Histol Histopathol (2012) 27: 235-240
DOI: 10.14670/HH-27.235

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Histological features and immune cell changes in skin lesions of engraftment syndrome of children undergoing hematopoietic stem cell transplantation

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Summary. Various skin eruptions are encountered during hematopoietic stem cell transplantation (HSCT) of children with hematologic malignancies. Engraftment syndrome (ES) is a disease characterized by fever, weight gain, maculopapular skin rash and noncardiogenic pulmonary edema. ES occurs during neutrophil recovery without identifiable causes of infection. Early detection of ES is critical to reduce mortality and morbidity, but identical morphologic changes found in skin lesions from ES and graft-versus-host disease (GVHD) are a challenging problem for histology-based diagnosis. To resolve this issue, immunopathologic changes in skin lesions of ES were studied. Five skin biopsies from patients with symptoms clinically compatible with ES were retrieved and compared to 15 age- and sex-matched cases of acute GVHD with antibodies to CD3, CD4, CD8 and CD1a. Mean numbers of epidermal $CD8^+$ cells and $CD1a^+$ cells were lower in ES than in GVHD. However, there were no significant differences in mean score of GVHD grade, mean numbers of lymphoid cells, $CD3^+$ cells, or $CD4^+$ cells. In the setting of HSCT in children, the dominance of $CD4^+$ cells and a decreased number of $CD1a^+$ cells in the epidermis are specific features for the skin lesions of ES.

Key words: Lichenoid eruptions, Hematopoietic stem cell transplantation, Graft vs. host disease, Engraftment syndrome

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Introduction

Hematopoietic stem cell transplantation (HSCT) is used as a curative modality for various kinds of malignant hematological diseases, thanks to the recent development in our understanding of the biology of both autologous and allogeneic stem cell transplantation (Craddock, 2000). However, a range of skin lesions encountered during these procedures present diagnostic challenges. Acute graft-versus-host disease (GVHD) in either autologous or allogeneic HSCT, drug eruption and skin infection are major differential categories that must be considered in such clinical settings.

A constellation of clinical signs such as fever, skin rash, and pulmonary injury have been described, originally in recipients of autologous (Lee et al., 1995; Ravoet et al., 1996), and more recently in allogeneic HSCT (Gorak et al., 2005). Engraftment syndrome (ES) is a complex disease of various signs and symptoms that typically occurs during the period of neutrophil recovery (Lee et al., 1995; Ravoet et al., 1996). Fever, weight gain, maculopapular skin rash and noncardiogenic pulmonary edema without identifiable causes of infection are consistently described (Lee et al., 1995; Ravoet et al., 1996). Since there is no uniform diagnostic criterion for ES, incidence, clinical features and risk factors of ES vary between reports (Lee et al., 1995; Cahill et al., 1996; Nurnberger et al., 1997). Other authors describe this syndrome under the term of capillary leak syndrome (CLS) (Cahill et al., 1996), or auto-aggression syndrome in the setting of autologous HSCT (Moreb et al., 1997). The pathophysiologic mechanism of ES is still poorly understood, but is considered multifactorial (Spitzer, 2001). Interactions between T cells and other effector cells,

proinflammatory cytokine production, and injury of epithelial and endothelial cells from toxic regimens are regarded as the main causes of the development of ES (Jadus and Wepsic, 1992; Rabinowitz et al., 1993; Takatsuka et al., 2000).

Early recognition and accurate diagnosis of ES is necessary, because a patient showing ES has greater chance of morbidity and mortality and systemic steroid treatment is indicated (Nurnberger et al., 1997; Edenfield et al., 2000).

In several reports, the description of histologic features of skin lesions in ES is identical to acute GVHD, grade 1 or 2 (Lee et al., 1995; Moreb et al., 1997). However, previous ES studies were focused mainly on clinical features, and studies on the pathologic features of skin lesions found in ES have not been performed to date. To resolve these issues, we retrieved skin biopsies from patients who underwent HSCT and reviewed the morphologic features of skin lesions found in patients clinically compatible with diagnostic criteria of ES. As a main differential diagnosis and control disease, skin biopsies from acute GVHD cases were also included and were compared with skin lesions in ES.

