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Increased ratio of FoxP3+ regulatory T cells/CD3+ T cells in skin lesions in drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms

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Summary

Background. Drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms (DIHS/DRESS) is a severe drug eruption accompanied by multiorgan disorders. Several unique aspects of DIHS/DRESS, including reactivation of herpesvirus, liver dysfunction and hypogammaglobulinemia, have similarities to graft-versus-host disease (GVHD).
Aim. In this study, we focused on the dynamics of regulatory T cells (Tregs) infiltrating into the skin lesions of DIHS/DRESS and GVHD.
Methods. Skin biopsies were taken from patients with DIHS/DRESS, GVHD, or maculopapular drug eruption. Tregs were detected using immunostaining with anti-FoxP3.
Results. The ratio of FoxP3+ T cells to CD3+ T cells was significantly higher in the skin lesions of DIHS/DRESS than in those of patients with GVHD and was positively correlated with the number of days from disease onset in the acute phase.
Conclusions. The dynamics of Tregs in skin lesions are different between DIHS/DRESS and GVHD, despite there being many similarities between these conditions.

Introduction

Drug-induced hypersensitivity syndrome /drug rash with eosinophilia with systemic symptoms (DIHS/DRESS) is a severe drug eruption accompanied by multiorgan disorders,[1] It may be related to reactivation of human herpesvirus (HHV), especially HHV-6 [2–4] and mild epidermal injury, in contrast to other severe adverse cutaneous drug reactions such as toxic epidermal necrosis (TEN) and Stevens–Johnson syndrome (SJS). However, the mechanisms of HHV reactivation and development of drug rashes are currently unknown. DIHS/DRESS has several notable features, such as delayed onset, worsening of clinical symptoms even after withdrawal of the causative drug, hypogammaglobulinemia,[5] reactivation of latent HHV during the acute stage of the disease, and autoimmune complications developing as short-term or long-term sequelae, such as autoimmune thyroiditis, positive reaction of antinuclear antibodies and fulminant type 1 diabetes mellitus.[6,7] Many aspects of this syndrome suggest close similarities between DIHS/DRESS and graft-versus-host disease (GVHD). We and other researchers have also revealed a relationship between HHV-6 reactivation and rash/GVHD after allogeneic stem cell transplantation,[8,9] Various complications frequently occurring in GVHD, such as autoimmune disease,[10,11] are frequently observed during the course of DIHS/DRESS, even long after its clinical resolution. However, there are clinical and histological differences between DIHS/DRESS and GVHD; for example, interface dermatitis and apoptotic keratinocytes can be

observed in both DIHS/DRESS and GVHD, but are more severe in the latter.

Recently, much attention has been focused on regulatory T cells (Tregs) and their roles in drug eruption/GVHD. However, the dynamics of Tregs in the skin lesions in DIHS/DRESS and GVHD are not fully understood. In this study, we focused on the dynamics of Tregs infiltrating into the skin, one of the major target organs in DIHS/DRESS and GVHD, to examine the involvement of Tregs in the development of DIHS/DRESS and GVHD skin lesions.

Methods

The study was approved by the medical ethics committee of Nara Medical University, and all patients gave informed consent.

Patients and samples

Our study consisted of three groups of patients: patients with DIHS/DRESS (n = 12), patients with acute GVHD (n = 12) and patients with maculopapular drug eruption (MDE) (n = 18). The eliciting drugs had been withdrawn by the time of diagnosis of DIHS/DRESS or drug eruption in all patients.

The DIHS/DRESS group consisted of 12 patients (5 men, 7 women; median age 59 years, range 13–75) who were enrolled consecutively during the period April 2003. The profiles of these patients are shown in Table 1. Diagnosis of DIHS/DRESS was based on criteria established by a Japanese consensus group[12] and RegiSCAR group.[13] Reactivation of HHV, including HHV-6 and HHV-7, was demonstrated by an increase in the titre of the specific serum IgG antibody and/or DNA levels in whole blood as detailed below. Skin biopsies were also taken from areas of maculopapular erythema in this group.

