Bioscience, Tokyo, Japan) and the 3-amino-9-ethylcarbazole (AEC) substrate kit (Nichirei Bioscience).

Paraffin-embedded skin sections were dewaxed and rehydrated. After antigen retrieval and blocking of nonspecific binding sites, sections were incubated with a monoclonal antibody against CD16 (Lab Vision, Fremont, CA, U.S.A.). Immunostaining was then carried out using Histofine Simple Stain AP (M) (Nichirei Bioscience) and a 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate kit (Dako Japan). After a second round of endogenous peroxidase inhibition and blocking of nonspecific binding sites, sections were incubated with a monoclonal antibody (CD45RO; Nichirei Bioscience) specifically recognizing mature T cells. Immunostaining was developed using Histofine Simple Stain MAX PO (M) and the AEC substrate kit.

Evaluation method

The degree of epidermal damage was classified according to the graft-versus-host reaction (GVHR) grades described by Lerner *et al.*,[15] as follows: grade 0, normal skin; grade I, mild vacuolization of epidermal cells with occasional dyskeratotic bodies; grade II, diffuse vacuolization of basal cells with scattered dyskeratotic bodies; grade III, subepidermal cleft formation; grade IV, complete epidermal separation. In this study, skin sections that showed dermal infiltration without epidermal change were classified as grade 0.

The number of CD16+ or CD45RO+ cells was evaluated by counting the total number of positive cells along a linear 500-μm section of the epidermis. Three or four typical lesions were examined, and mean cell counts were obtained by calculating the average number of positive cells.

The grade of epidermal damage, number of cells and immunofluorescence stains were blindly evaluated by two of the investigators (M.T. and S.M.). Significant variations in the results were not observed.

Statistical analysis

Statistical significance was determined using Student's paired t-tests. Differences were considered statistically significant at $P < 0{\cdot}05$.

Results

CDI4+ CD16+ cells infiltrate in the epidermis and the dermoepidermal junction of Stevens–Johnson syndrome/toxic epidermal necrolysis lesions

Consistent with previous reports, CD14+ cells were observed in the epidermis, at the dermoepidermal junction, and in the upper dermis of skin biopsies from all 11 patients with SJS/TEN. Interestingly, > 80% of the CD14+ cells coexpressed CD16 (Fig. 1B) whereas > 90% of the CD16+ cells expressed CD14 (Table 2). CD16 is typically expressed not only by CD14+ cells, but also by natural killer cells. In the SJS/TEN skin lesions examined in this study, a small number of CD16+ cells coexpressed CD2 but not CD56 (data not shown).

To examine the characteristics of CD14+ CD16+ cells further, expression of CD11c, HLA-DR and CD163 was examined (Table 2). CD11c, a marker for monocytes and dendritic cells (DCs), was expressed in almost all CD16+ cells located in the epidermis and at the dermoepidermal junction (Fig. 1Ca). HLA-DR expression was observed in CD16+ cells and in other cells, including keratinocytes (Fig. 1Cb). CD163, a macrophage marker, was weakly expressed on CD16+ cells at the dermoepidermal junction and in the upper dermis (Fig. 1Cc).

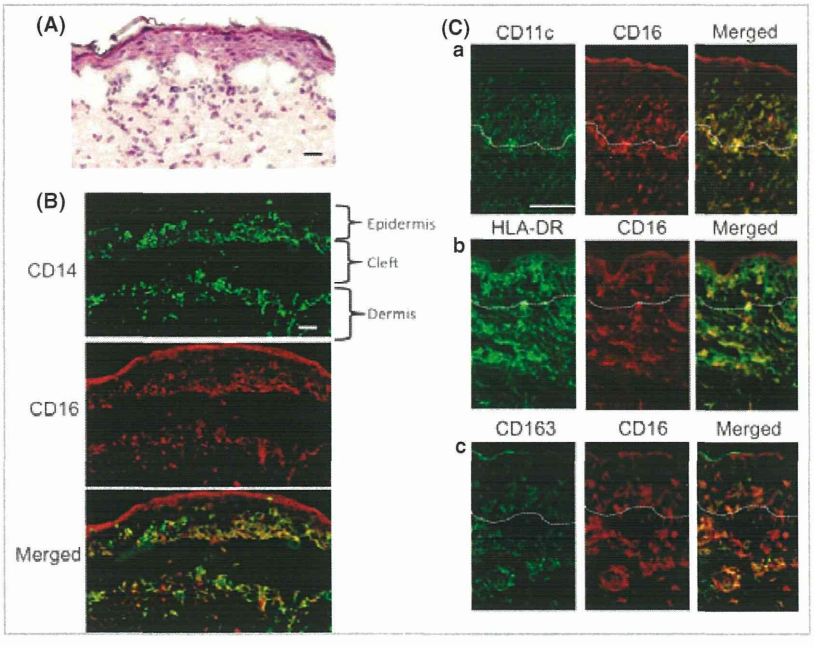

Fig 1. Immunostaining of monocyte, dendritic cell and macrophage markers in skin biopsies. (A) Haematoxylin and eosin staining in cryosection of patient 2 with toxic epidermal necrolysis (TEN). (B) Costaining of CD16 (red) and CD14 (green) in cryosections of skin lesions from patient 2 with TEN. (C) Costaining of CD16 (red) and CD11c, HLA-DR and CD163 (green) in cryosections of skin lesions from patients with Stevens–Johnson syndrome (patients 6, 5 and 9, respectively). Dotted line indicates the dermoepidermal junction. Black or white bar = 100 μm.