Materials and methods

Patients

From January 2000 to December 2006, 106 cases of punch biopsy of skin were submitted to the department of pathology from pediatric patients with hematological malignancies who underwent autologous or allogeneic HSCT at Seoul National University Children's Hospital and Samsung Medical Center in Seoul, Korea. Cases compatible with ES ware selected according to the diagnostic criteria described below (Spitzer, 2001).

Major criteria

- Temperature of ≥38.3°C with no identifiable infectious etiology.
- Erythrodermatous rash involving more than 25% of body surface area and not attributable to a medication.
- Noncardiogenic pulmonary edema, manifested by diffuse pulmonary infiltrates consistent with this diagnosis, and hypoxia

Minor criteria

- Hepatic dysfunction with either total bilirubin ≥2 mg/dl or transaminase levels >2 x normal.
- Renal insufficiency (serum creatinine of ≥2 xbaseline level)
- Weight gain ≥2.5% of baseline body weight
- Transient encephalopathy unexplainable by other causes.

A diagnosis of ES is made by the presence of all three major criteria or two major criteria and one or more minor criteria. ES should occur within 96 hours of engraftment (neutrophil count of ≥500/μl for 2 consecutive days) (Spitzer, 2001). Clinical characteristics of cases compatible with the above criteria are summarized in Table 1. As a control group, we randomly selected sex- and age-matched (±1 year) 15

Table 1. Summary of clinical findings of patients with engraftment syndrome.

Features	Case				
	1	2	3	4	5
Sex/age	F/17	F/3	M/10	F/15	M/14
Underlying disorder	ABL	ALL	ALL	ALL	ALL
Onset (day)[a]	21	13	13	16	13
Fever[b]/rash[c]/weight gain[d]	Yes	Yes	Yes	Yes	Yes
Skin lesion (site of involvement)	maculopapule (back, upper and lower extremities)	maculopapule (trunk, back, face upper and lower extremities)	maculopapule (trunk, upper and lower extremities)	maculopapule (site not described)	macule (back, face, upper and lower extremities)
Pulmonary infiltrate	Yes	No	Yes	Yes	Yes
Hepatic dysfunction[e]	Yes	Yes	Yes	No	Yes
ANC >500/μl (days)[f]	21	13	11	15	12
Source of HSCT	allogeneic	allogeneic	allogeneic	allogeneic	allogeneic
Number of CD34$^+$cell Infused	3.37x10^5/kg	2.8x10^5/kg	2.14x10^5/kg	2.55x10^5/kg	NA
Treatment response	response to steroid	response to steroid	response to steroid → desquamation	response to steroid	response to steroid → desquamation
Clinical course	subsided	subsided	GVHD on day 34[f]	GVHD on day 47[f]	subsided

[a]: days after HSCT; [b]: elevation of body temperature ≥38.3°C; [c]: skin lesion involving more than 25% of body surface area; [d]: weight gain ≥2.5% of baseline body weight; [e]: hepatic dysfunction either total bilirubin ≥2mg/dl or transaminase levels ≥2x normal; [f]: days after HSCT. ABL, acute biphenotypic leukemia; ALL, acute lymphoblastic leukemia; NA, data not available; ANC, absolute neutrophil count; HSCT, hematopoietic stem cell transplantation; GVHD, graft-versus-host disease.

cases diagnosed with acute GVHD during the same period. Histologic features found in skin from ES and acute GVHD were compared by routine H&E staining. The study was reviewed by the ethical committees of both hospitals.