Table 2 details the characteristics of 12 consecutive patients with clinical signs of acute GVHD (3 men, 9 women; median age 52 years, range 7–66) who received allogeneic stem cell transplantation for haematological malignancy during the period November 2002 to August 2011. All 12 patients had received standard prophylaxis (ciclosporin in 10 patients and mycophenolate mofetil in 2 patients) prior to transplantation. Skin biopsies were taken from areas of erythematous maculopapular rash in all 12 patients, which were clinically graded according to standard criteria.[14]

The final group consisted of 18 patients (10 men, 8 women; median age 61 years, range 32–81). Skin biopsies were also taken from areas of cutaneous rash of patients without allografts or DIHS/DRESS (n = 18) that was clinically and histopathologically considered as MDE.

Assessment of herpesvirus DNA

DNA levels were assessed by PCR. DNA was extracted from whole blood using a QIAamp DNA Blood Mini-kit (Qiagen Inc., Tokyo, Japan) in accordance with the manufacturer's instructions, and then used for PCR. For assessment of HHV-6 and HHV-7 DNA levels in peripheral blood, real-time PCR was performed as described in a previous report,[15] and results expressed as viral DNA genome equivalents per 1 mL of whole blood. In DIHS/DRESS, HHV-6 DNA is usually detected during days 14–21 after the onset of skin eruption, whereas it is usually increased in accordance with the skin eruption in GVHD, as described previously.[9]

Immunohistochemistry

Tissues were fixed in formalin, embedded in paraffin wax, and cut into sections 4μm thick. Immunostaining was performed using anti-CD3 (code A0452; Dako, Glostrup, Denmark) polyclonal antibody, anti-FoxP3 (clone 236 A/E7; BD Biosciences Inc., San Jose, CA, USA), and anti-CD4 (NCL-CD4-368, clone 4B12), anti-CD8 (NCL-C8-295, clone 1A5) (both Novocastra Ltd, Newcastle upon Tyne, UK) monoclonal antibodies as primary antibodies. Biotinylated antimouse IgG was used as secondary antibody, and bound antibody was evaluated using streptavidin-biotinylated peroxidase complex. After washing, sections were exposed to the chromogen and counterstained with haematoxylin. The numbers of immunostained cells in the dermis were counted in five high-power fields (HPF) and expressed as the mean number. The ratios of FoxP3+ Tregs, CD4+ T cells, and CD8+ T cells to CD3+ T cells in the dermis were then calculated.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using the Student t-test. Pearson correlation coefficient was used to evaluate the correlation between the FoxP3+ Treg/CD3+ T-cell ratio in lesional skin and the number of days from onset. $P < 0.05$ was considered statistically significant.

Results

Histopathological examination

Histopathological examination of skin biopsies obtained from the erythematous maculopapular rashes of patients with DIHS/DRESS showed perivascular lymphocytic infiltration with eosinophils (8 cases; 66.7%), interface dermatitis with vacuolar degeneration (2 cases; 16.7%) and spongiotic dermatitis with vacuolar degeneration (2 cases; 16.7%). Skin biopsies from rashes in patients with acute GVHD were graded according to the criteria by Lerner *et al.*[15,16] and showed vacuolar degeneration (histological grade I; 6 cases; 50%) and spongiosis with apoptotic cells (histological grade II; 6 cases; 50%). None of the cases showed a cleft between the epidermis and dermis (histological grade III or IV). Tissue from MDE mainly exhibited perivascular lymphocytic inflammation, occasionally with eosinophils.

Increased FoxP3+ Treg/CD3+ T-cell ratio in the skin lesions of DIHS/DRESS

The FoxP3+ Treg/CD3+ T-cell ratio was significantly higher in DIHS/DRESS rashes than in GVHD and MDE tissue (Figs 1 and 2), but the ratio in GVHD was not significantly different from that in MDE. In skin biopsy specimens from GVHD rashes and MDEs, we found small numbers of FoxP3+ Tregs. By contrast, CD4+/CD3+ and CD8+/CD3+ T-cell ratios in the skin lesions were similar for the three groups (Figs 1 and 2). The numbers of CD3+ T cells per 5 high-power fields in skin biopsies of those patients were also not significantly different.