Table 2 Expression of markers in CD16+ cells infiltrating in the epidermis and at the dermoepidermal junction of Stevens–Johnson syndrome/toxic epidermal necrolysis

Patient	CD14	CD11c	HLA-DR	CD163	CD80	CD86	CD137 ligand
1	+++	+++	+++	(+++)	+++	+++	++
2	+++	+++	+++	(+++)	+++	+++	+++
3	+++	+++	+++	+++	+	++	+++
4	+++	+++	+++	+++	–	+	+++
5	+++	+++	+++	(++)	+++	+	+++
6	+++	+++	+++	(++)	+++	+++	+++
7	+++	+++	+++	(+)	+	+	+++
8	+++	+++	+++	(+++)	++	++	+++
9	+++	+++	+++	(+)	+	+	+++
10	+++	+++	+++	+	–	+	++
11	+++	+++	+++	+++	–	+	+

–, completely negative; +, positive in < 50% cells; ++, positive in > 50% and < 90% cells; +++, positive in > 90% cells; (+), weakly positive in < 50% cells; (++), weakly positive in > 50% and < 90% cells; (+++), weakly positive in > 90% cells.

However, in patients 3, 4, 10 and 11, in whom skin biopsies were performed later than in the other patients, strong CD163 expression was noted (Table 2). These findings indicated that the CD14+ CD16+ cells were of monocyte lineage, and that CD163 expression increased with time after disease onset.

The presence of a smaller number of CD14+ CD16+ cells was also observed in the lower dermis. These cells expressed either no or lower levels of CD11c, but higher levels of CD163, properties typical of macrophages (Fig. 1Ca, c).

CD16+ cells express CD80, CD86 and CD137 ligand

Monocyte lineage cells, such as macrophages and DCs, activate T cells through engagement of the T-cell receptor with a cognate peptide–MHC complex and an additional costimulatory signal, such as CD80, CD86 or CD137L. We examined whether CD16+ cells also express these costimulatory factors. CD80 was expressed on CD16+ cells in eight of the 11 SJS/TEN skin samples, and CD86 on CD16+ cells in all samples, albeit to varying degrees (Fig. 2A, B and Table 2).

CD137L is an important costimulatory factor for activating CD8+ T cells.[13,14] We found that CD137L was strongly expressed on CD16+ cells in the SJS/TEN skin samples (Fig. 2C, Table 2). The expression of CD137, a receptor for CD137L, was revealed on CD8+ T cells infiltrating the lesion skin (Fig. 2D). The CD137L-expressing cells were seen bound to CD8+ T cells expressing CD137 in the dermis (Fig. 2E).

CD16+ cells appear at the dermoepidermal junction in the very early phase of Stevens–Johnson syndrome/toxic epidermal necrolysis

Infiltration of CD16+ cells may be an early event in the pathogenesis of SJS/TEN, indicating that CD16+ cells do not accumulate as a result of epidermal damage, but instead precede it. This scenario was confirmed in an evaluation of skin samples from the normal-appearing skin of eight patients with SJS/TEN. Samples were taken from sites near skin lesions, before the development of epidermal detachment, and paraffin embedded for immunohistological analysis. The results showed a small number of CD16-expressing cells at the dermoepidermal junction and/or in the dermis in five of the eight samples (Fig. 3A). The skin lesions of three of these five patients (Fig. 3Aa, b, d) had progressed to epidermal detachment, despite intensive therapy. Moreover, epidermal detachment developed at the sites where samples were obtained from normal-appearing skin.

Detailed information for patient 6 (Table 1 and Fig. 3Aa) is shown in Figure 3B. Skin biopsies were obtained from an erythematous lesion and from a nonlesional area on the patient's abdomen (Fig. 3Ba). The lesion expanded rapidly, and normal-appearing skin had progressed to epidermal detachment 2 days postbiopsy. Within the lesion, numerous apoptotic keratinocytes, subepidermal blister formation, and mononuclear cell infiltration were observed (Fig. 3Bb). Normal-appearing nonlesional skin showed no sign of epidermal damage and the infiltration of only a small number of mononuclear cells into the dermis (Fig. 3Bc). A slight separation was noted at the dermoepidermal junction. In cryosections of the nonlesional skin, immunostaining revealed HLA-DR expression in epidermal keratinocytes and in the dermal component (Fig. 3Bd). Moreover, it was readily apparent that CD16+ cells had already appeared at the dermoepidermal junction (Fig. 3Be). CD16+ cells expressing CD137L (data not shown) were seen bound to CD137-expressing cells at the dermoepidermal junction (Fig. 3Bf). These findings suggest that CD16+ cells are involved in the development of epidermal damage.