Histologic and immunohistochemical analysis

Skin biopsies were evaluated using Lerner's grading criteria for acute GVHD (Lerner et al., 1974): grade 1, lichenoid mononuclear infiltration of the upper dermis and epidermis with vacuolation of basal cells; grade 2, more severe damage resulting in individual basal cell death (apoptosis); grade 3, coalescence of dead basal cells to produce clefts at the dermal-epidermal junction; and grade 4, sloughing of the epidermis. In addition, the numbers of lymphoid cells and immune cell subsets in skin from ES and acute GVHD were quantitated by H&E staining and immunohistochemical staining. Paraffin sections were deparaffinized, rehydrated and blocked before incubation with primary antibodies to CD3, CD4, CD8, CD1a (clone F7.2.38, dilution 1:50; 4B12, 1:20; 144B, 1:25; clone O10, 1:50, DAKO Corp., Glostrup, Denmark). Primary antibodies were incubated with secondary goat or rabbit anti-mouse antibody and visualized using an LSAB peroxidase kit (DAKO Corp., Glostrup, Denmark) according to the manufacturer's instructions. In the case of CD4 and CD8, antigen retrieval was conducted by treatment with a microwave for 10 minutes in citrate buffer. Quantitative morphometric analysis was done according to the method described by Jerome et al., (1998). For the comparison of tissue sections showing different shapes and sizes, the quantitative data were normalized to the area (in mm^2) of epidermis present in the section. "Epidermal area" was measured from the granular layer to the basement membrane.

Statistical analysis

For the comparison of dichotomous variables, χ^2-tests were performed, and to compare continuous variables the Mann-Whitney *U* test was used. Statistical tests were carried out using SPSS12.0 software (SPSS Inc., Illinois, USA). Variables with a *p*-value <0.05 were considered significant. All data are represented as mean ± standard deviation.

Results

Clinical findings

Among 106 patients who underwent skin biopsy, clinical features of five cases (4.7%) met the diagnostic criteria of ES. Detailed clinical features are summarized in Table 1. Two patients were male and three female, the mean age was 11.8 years (range 3-17). The underlying disease of four patients was acute lymphoblastic leukemia, and one had acute biphenotypic leukemia. The source of HSCT was allogeneic in all five patients. Onset of skin lesion was 15.2 days after HSCT (range 13-21) and 1.6 days (range 0-3) after absolute neutrophil count (ANC) was elevated above 500/μl. Skin lesions were erythematous macular and papular in four patients (80%), and macular in one (20%) (Fig. 1A). Upper and lower extremities were involved in all cases except case 4. Three patients had skin rash on the back. The face was involved in two patients. Original pathologic diagnoses