Relationships between FoxP3+ Tregs/CD3+ T cells and the period from onset

Figure 3 shows the relationships between the ratio of FoxP3+ Tregs/CD3+ T cells in the lesional skin and the number of days from disease onset. All patients with DIHS/DRESS in this study had received no major treatment such as high-dose corticosteroid before the skin biopsies were taken. The FoxP3+ Treg/CD3+ T-cell ratio was positively correlated with the number of days from disease onset during the acute phase in DIHS/DRESS, but there was no correlation in either GVHD or MDE.

Discussion

Although DIHS/DRESS and GVHD can have similar presentations, there are a some clinical and histological differences between them. The cutaneous presentation of DIHS/DRESS often involves a maculopapular rash or erythroderma, but not blister formation or erosion. The common pathological findings of DIHS/DRESS are superficial perivascular lymphocytic infiltration with extravascular eosinophils, but histologically, severe liquefaction degeneration of the basal layer or epidermal necrosis is rarely found. By contrast, GVHD often presents with blister formation and erosion, and histologically shows lichenoid reaction with epidermal necrosis and/or epidermolysis.

Previous research on the dynamics of skin-infiltrating Tregs in GVHD showed that a decreased number of skin-infiltrating Tregs was associated with severity of GVHD;[17] however, another study showed that Tregs increased with degree of inflammation and grade of GVHD.[18]

Patients with DIHS/DRESS in the acute stage were found to exhibit increased frequencies of Tregs and gradual loss of their function after resolution in peripheral blood mononuclear cells (PBMCs).[19] However, there have been no studies about the dynamics of skin-infiltrating Tregs in DIHS/DRESS. Therefore, we focused on the dynamics of infiltrating Tregs in the skin lesions of these diseases, and found considerable differences between DIHS/DRESS and GVHD.

In the current study, the FoxP3+ Treg cell/CD3+ T-cell ratio was significantly higher in lesions from DIHS/DRESS than in those from GVHD and MDE, whereas the numbers of CD3+ T cells infiltrating into the skin lesions were similar in all three conditions (Figs 1 and 2). We also found that the ratio was positively correlated with the number of days from disease onset during the acute phase of DIHS/DRESS (Fig. 3). However, each dot in Fig. 3 represents the FoxP3+/CD3+ ratio from different patient samples, so the data does not show sequential data from individual patients, and thus results must be interpreted with caution. By contrast, the ratios of CD4+CD3+ T cells and CD8+CD3+ T cells in cutaneous lesions were similar for DIHS/DRESS, GVHD and MDE (Fig. 2). These findings suggest that clinical and histological differences between DIHS/DRESS and GVHD may result from differences in the frequency of FoxP3+ Tregs infiltrating into the skin lesions of these diseases. Tregs play a significant role in suppression of various diseases, including allergic responses, autoimmune and infectious disease, and cancers.[20,21] Accordingly, it is likely that an increased number of FoxP3+ T cells infiltrating into DIHS/DRESS skin lesions can protect the epidermis from severe damage compared with that in GVHD skin lesions.

Conclusion

In conclusion, the present study suggests that, despite many similarities, the dynamics of Tregs are different between DIHS/DRESS and GVHD in skin lesions, and that this difference may exert a considerable influence on the development of skin presentations in the two diseases.

What's already known about this topic?

- There are close similarities between DIHS/DRESS and GVHD, including HHV-6 reactivation, skin eruption, and autoimmune disease-like complications.
- However, there are also some clinical and histological differences between these two conditions.
- There are conflicting reports about the dynamics of skin-infiltrating Tregs in GVHD: severity of disease has been associated with both a decreased and an increased number of skin-infiltrating Tregs.
- Patients with DIHS/DRESS patients exhibit increased frequencies of Tregs in PBMCs at the acute stage; however, the dynamics of skin-infiltrating Tregs in DIHS/DRESS are currently unknown.