Correlation between the number of CD16+ cells and epidermal damage

Mild epidermal damage is often observed in drug rashes other than SJS/TEN. In EM or MP, there is a slight degree of epidermal damage, such as scattered apoptotic keratinocytes and/or liquefaction degeneration of basal cell layer. We assessed the relationship between the degree of epidermal damage and the number of CD16+ cells in 47 patients with drug rash, including those with SJS, TEN, MP and EM. Because the grade of epidermal damage varies according to the timing and location of the skin biopsy, in this study the degree of epidermal damage in all skin samples was classified according to GVHR grade. Thus, MP/EM samples were classified as grade 0 or I, except for one case of grade II. In contrast, all cases of SJS/TEN were classified as grades II–IV. In grade 0 skin samples, no CD16+ or CD45RO+ cells were observed in the epidermis, similar to normal skin (Fig. 4Aa). In grade I, no or only a few CD16+ cells had infiltrated the dermoepidermal junction, as was the case in MP and EM (Fig. 4Ab). In the diseases with higher GVHD grades, the number of CD16+ cells increased in parallel with the degree of epidermal damage

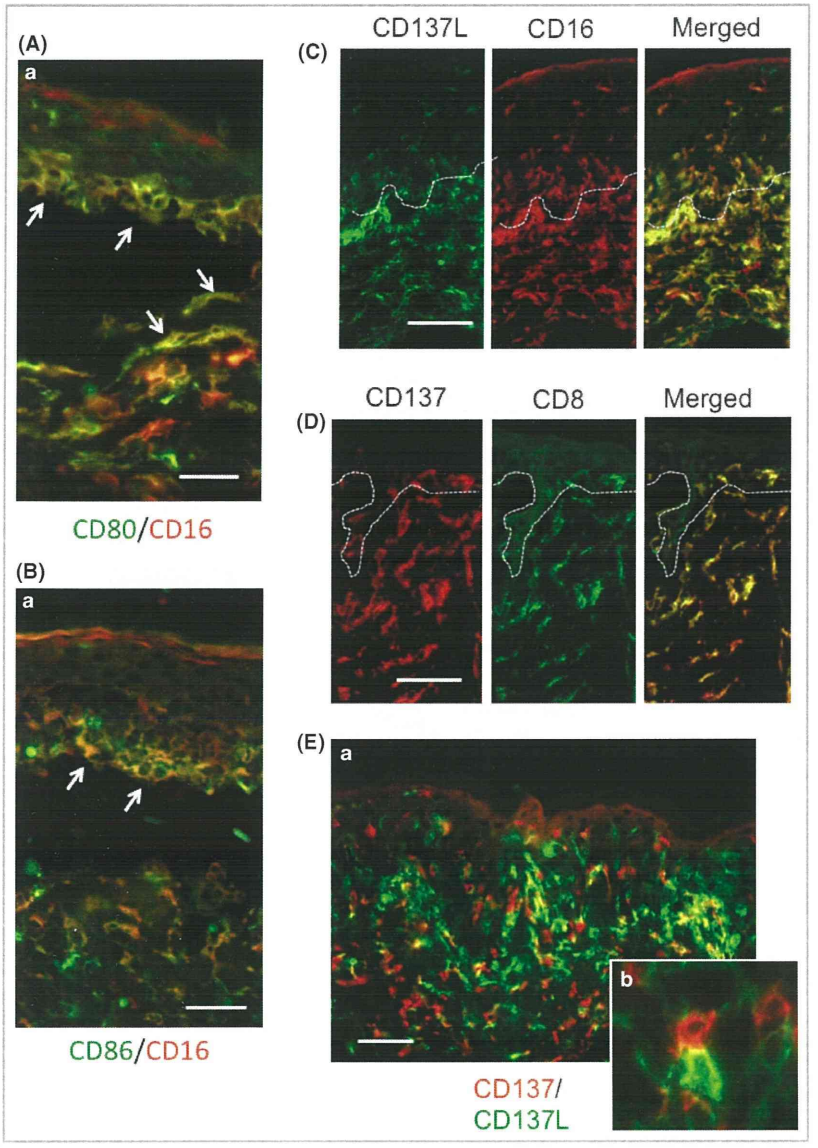

Fig 2. CD80, CD86 and CD137 ligand (CD137L) expression on CD16+ cells. (A) Costaining of CD16 (red) and CD80 (green) in cryosections of skin lesions from patient 2 with toxic epidermal necrolysis (TEN). (B) Costaining of CD16 (red) and CD86 (green) in cryosections of skin lesions from patient 1 with TEN. Arrows indicate cells coexpressing CD16 and CD80/CD86. (C) Costaining of CD16 (red) and CD137L (green) in cryosections of skin lesions from patient 6 with Stevens–Johnson syndrome (SJS). (D) Costaining of CD137 (red) and CD8 (green) in cryosections of skin lesions from patient 5 with SJS. (E) Costaining of CD137 (red) and CD137L (green) in cryosections of skin lesions from patient 9 with SJS (a). CD137-expressing cells (red) are bound to CD137L-expressing cells (green) (patient 7) (b). Dotted lines indicate the dermoepidermal junction. White bar = 100 μm.

(Fig. 4Ac, d, e). The number of CD16+ or CD45RO+ cells that infiltrated the epidermis, including the dermoepidermal junction, was counted and the result compared according to the respective grade. The number of CD16+ cells increased significantly with increasing grade, while there was no difference in the number of CD45RO+ cells among grades I–IV (Fig. 4B).

Discussion

The present study focused on the numerous CD14+ CD16+ monocyte lineage cells that infiltrate the epidermis and upper dermis of SJS/TEN skin lesions. Abundant infiltration of monocytes in the epidermis of TEN skin lesions was demonstrated in earlier studies using immunohistochemical analysis.[9–11] On the other hand, recent studies have examined cells collected from the bullous fluid of patients with TEN. Monocytes were found to be a minor component, especially in the early phase of the disease.[7] Thus, there is a discrepancy between the cellular composition of TEN lesions observed by histological examination vs. that determined using flow cytometric analysis of bullous fluid cells. Although the reason for this difference is unclear, morphological changes occurring in monocytes after stimulation may make it difficult to collect these cells from blister fluid.