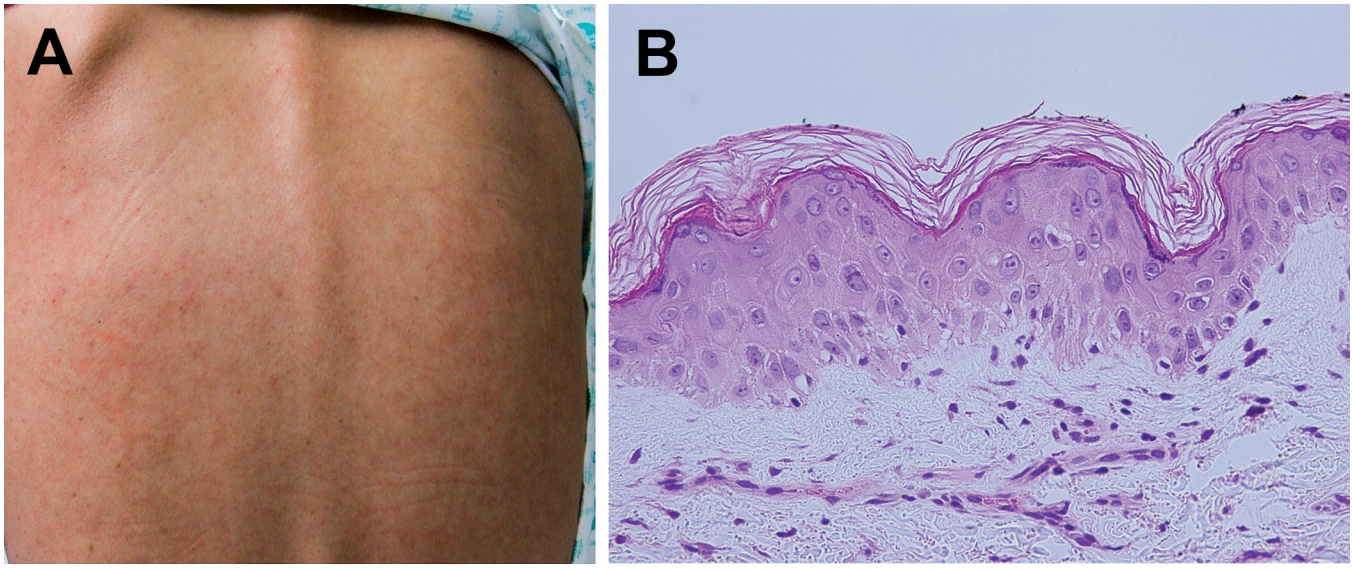

Fig. 1. Clinical and pathologic features of engraftment syndrome (ES). **A.** Erythematous macules with varying size are noted on the whole area of the back. **B.** Vacuolar interface change of basal layer is observed with sparse lymphoid infiltrate in superficial dermis. x 200

were acute GVHD grade 1 or 2 in four cases, and interface dermatitis in one (Fig. 1B). Skin lesions subsided after steroid treatment in all cases. Two cases were followed by desquamation. Acute GVHD involving skin without signs or symptoms of ES were encountered in two cases during clinical course on day 34 and 47, respectively.

Fifteen age- and sex-matched cases of acute GVHD were compared with the five ES patients. The underlying disorder of thirteen cases was acute lymphoblastic leukemia, and two had acute myeloid leukemia. The onset of skin lesion was 33.6 days after HSCT (range 18-54), which was later than that of ES group (33.6±11.9 in GVHD vs. 15.2±3.5 in ES, $p<0.01$). ANC (cells/μl) measured at presentation of skin lesion was higher in GVHD than ES (2056.8±1381.5 vs. 545.6±32.3,

Table 2. Comparison of histologic and immune cell features of skin lesions in ES and acute GVHD.

Morphologic features		Diagnostic category		*p*-value
		ES (n=5)	GVHD (n=15)	
GHVD score	grade	0.8±0.4	1.1±0.8	0.537
Lymphoid cells[a]	Epidermis	148.6±114.7	290.0±172.0	0.106
	Dermis	689.6±298.8	798.9±379.4	0.570
CD3$^+$ cells[a]	Epidermis	128.6±101.9	233.4±151.8	0.190
	Dermis	590.0±245.6	681.3±317.5	0.541
CD4$^+$ cells[a]	Epidermis	89.4±69.9	101.0±63.6	0.861
	Dermis	271.0±122.9	265.1±124.3	0.760
CD8$^+$ cells[a]	Epidermis	34.8±25.7	131.1±84.2	0.013
	Dermis	268.8±147.0	327.7±161.5	0.458
CD1a$^+$ cells[a]	Epidermis	6.4±4.3	26.2±10.1	0.002
	Dermis	0.8±0.8	1.4±1.3	0.466
CD4$^+$/CD3$^+$ (%)	Epidermis	70.7±4.2	42.8±9.2	0.003
	Dermis	45.6±3.2	40.5±9.8	0.089
CD8$^+$/CD3$^+$ (%)	Epidermis	29.0±6.5	57.7±10.9	0.003
	Dermis	44.0±6.2	47.4±5.9	0.275
CD4$^+$/CD8$^+$ ratio	Epidermis	2.5±0.4	0.8±0.3	0.003
	Dermis	1.0±0.1	0.9±0.3	0.150

[a]: To allow comparison of tissue sections of different sizes, the cell numbers counted were normalized to the area (in mm^2) of epidermis present in the section. All data are compared by Mann-Whitney *U*-test and represented in mean ± standard deviation. ES, engraftment syndrome; GVHD, graft-versus-host disease.