What does this study add?

- In the current study, levels of FoxP3+ Tregs were significantly higher in the skin lesions of DIHS/DRESS than in those of GVHD.
- The FoxP3+ Treg cell/CD3+ T-cell ratio was positively correlated with the number of days from disease onset during the acute phase of DIHS/DRESS, but not in GVHD or MDE.

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Figure 1 Expression of FoxP3+, CD3+, CD4+ and CD8+ T cells in drug-induced hypersensitivity syndrome (DIHS/DRESS), graft-versus-host disease (GVHD) and maculopapular drug eruption (MDE). Skin biopsies from patients with DIHS/DRESS showed a high number of FoxP3+ T cells in the epidermal-dermal junction and upper dermis compared with those in GVHD and MDE. Sections were counterstained with haematoxylin, and images show representative serial sections from the same lesion of a patient with each disease (original magnification × 200). Patient numbers correspond with those in the tables.

Figure 2 Ratios of FoxP3+ regulatory T cells, CD4+ T cells, and CD8+ T cells to CD3+ cells in paraffin wax-embedded biopsies taken from patients with drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms (DIHS/DRESS; $n = 12$), graft-versus-host disease (GVHD; $n = 12$) and maculopapular drug eruption (MDE; $n = 18$) are shown. (a) In DIHS/DRESS, a high ratio of FoxP3+ T cells per 100 CD3+ T cells was observed. (b, c) The ratios of CD4+/CD3+ and CD8+/CD3+ T cells infiltrating into the lesional skin of DIHS/DRESS were not statistically different from those in GVHD and MDE. (d) Numbers of infiltrating CD3+ T cells were quite similar in DIHS/DRESS, GVHD and MDE ($^{*}P < 0.05$, $^{**}P < 0.01$).

Figure 3 There was a correlation between the FoxP3+ Treg/CD3+ T-cell ratio and the time from disease onset in skin biopsies from patients with drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms (DIHS/DRESS).

Table1
Characteristics of DIHS/DRESS patients

Patient	Age/Sex	Causative drug	Viral reactivation	Viral DNA loads (of whole blood) or titers	Days from onset to skin biopsy	Immunosuppressive treatments at the time of skin biopsy	Timing of viral reactivation (days from skin biopsy)	FoxP3$^+$/ CD3$^+$ cells in the skin lesion (%)	Skin rash	Eosinophil (/μl)	Liver dysfunction (IU/L)
1	62/M	Carbamazepine	HHV-7	1.2×10^4copies/ml	5	none	-1	14.7	Maculopapular erythema	980	286/723
2	68/F	Carbamazepine	HHV-6	8.8×10^3copies/ml	15	none	2	17	Maculopapular erythema	1560	34/45
3	75/F	Allopurinol	HHV-6, HHV-7	1.3×10^3 copies/ml (HHV-6)	13	none	3	27.2	Maculopapular erythema, purpura	1200	57/84
4	61/F	Salazosulfapyridine	HHV-6	7.2×10^4 copies/ml	13	Prednisolone 10 mg/day	4	23.8	Erythroderma	1200	49/107
5	64/F	Mexiletine	HHV-6	3.4×10^5 copies/ml	10	Betamethasone 1.0 mg/day	5	17.3	Maculopapular erythema, purpura	3000	100/182
6	44/M	Carbamazepine	HHV-6, HHV-7	7.4×10^3 copies/ml (HHV-6)	11	Betamethasone 1.0 mg/day	6	14.7	Erythroderma, pustule	7300	91/130
7	62/M	Lamotrigine	HHV-7	IgG (1:20) (day15) IgG (1:1280) (day29)	13	none	7	13.3	Maculopapular erythema	700	28/104
8	32/M	Allopurinol	HHV-6	4.8×10^3 copies/ml	8	none	9	9.9	Maculopapular erythema	2200	52/304
9	56/M	Cyanamide	HHV-6	2.4×10^4 copies/ml	4	none	10	15.6	Maculopapular erythema	3200	101/119
10	57/F	Salazosulfapyridine	HHV-6	2.6×10^3 copies/ml	10	Prednisolone 10 mg/day	10	7.5	Erythroderma, pustule	5800	257/383
11	13/F	Carbamazepine	HHV-6	2.0×10^4 copies/ml	2	none	13	7.8	Maculopapular erythema	2100	124/295
12	36/F	Lamotrigine	HHV-6	1.4×10^5 copies/ml	6	none	13	6.2	Erythroderma	2400	40/108