In peripheral blood, CD14+ CD16+ monocytes are a minor component, accounting for approximately 10% of monocytes.[12] These cells are referred to as proinflammatory monocytes due to their production of higher levels of TNF-α and lower levels of interleukin-10 after stimulation.[16,17] Additionally, these cells express CD11c and HLA-DR, and have been recognized as circulating DC precursors.[18,19] In our study, CD14+ CD16+ cells in the skin lesions of patients with SJS/TEN expressed CD11c and HLA-DR, similar to CD14+ CD16+ monocytes in blood. This finding provides new insight into the role of monocytes in SJS/TEN lesions.

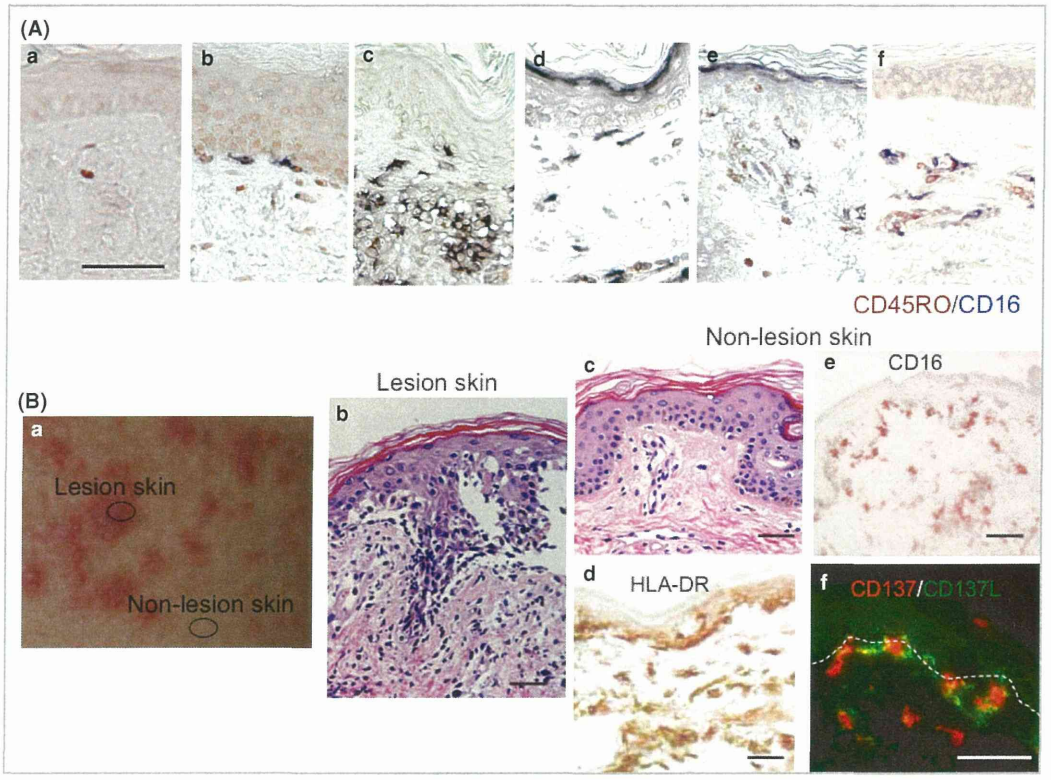

Fig 3. The very early phase of toxic epidermal necrolysis (TEN). (A) Immunostaining for CD16 and CD45RO in paraffin-embedded skin from normal skin (a) and the normal-appearing skin of four patients with Stevens–Johnson syndrome/TEN (b–f). (B) Abdominal skin biopsies were taken from an area of macular erythema and the adjacent nonlesional skin (a). Haematoxylin and eosin staining of the erythematous skin lesion (b) and the nonlesional skin (c). Immunostaining for HLA-DR (d) and CD16 (e) in a cryosection from nonlesional skin. CD137-expressing cells bind to CD137 ligand (CD137L)-expressing cells at the dermoepidermal junction (f). Dotted line indicates the dermoepidermal junction. Black or white bar = 100 μm.

CD14+ CD16+ cells in the epidermis and at the dermoepidermal junction of SJS/TEN lesions were found to express costimulatory ligands, such as CD80, CD86 and CD137L. CD80/CD86 and CD137L activate T cells through their receptors, CD28 or CD137, respectively, in a process involving engagement of the T-cell receptor and a cognate peptide–MHC complex.[13,20] CD80–CD28 and CD86–CD28 are widely considered to be the most important costimulatory pathways for CD4+ T-cell activation and expansion,[21–23] while CD137L–CD137 signalling plays an important role in the expansion of CD8+ T cells.[13,24,25] CD137, a member of the TNF receptor family, is absent from resting T cells but is rapidly expressed on antigenic stimulation.[26–28] CD137 signalling could avoid the activation-induced cell death of CD8+ T cells and prolong their proliferation.[29–32] Even if CD8+ T cells become anergic after stimulation, CD137L could reactivate these T cells to proliferate.[32] Moreover, CD137 signalling directly augments the cytotoxic function of CD8+ T cells,[33–37] with costimulation by CD137L increasing perforin and granzyme A expression.[25] Thus, it is conceivable that activation of the CD137L–CD137 system enhances CD8+ T cell-mediated immune responses. In fact, the systemic administration of agonistic anti-CD137 or endogenous CD137L has been shown to cause a potent cell-mediated immune response against tumours[38–42] or an enhanced virus-specific CD8+ T-cell response, in the latter case resulting in rapid viral clearance.[26,43–46] Together, these observations provide evidence for the importance of the CD137L–CD137 system in CD14+ CD16+ cells and for a key role of CD8+ T cells in the skin in the development of SJS/TEN.