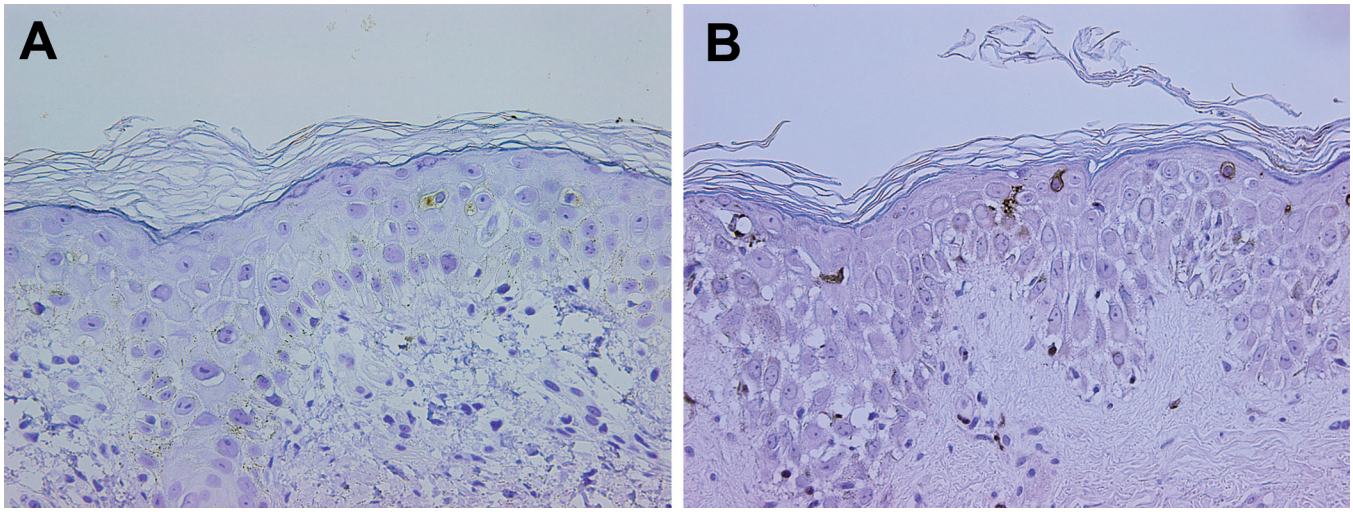

Fig. 2. Immunohistochemical features of ES. CD1a$^+$ Langerhans cells in the epidermis in ES **(A)** are decreased in number compared to skin from acute GHVD **(B)**. x 200

$p<0.05$). Neurologic symptoms or weight gain were not observed in GVHD. The source of HSC, number of $CD34^+$ cells infused or use of G-CSF or GM-CSF did not differ between the ES and GVHD patients.

Histologic and immunohistochemical findings.

Mean score of GVHD was the same in both diseases (0.8±0.4 in ES vs. 1.1±0.8 in GVHD). To identify differences in immune cell response, the numbers of lymphoid cells in each subset, and $CD1a^+$ cells, were quantitated in epidermis and dermis (Table 2). Total numbers of lymphoid cells, $CD3^+$ lymphocyte subsets and $CD4^+$ lymphoid subsets were the same in both ES and GVHD. However, the mean number of $CD8^+$ cells in the epidermis was higher in GVHD than in ES (131.1±84.2 vs. 34.86±25.7, $p<0.05$). The numbers of $CD1a^+$ Langerhans cell in the epidermis were also higher in GHVD than in ES (26.2±10.1 vs. 6.4±4.34, $p<0.05$) (Fig. 2A,B).