Table 1 shows the maximum value in the category of eosinophil and AST/ALT during the course of DIHS/DRESS.

Table2

Profiles of patients of GVHD after allogenic stem cell transplantation

Patient	Age/Sex	Underlying disease	Transplantation	Pretransplant conditioning	Viral reactivation	Viral DNA loads (of whole blood)	Days from onset to skin biopsy	$FoxP3^+$/ $CD3^+$ cells in the skin lesion (%)	Grade of GVHD
1	37/F	ALL	CBCT	TBI, FLU, BU	HHV-6	1.6×10^4 copies/ml	7	9.5	I
2	51/F	MDS	PBSCT	TBI, FLU, CPA, Mesna	HHV-6, CMV	5.2×10^3 copies/ml	3	4.2	I
3	63/F	MDS	CBCT	TBI, FLU, CPA, Mesna	HHV-6, CMV	8.0×10^4 copies/ml	3	3	I
4	45/F	AML	PBSCT	TBI, FLU, BU	HHV-6	9.2×10^3 copies/ml	2	2.5	IV
5	46/F	MDS	PBSCT	FLU, BU	CMV	4.4×10^3 copies/ml	5	4.8	II
6	52/M	ALL	CBCT	TBI, CPA, VP-16	HHV-6	1.2×10^3 copies/ml	6	8.2	II
7	62/F	ALL	PBSCT	FLU, BU	ND	ND	4	0.7	IV
8	36/F	ALL	BMT	TBI, CPA, BU, Mesna	ND	ND	29	3.3	III
9	7/M	ALL	BMT	TBI, L-PAM	ND	ND	27	0.6	IV
10	66/F	MM	PBSCT	L-PAM, BTZ	HHV-6	3.4×10^3 copies/ml	6	4.2	II
11	64/F	CML	BMT	TBI, FLU, BU, ATG	HHV-7	8.4×10^3 copies/ml	3	9.8	I
12	60/M	AML	CBCT	FLU, BU, Ara-C	HHV-6	7.2×10^3 copies/ml	4	6.7	I

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ara-C, cytosine arabinoside; ATG, antithymocyte globulin; BMT, bone marrow transplantation; BTZ, bortezomib; BU, busulfan;
CBCT, cord blood cell transplantation; CLL, chronic lymphoblastic leukemia; CML, chronic myeloid leukemia; CPA, cyclophosphamide; FLU, fludarabine; GVHD, graft-versus-host disease;
L-PAM, L-phenylalanine monohydrochloride; MDS, myelodysplastic syndrome: MM, multiple myeloma; ND, no data; PBST, peripheral blood cell stem cell transplantation; TBI, total body irradiation; VP-16, etoposide