In contrast to CD137L expression levels, which did not differ widely among SJS/TEN samples, CD80 and/or CD86 expression by CD16+ cells tended to be stronger in the early than in the late phase of SJS/TEN. In particular, strong CD80 and CD86 expression was observed in the skin of patients in whom the area of epidermal detachment progressed after the biopsy was performed and treatment was started (patients 1, 2, 5 and 6). Costimulation with CD80/CD86 and CD137L has been reported to promote the proliferation and cytotoxicity of CD8+ T cells more strongly than CD137L alone.[32] Thus, CD80 and CD86 expression levels may be involved in enhancing the activity of CD8+ T cells and thereby contribute to disease progression. However, because this study was based on only a few cases, further research is necessary.

Our results also demonstrated that the number of CD16+ cells infiltrating the epidermis and dermoepidermal junction

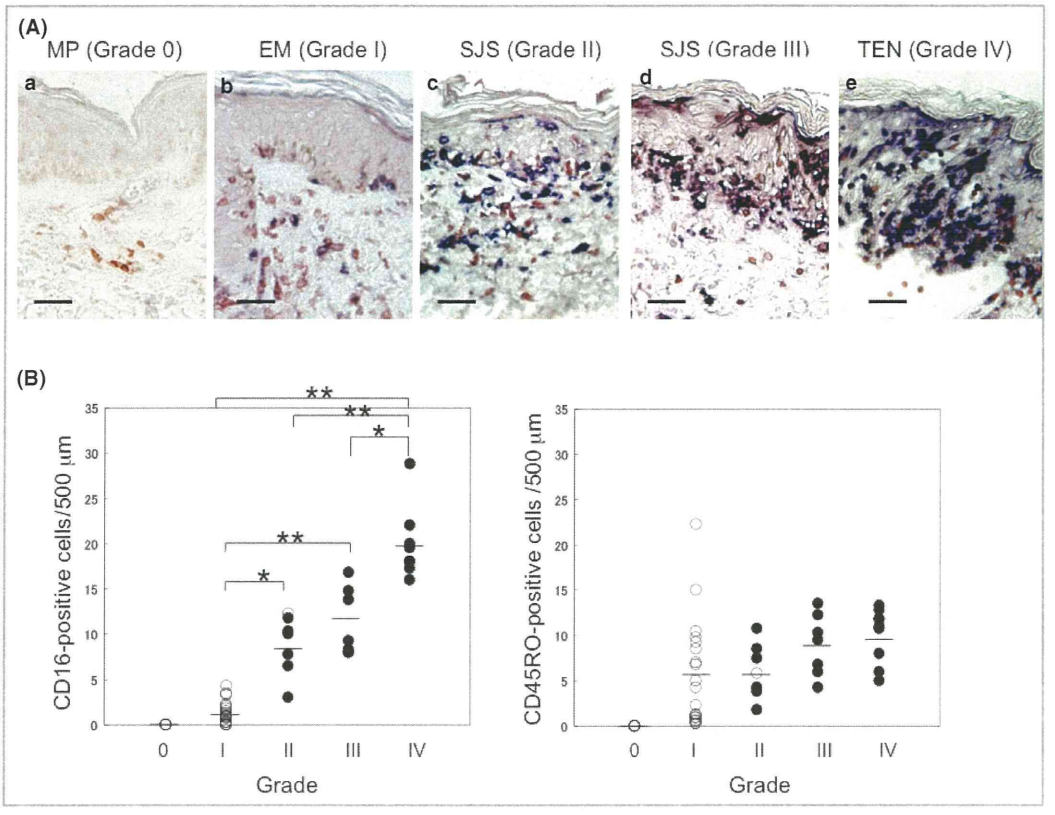

Fig 4. Correlation between CD16+ or CD45RO+ cell number and histopathological graft-versus-host reaction grade of epidermal damage. (A) Immunostaining of CD16 (blue) and CD45RO (red) in skin biopsies. Skin biopsy from patients with grade 0 maculopapular-type drug rash (MP) (a), grade I erythema multiforme-type drug rash (EM) (b), grade II and grade III Stevens-Johnson syndrome (SJS) (c and d, respectively), and grade IV toxic epidermal necrolysis (TEN) (e). Black bar = 100 μm. (B) CD16+ and CD45RO+ cells in the epidermis and at the dermoepidermal junction were counted in skin sections prepared from patients with MP/EM (open circles) and SJS/TEN (closed circles). *P < 0·05; **P < 0·001.

