M/35	1	37	107	51.28	53.65	62.92
4	M/44	2	22	119	40.26	38.88	61.46

BSA: body surface area involved during active stage of disease; sFasL of PBMCs after stimulation with 10 μg/ml or 100 μg/ml carbamazepine; IgE: serum IgE level during active stage of disease.

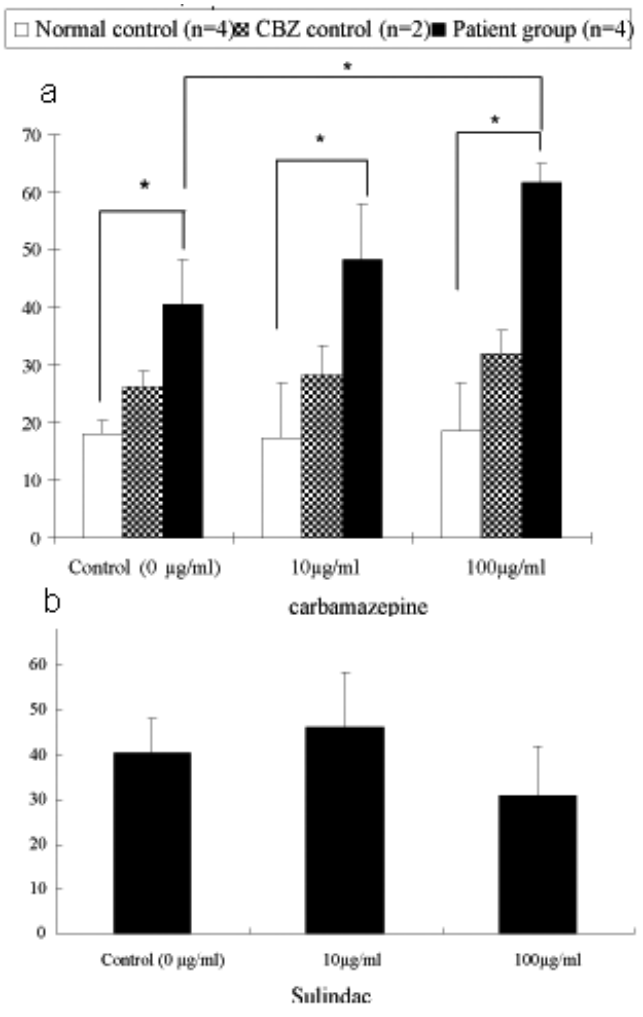

Fig. 1. Soluble Fas ligand (sFasL) secretion by the peripheral blood mononuclear cells (PBMC) after (a) treating with 0, 10, or 100 μg/ml carbamazepine (CBZ) for 24 h in normal control group (chequered bar), CBZ control group (black bar), and patient group (checkered bar); and (b) with 0, 10 or 100 μg sulindac in the patient group. The PBMCs of control groups showed no significant change in sFasL secretion after stimulating with CBZ (p <0.05). The PBMCs from the patient group showed significant increase in sFasL secretion after stimulating with 100 μg/ml CBZ (p<0.05). In addition, the patient group showed significantly higher sFasL secretion by PBMCs compared with the normal control group at all experimental conditions (p <0.05). As demonstrated in (b), treatment with sulindac did not result in significant change in sFasL secretion by PBMCs in the patient group (p >0.05).

patients with TEN/SJS contained high levels of sFasL during the active stage of disease. Moreover, they have shown that the secretion of sFasL by PBMCs from a patient with active TEN/SJS could be significantly increased by stimulating with the causal drug. Our result suggested that sFasL secretion by PBMCs may also be increased after incubating with the offending drug years after active stage of disease. As demonstrated in our result, sulindac failed to stimulate sFasL secretion from the patient group, indicating that the increase in sFasL secretion by PBMCs was a drug-specific response. However, it should be noted that although the increase in sFasL secretion after incubation with the offending drug reached statistical significance, the change was small and requires further discussion.

As mentioned previously, one of the patient (patient 4) showed up-regulation of sFasL secretion by PBMCs only after stimulation with 100 μg/ml CBZ. According to Sachs et al. (14), the levels of interleukin (IL)-5, -10, and interferon-alpha in the culture supernatant by drug-specific stimulated PBMCs from patients with drug allergy were time-dependent and peaked on days 4–6 after incubation with causal drug. In addition, a greater increase in IL-5 concentration was associated with higher drug concentration. Therefore, the sFasL secretion may have increased in our patient after stimulating with 10 μg/ml CBZ if the incubation time with the causal drug was prolonged. Another issue that warrants some discussion is that while the patient in the study by Abe et al. (12) showed large increases in sFasL secretion after stimulation with the causal drug, the patients in our study showed approximately two-fold increases in sFasL secretion. Inter-individual differences and state of disease activity could have resulted in this discrepancy. First of all, as demonstrated in the study by Abe et al. (12), the serum sFasL varies significantly among patients with TEN/SJS. In addition, the PBMC stimulation test in their study consisted of only one patient with active disease. Therefore, inter-individual variation in the levels of sFasL secretion may have caused this discrepancy. It should be pointed out that Sachs et al. (14) also suggested that there may be a significant inter-individual variation in the levels of drug-specific IL-5 secretion by PBMCs from patients with drug-specific exanthem, although a consistent increase in IL-5 secretion was observed after stimulation (14). Secondly, the patient in the study by Abe et al. (12) was in active state of disease while in our series, the patients had already completely recovered from TEN/SJS for at least 1 year. The difference in the status of disease activity may also contribute to the differences noted between the two studies. To investigate the potential clinical role for using sFasL secretion by drug-stimulated PBMCs as a tool for determining offending agent, modifications of our laboratory protocol and further studies are required. Currently, its use has limited clinical significance due to the small, though statistically significant, change after specific drug stimulation.

It has been reported that the serum levels of sFas and sFasL were elevated in certain rheumatic diseases (15). Furthermore, the elevation of sFas correlated with HLA status but not disease activity (16). A recent report by Chung et al. (17) on Taiwanese patients with CBZ-induced SJS has revealed a striking association between HLA-B*1502 with carbamezepine-induced SJS. Since the basal PBMC secretion profiles of sFasL from patients with previous CBZ induced TEN/SJS were significantly higher than the control groups, our result

suggests that certain HLA haplotypes may be associated with the predisposition to produce higher sFasL and therefore prone to the development of TEN/SJS.

In summary, we have shown that individuals whose PBMCs are high sFasL secretors are prone to the development of severe drug-induced TEN/SJS. Moreover, in patients with medical history suggesting previous drug-induced blistering disease, the sFasL secretion by PBMCs may also serve as an auxiliary diagnostic tool for confirming the diagnosis. There is no doubt that additional larger scale studies involving various kinds of drugs and modifications of laboratory protocols are warranted in order to establish the potential clinical roles for using sFasL secretion by the drug-stimulated PBMCs as a diagnostic tool. Our report, however, indicates a new possibility for identifying individuals who are prone to the development of severe drug-induced blistering disease.

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