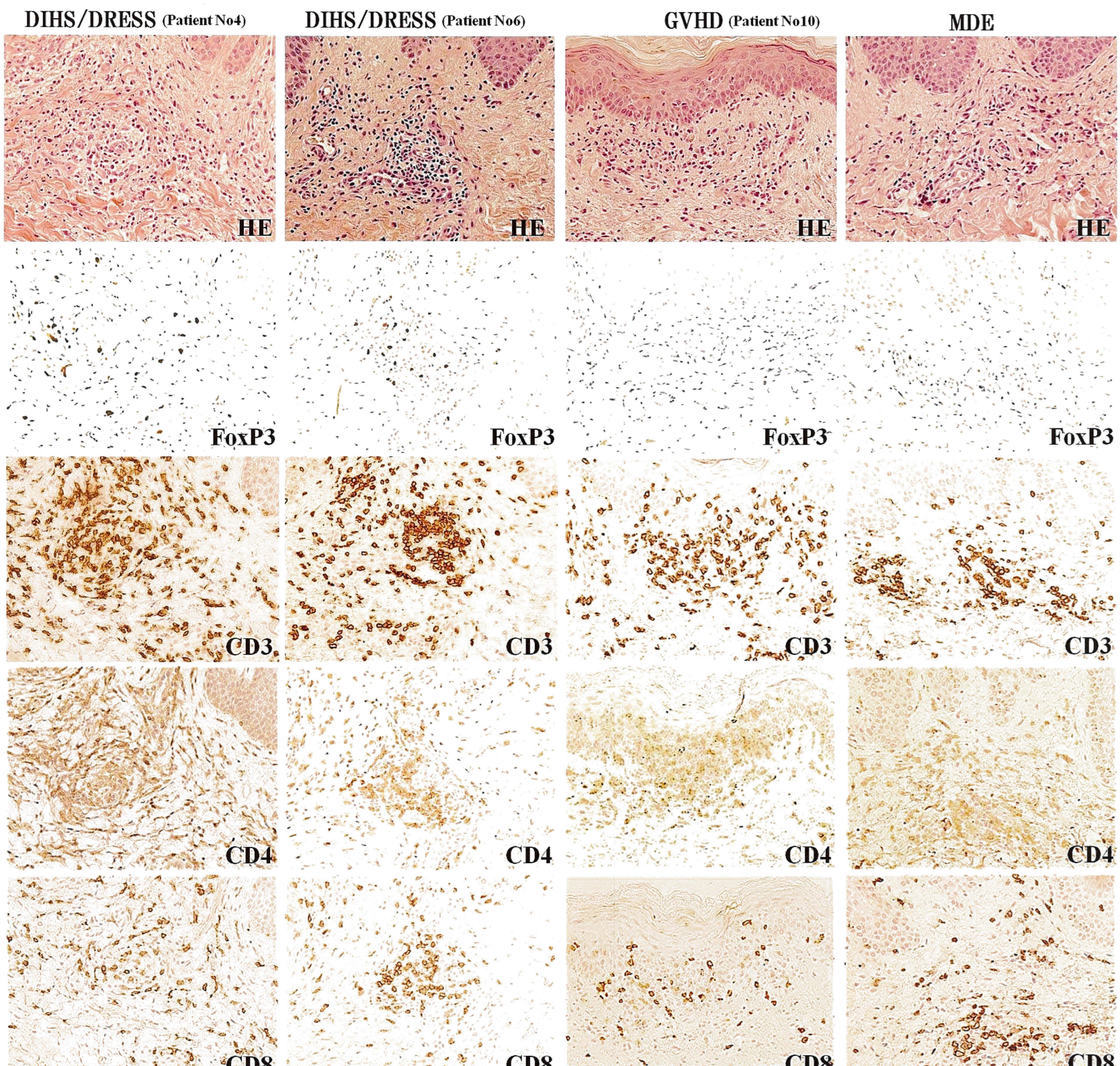

DIHS/DRESS (Patient No4)
DIHS/DRESS (Patient No6)
GVHD (Patient No10)
MDE
HE
FoxP3
CD3
CD4
CD8