correlated with the grade of epidermal damage. Because the number of CD16+ cells was higher than that of CD45RO-expressing T cells in grade IV disease, it seems likely that CD16+ cells not only enhance CD8+ T-cell activity but also contribute directly to the epidermal damage. Cytotoxic effects of CD16+ monocytes against mesangial cells or tumour cells have been demonstrated in *in vitro* studies.[17,47] Because CD16+ monocytes produce abundant TNF-α,[16,17] cytokines may, at least in part, be responsible for the epidermal damage. Significant epidermal damage due to combined TNF-α and IFN-γ signalling was demonstrated in a skin explant model developed for the analysis of acute graft-versus-host disease following bone marrow transplantation.[48] In another study, peripheral blood mononuclear cells (PBMC) from bone marrow transplant donors were cultured with the recipient's irradiated lymphocytes and then cocultured with skin explants obtained from the recipient. Significant epidermal damage, including apoptosis of epidermal keratinocytes, resembling that in drug rash or SJS/TEN was observed.[49,50] This phenomenon could be reproduced in cell-free supernatants from allostimulated PBMC, and the epidermal damage inhibited by the addition of anti-TNF-α or anti-IFN-γ antibody.[50] TNF-α and IFN-γ levels in the supernatants correlated with the severity of cellular damage in the epidermis.[50] The infiltration of CD14+ CD16+ cells of monocyte lineage into the epidermis along with CD8+ T cells causes TNF-α levels to increase, which may lead to the induction of apoptosis in keratinocytes in response to IFN-γ produced by T cells, leading to epidermal damage. Clinically, the efficacy of anti-TNF-α therapy in SJS/TEN treatment has been reported,[51,52] but the direct cytotoxicity of CD14+ CD16+ cells of monocyte lineage against keratinocytes remains to be investigated.

In conclusion, our data suggest that CD14+ CD16+ monocyte lineage cells contribute to the epidermal damage characteristic of SJS/TEN. These cells may enhance the proliferation and cytotoxicity of CD8+ T cells in addition to prolonging their activation in SJS/TEN via CD137, causing severe epidermal damage. Thus, inhibition of the interaction between CD14+ CD16+ cells and CD8+ T cells may be a useful strategy in the treatment of SJS/TEN. Blockage of the CD137/CD137L system is also expected to reduce the cytotoxicity of CD8+ T cells. A previous report demonstrated that, in the mouse, blockage of the CD137–CD137L pathway reduced the number and cytotoxicity of CD8+ T cells and ameliorated the acute GVHR.[35,53] The mechanism underlying the accumulation and function of CD14+ CD16+ monocyte lineage cells in SJS/TEN remains to be elucidated.

What's already known about this topic?

- In the skin lesions of patients with Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), monocytes are abundantly observed in the epidermis and at the dermoepidermal junction.

What does this study add?

- CD14+ monocytes in the lesional skin of SJS/TEN coexpress CD16, CD11c and HLA-DR, similar to proinflammatory monocytes or dendritic cell precursors in blood.
- These cells may enhance the cytotoxicity of CD8+ T cells through expression of CD137 ligand, CD80 and CD86.
- These cells might be implicated directly in the epidermal damage, because the number of CD16+ cells infiltrating the epidermis increases depending on the grade of epidermal damage.

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CORRESPONDENCE

Multiple fixed drug eruption caused by cyclophosphamide and its metabolite

Fixed drug eruption (FDE) is a cutaneous adverse drug reaction characterized by recurrence at the same skin or mucous membrane sites upon re-administration of causative drugs [1]. Cyclophosphamide is converted in the liver to an active form which can inhibit DNA synthesis and modulate immune system responses [2]. To the best of our knowledge, there have only been two reports describing cyclophosphamide-induced FDE [3, 4]. Here we report a case of multiple FDE due to cyclophosphamide, diagnosed by not only clinical features but also a patch test.

An 18-year-old Japanese woman suffering from systemic sclerosis (SSc) and interstitial pneumonia for 5 years was referred to our department because of multiple painful eruptions, mainly on her bilateral inner thighs *(figure 1A)*. At the time, she had undergone 15 courses of cyclophosphamide pulse therapy (500 mg/m^2). After the ninth course however, she developed several painful brownish annular patches about 10 h after cyclophosphamide administration. The eruptions gradually resolved with residual pigmentation but eruptions flared up at the same sites after every pulse therapy session. In addition, the number of patches increased in response to repeated administration of cyclophosphamide. She was therefore suspected of having FDE caused by cyclophosphamide. A skin biopsy of a plaque on her left inner thigh was performed, and histopathological findings were consistent with FDE *(figure 1B)*.

To make a definite diagnosis, a patch test and a lymphocyte stimulation test were performed after informed consent was obtained. Cyclophosphamide and its metabolites – cyclophosphamide monohydrate and carboxyphosphamide – were dissolved and diluted with saline to 5% and 0.5% concentrations, which were the usage concentrations of high-dose pulse therapy (5%) and diluted at 10% (0.5%) respectively, and applied to lesional skin and normal skin of the inner thigh for patch testing. After 48 h, well-defined erythema was observed on the lesional skin areas treated with the 5% cyclophosphamide and 5% cyclophosphamide monohydrate solutions but no reactions were evident on normal skin. The lymphocyte stimulation test was negative because cell proliferation was suppressed with increased drug concentration *(figure 1C)*.