The relative proportions of cellular subsets expressing either CD4 or CD8 among $CD3^+$ lymphoid cells were compared. The relative proportion of $CD4^+$ cells to $CD3^+$ cells in the epidermis was higher in ES than in GVHD (70.7±4.2 % vs. 42.8±9.2 %, $p<0.05$). However, the relative proportion of $CD8^+$ cells to $CD3^+$ cells in the epidermis was higher in GVHD than in ES (29.0±6.5 % vs. 57.7±10.9 %, $p<0.05$). When calculated for cellular subsets in the dermal compartment, these proportions were the same in both conditions.

Discussion

Engraftment syndrome is a not uncommon condition, with incidence ranging 7-21%, among patients receiving autologous and allogeneic HSCT (Nurnberger et al., 1997; Edenfield et al., 2000). However, skin biopsy is not frequently performed in those settings. In the largest series by Lee (1995), biopsy was performed in 23 of patients (9%) out of 248 patients with ES. In our series, histologic materials clinically compatible with ES were obtained from only five cases (4.7%) out of 106 skin biopsies during 7 years. In our series, cases that underwent skin biopsy were selectively reviewed. Thus the exact incidence of ES could not be estimated or compared based on this data.

Histologic features of ES and acute GVHD are considered identical (Lee et al., 1995; Moreb et al., 1997). Superficial perivascular infiltration of lymphoid cells and interface vacuolar change are reported in most cases (Lee et al., 1995; Moreb et al., 1997). However, subtle but definite alterations were observed in immune cell subsets in skin lesions. The absolute number of lymphoid cells and $CD3^+$ cells was basically the same in both conditions. However, several calculated values in our study (Table 2) showed a predominance of $CD4^+$ cells in epidermis in skin from ES. This finding is consistent with the study by Lee et al., where $CD4^+$ cells were major cellular subsets in ES (Lee et al., 1995). These features also suggest that the underlying mechanism of skin lesion will be different between ES and GVHD, although common morphologic features are shared by the two conditions.

To evaluate the number of Langerhans cells, $CD1a^+$ cells were counted. Number of these cells were significantly lower in ES than in GVHD. Langerhans cells have been found to decrease in number with exposure to ionizing radiation (Breathnach and Katz, 1985) and in drug reaction (Dascalu et al., 1992). Thus, this phenomenon might reflect the change due to the preparative procedure of myoablation by total body irradiation and chemotherapy. In a mouse experiment on the effects of irradiation on epidermal Langerhans cells, 8 Gy whole-body irradiation induced a 57% decrease of Langerhans cells in epidermis and recovery was delayed for 3 weeks (Cole et al., 1984). Though extrapolating data from mouse to human is limited, our data seem to reflect a similar change in number of Langerhans cells in human tissue on the way to recovery in response to irradiation. The onset of ES is earlier than that of acute GVHD, in that mean onset of ES was 15.2 days and that of acute GVHD was 33.6 days after HSCT. Thus, morphologic features of ES might partly reflect earlier changes in skin after myoablation, with alterations caused by neutrophil recovery of transplanted stem cells. Cole et al. (1984) also reported that morphologic alterations, such as loss of dendritic features and bizarre appearance, were observed in association with change in number and distribution pattern. Further studies including morphologic and ultrastructural examination of Langerhans cells, might reveal more helpful findings.

Various skin lesions can be encountered by patients undergoing myoablation and following HSCT. In the case of skin biopsy showing interface dermatitis after HSCT in pediatric patients, evaluation of the predominance of $CD4^+$ or $CD8^+$ cells and the number of Langerhans cells in the epidermis might be helpful differential features. Clinical considerations, such as early onset after HSCT, low ANC, the presence of systemic leak phenomenon or neurologic symptoms, will also increase the accuracy of pathologic diagnosis.

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Accepted September 23, 2011