Fig 2

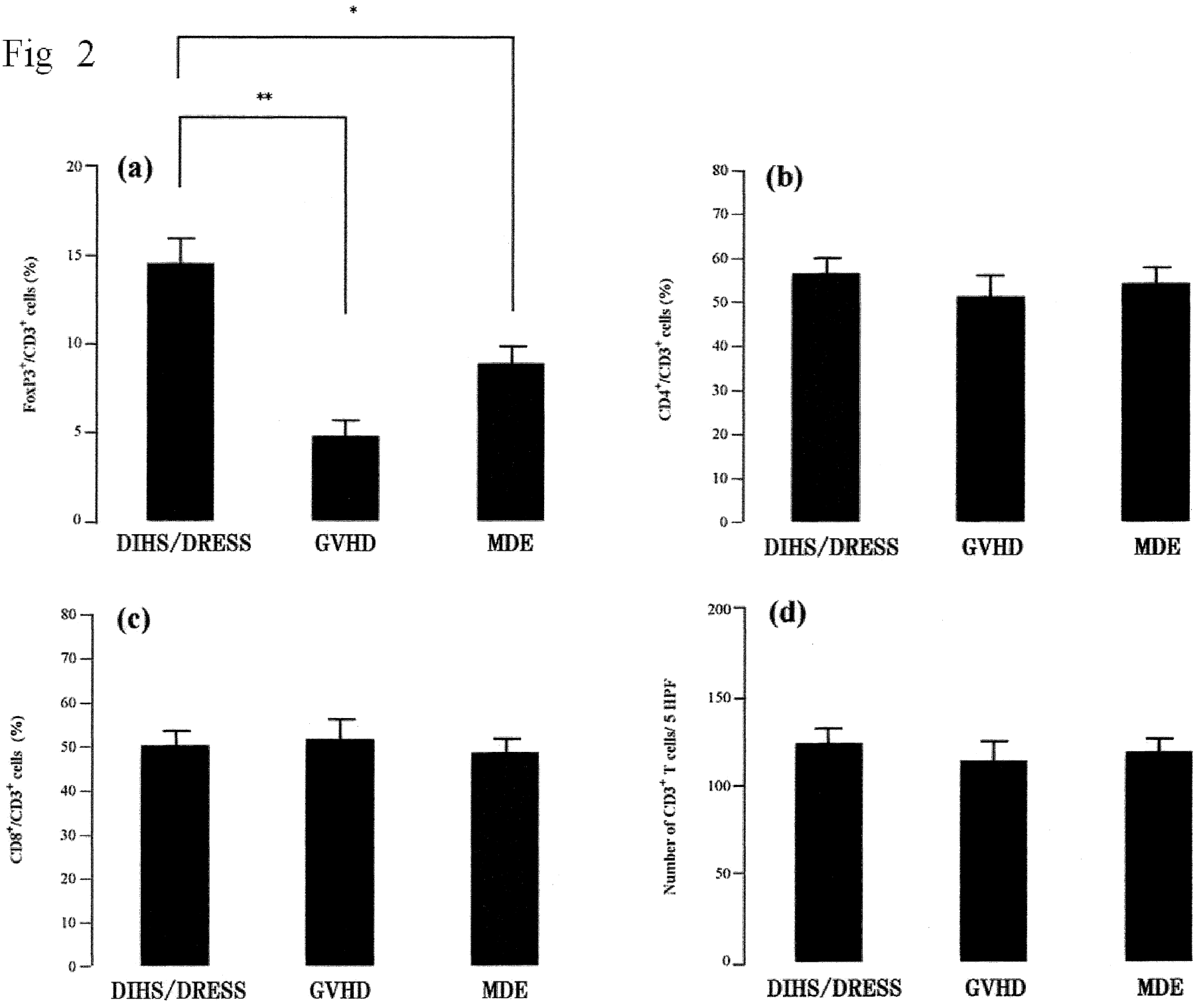
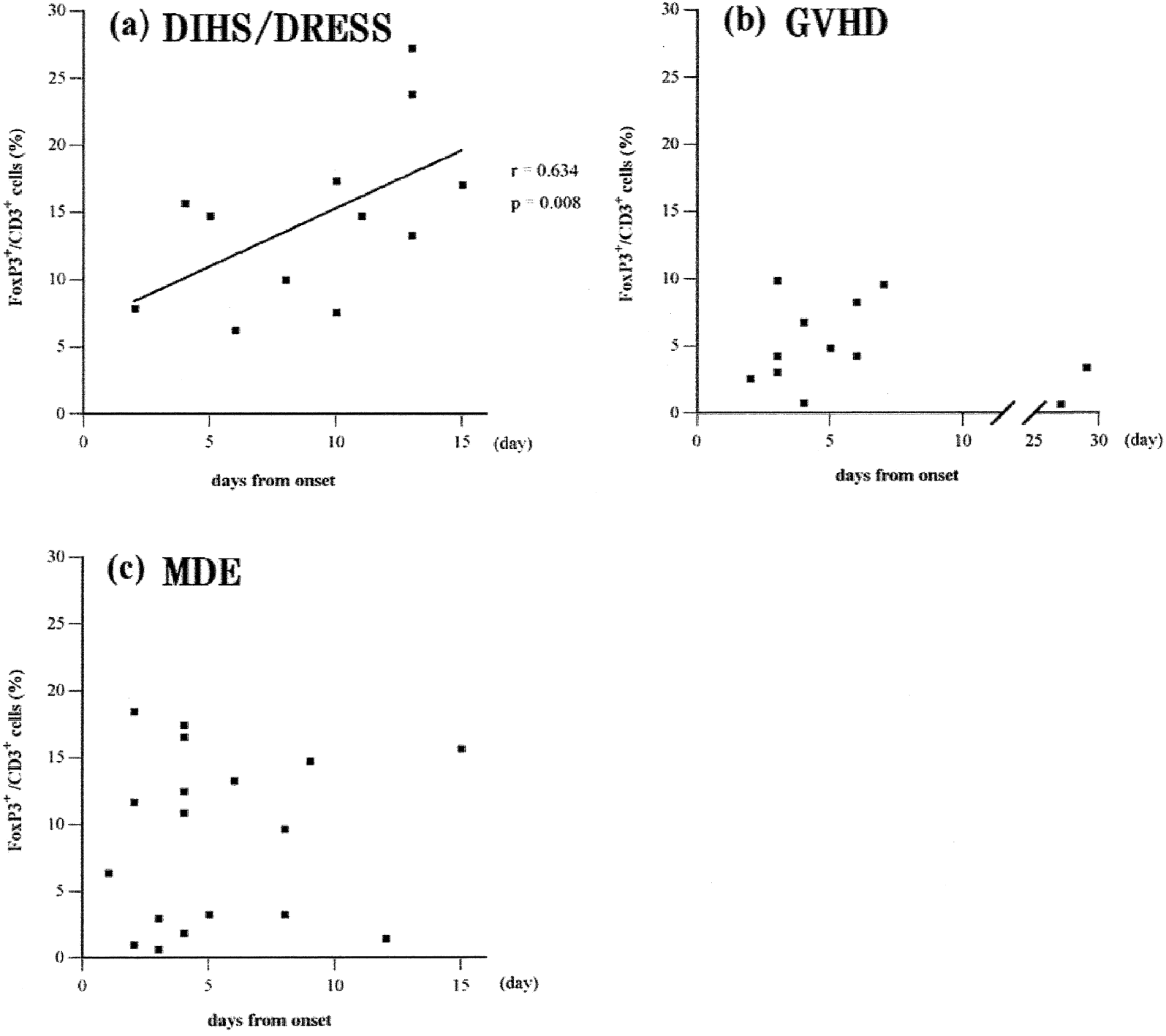

*
**
(a)
FoxP3+/CD3+ cells (%)
20
15
10
5
0
DIHS/DRESS
GVHD
MDE
(b)
CD4+/CD3+ cells (%)
80
70
60
50
40
30
20
10
0
DIHS/DRESS
GVHD
MDE
(c)
CD8+/CD3+ cells (%)
80
70
60
50
40
30
20
10
0
DIHS/DRESS
GVHD
MDE
(d)
Number of CD3+ T cells/ 5 HPF
200
150
100
50
0
DIHS/DRESS
GVHD
MDE

Fig 3


(a) DIHS/DRESS
r = 0.634
p = 0.008
FoxP3+/CD3+ cells (%)
days from onset
(day)
(b) GVHD
FoxP3+/CD3+ cells (%)
days from onset
(day)
(c) MDE
FoxP3+ /CD3+ cells (%)
days from onset
(day)