This is the third report of FDE caused by cyclophosphamide. All cases occurred in patients with autoimmune disorders treated with repeated high-dose pulse therapy

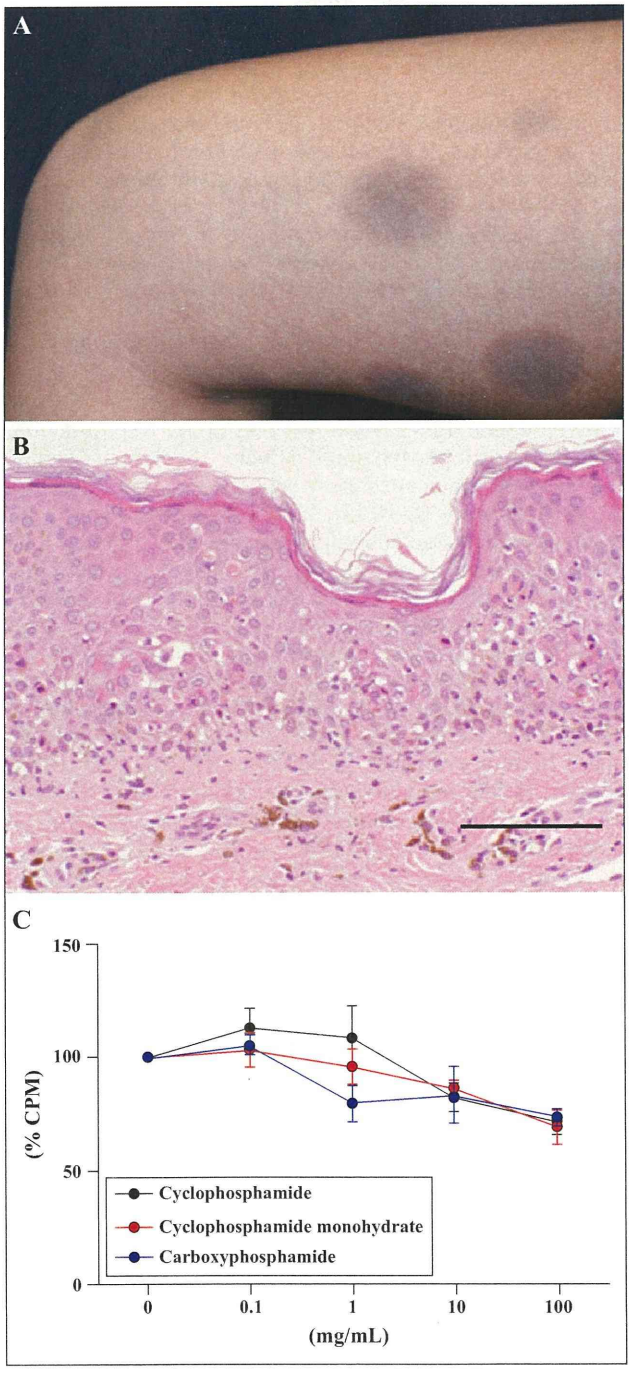

Figure 1.

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Figure 1. A) Clinical findings of FDE. Multiple annular brownish patches appeared on bilateral inner thighs about 10 hours after the administration of cyclophosphamide. **B)** Histological findings of FDE. Skin biopsy was performed from a patch on left inner thigh. Hematoxylin-Eosin stain revealed features of FDE. Scale bar represents 0.1 mm. **C)** Result of the lymphocyte stimulation test. The lymphocyte stimulation test revealed the suppression of cell proliferation with an increase in the concentration of cyclophosphamide and its metabolites. The reference level (100%) is the unstimulated condition.

after the seventh to fourteenth course in the previous cases and after the ninth course in the present case [3, 4]. Cyclophosphamide has been used for the treatment of malignancies and autoimmune disorders for a long time and it has recently been approved for the treatment of SSc in Japan. Cyclophosphamide has both cytotoxic and multiple immunomodulatory effects, whose biological activities are dose dependent. The present case was diagnosed according to the clinical course and the results of the patch tests, despite the absence of proliferation by the culprit drug and its metabolites in the lymphocyte stimulation test, which was likely due to the inhibitory effect of cyclophosphamide on DNA synthesis.

The immunomodulatory effects of cyclophosphamide influence various types of immune cells. Cyclophosphamide promotes proliferation, survival, and activation of Th1 cells which produce IFN-γ while suppressing IL-10 production. Moreover, cyclophosphamide depletes regulatory T and B cell subsets which mediate peripheral tolerance through the production of IL-10 [5]. Intraepidermal $CD8^+$ T cells, which produce large amounts of IFN-γ, play a critical role in the pathogenesis of FDE [1]. Teraki *et al.* reported that the number of $CD4^+$ and $CD8^+$ T cells capable of producing IL-10 significantly increases after oral challenge with the causative drug, suggesting that expansion of IL-10-producing T cells may be responsible for the spontaneous resolution of FDE [6]. Indeed, infiltrated intraepidermal lymphocytes were mainly positive for CD8 during the acute phase (data not shown). In addition, although the pathogenesis of SSc remains unknown, an imbalance between Th1/Th2 or Th17/regulatory T cytokines is closely involved in the development of SSc [7].

These findings can explain why FDE caused by cyclophosphamide has been reported in patients with SSc despite no case of FDE in patients with malignancy. Frequent repetitive administration of cyclophosphamide certainly increases the risk of sensitization, and especially in patients with autoimmune disorders such as SSc, the immunomodulatory effects of high-dose cyclophosphamide may suppress the peripheral tolerance which is prominently involved in IL-10, not only by increasing effector cells but also by suppressing regulatory cells. Thus, physicians who prescribe cyclophosphamide should consider the potential for adverse drug reactions. ■

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SHORT COMMUNICATION

A Case of Toxic Epidermal Necrolysis Induced by Allopurinol with Human Herpesvirus-6 Reactivation

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Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms (DRESS) (1, 2) are severe adverse drug reactions (ADRs). Recently, human herpesvirus 6 (HHV-6) reactivation has been observed frequently in patients with DIHS/DRESS, but not in SJS/TEN (3–5). Therefore, it has been suggested that HHV-6 is closely related to the pathogenesis of DIHS, but not to that of SJS/TEN (6, 7). We report here a case of TEN induced by allopurinol, accompanied by HHV-6 reactivation.

CASE REPORT

A 73-year-old woman was treated for gout with allopurinol (300 mg/day). Twelve days later, she developed a rash with sore throat and fever. Three days after that, on day 4, erosions appeared on her lips and oral mucosa. She had had a past history of rash induced by allopurinol. She was diagnosed as having SJS, and allopurinol was discontinued. Although systematic betamethasone (6 mg/day) was started the next day, the rash increased rapidly and became confluent. She was referred to our hospital on day 9. Physical examination revealed high fever and haemorrhagic erosions on the lips, oral mucosa (Fig. S1; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1610), and genital region, as well as skin rash. Multiple erythematous lesions, including atypical target lesions, were observed on her entire body, and were confluent with blisters and erosions (Fig. 1a). No lymphoadenopathy was observed. Approximately 80% of the body surface area was detached. Laboratory investigations disclosed a high white blood cell count (12.49×10^9/l) with atypical lymphocytes (1.5%), hypoproteinaemia (4.0 g/dl), increased serum creatinine (1.24 mg/dl), hypoimmunoglobulinaemia (IgG, 352 mg/dl), and a high CD4/CD8 ratio (2.9). There was no liver dysfunction (aspartate aminotransferases (AST) 8 U/l and alanine aminotransferase (ALT) 8 U/l), but there was pulmonary oedema. Eosinophil was not detected in the blood, but increased later (Fig. 2). A skin biopsy obtained from the left thigh showed epidermal necrosis and subepidermal blisters. Infiltration with mononuclear cells was observed in the upper dermis (Fig. 1b). Taken together, the diagnosis on admission was TEN due to allopurinol. Later, it was established that she had the HLA-B*58:01 leukocyte antigen type.

The patient was treated with steroid pulse therapy with methylprednisolone at 1,000 mg/day for 3 days, and twice with plasma exchange. In addition, 5 g/day of immunoglobulin was administered for 3 days because of hypoimmunoglobulinaemia.

With these treatments, progression of the rash stopped, and re-epithelialization began. Eye lesions, which are often observed in TEN, such as conjunctival injection and pseudomembranes appeared after day 51. The anticytomegalovirus IgG titre was as high as 128 by enzyme-linked immunosorbent assay (ELISA) on day 66.

Peripheral blood samples were obtained for virological examination. Titres of IgG antibodies to HHV-6 were determined using an immunofluorescent (IF) antibody assay. The HHV-6 IgG antibody titre increased from 1:40 on day 12 to 1:1,240 on day 21. The HHV-6 DNA level in a sample of peripheral blood was 3.0×10^4 copies in 10^6 peripheral blood mononuclear cells and 3.5×10^5 copies/ml serum on day 12 by real-time quantitative PCR. There were no significant changes in specific IgG titres for herpes simplex virus, HHV-7, or Epstein–Barr virus during the course of the study. The DNA of these viruses was not detected in the serum.

The results of lymphocyte transformation tests and patch tests were negative for allopurinol and oxypurinol. On day 66, the patient was transferred to another hospital near her home. After that, the patient's erosive rash recurred, and eventually, she died of sepsis.

DISCUSSION

The patient was diagnosed with an ADR due to allopurinol. This diagnosis was supported by the development of a rash after administration of allopurinol, by the past history of allopurinol-induced rash, and by HLA-

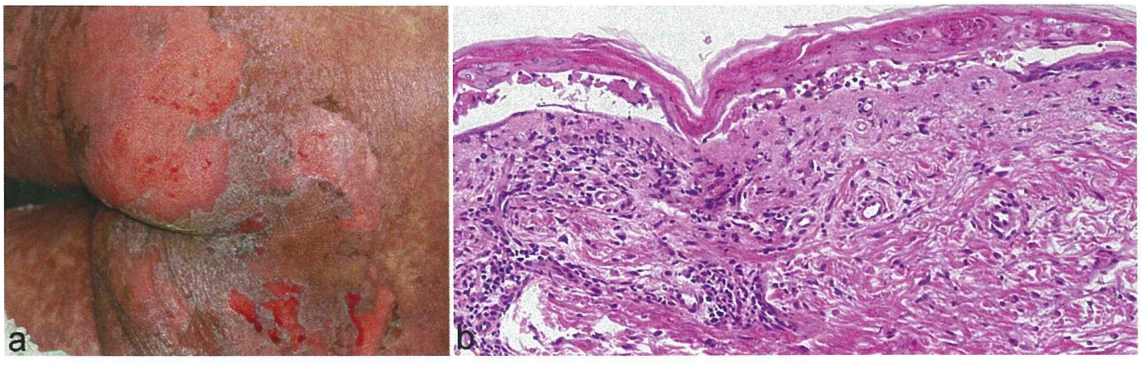

Fig. 1. Clinical features: (a) multiple erythematous lesions were confluent and widely detached. Histopathological features: (b) a skin biopsy showed necrosis of the epidermis and subepidermal blisters.