J Oral Pathol Med (2006) 35: 33–6

www.blackwellmunksgaard.com/jopm

Immunohistopathological study of the oral lichenoid lesions of chronic GVHD

M. Sato[1], N. Tokuda[2], T. Fukumoto[2], T. Mano[1], T. Sato[1], Y. Ueyama[1]

[1]*Department of Oral and Maxillofacial Surgery, Yamaguchi University, School of Medicine, Yamaguchi;* [2]*The First Department of Anatomy, Yamaguchi University, School of Medicine, Yamaguchi, Japan*

BACKGROUND: Chronic graft-vs.-host disease (cGVHD) is a common and serious complication after bone marrow transplantation (BMT). However, the detailed process of oral lichenoid lesions of cGVHD is still unknown. Therefore, we investigated the immunohistopathological features of cGVHD compared with oral lichen planus (OLP) and healthy controls.

METHODS: Nineteen allogenic BMT recipients with a histopathological diagnosis of cGVHD were investigated. We investigated the immunohistopathological features of cGVHD compared with OLP and healthy controls.

RESULTS: Immunohistopathological features showed that the infiltrations of CD4-positive T cells of cGVHD and OLP were significantly larger than those of the normal oral mucosa ($P < 0.005$). A larger number of CD8-positive T cells was infiltrated in cGVHD and OLP compared with the normal oral mucosa ($P < 0.001$). The difference in the number of CD4- and CD8-positive T cells between cGVHD and OLP was not significant. The infiltrations of Langerhans cells (CD1a) in cGVHD and OLP were significantly larger than those in the normal oral mucosa ($P < 0.005$). The difference in the number of Langerhans cells between cGVHD and OLP was not significant. CD68-positive macrophages were more frequently seen in cGVHD and OLP than in the normal oral mucosa ($P < 0.0001$). The difference in the number of CD68-positive macrophages between cGVHD and OLP was not significant.

CONCLUSIONS: It is suggested that Langerhans cells and CD8-positive T cell may play a major role in the pathogenesis of the oral lichenoid lesions of cGVHD, and the immune response was inducted in OLP as well as the oral lichenoid lesion of cGVHD in this study.

J Oral Pathol Med (2006) **35**: 33–6

Keywords: chronic graft-vs.-host disease; immunohistopathological; oral lichen planus; oral lichenoid lesion; oral manifestation

Correspondence: Miki Sato, 1-1-1 Minami Kogushi, Ube, Yamaguchi 755-8505, Japan. Tel: +81-836-22-2299. Fax: +81-836-22-2298. E-mail: m-sato@po.cc.yamaguchi-u.ac.jp
Accepted for publication August 15, 2005

Introduction

Recently, bone marrow transplantation (BMT) has been frequently performed in the treatment of severe aplastic anaemia and acute leukaemia. Graft-vs.-host disease (GVHD) is one of the most important complications of BMT. Specific target tissue includes skin, liver, gastrointestinal tract, and oral mucosa and salivary glands. Especially, oral manifestations of chronic GVHD (cGVHD) is the most serious long-term complications that influence quality of life (QOL) of patients.

Chronic GVHD involving the skin and mouth exhibits a clinical feature with lichenoid eruptions that is considered to reflect disturbances within the immune system (1).

In addition, it is reported that cytotoxic T cells play a major part in the pathogenesis of oral lichenoid lesions in patients with cGVHD (2). In addition to T lymphocytes reacting against host histocompatibility antigen, infectious agents might be predisposing factors for the development of GVHD (3). Furthermore, some studies of skin GVHD in mice models reported that host Langerhans cells that persist in the skin following BMT are responsible for the induction of GVHD (4, 5). However, the detailed mechanisms of oral lichenoid changes of cGVHD is still unknown.

The purpose of this study was to examine by immunohistopathology biopsies from the oral mucosa of patients with cGVHD and compared to those from patients with OLP, as well as healthy control groups with normal oral mucosa.

Patients and methods

Patients and biopsy specimens

Sixteen allogenic BMT recipients with a histopathological diagnosis of cGVHD were investigated. The biopsy was performed under local anaesthesia at Oral and Maxillofacial Surgery, Yamaguchi University Hospital of Medicine. Two lip biopsies were taken from lower lip. Other biopsy specimens were taken from oral lichenoid lesions (10 buccal mucosa, three gingiva and one tongue). All biopsy specimens were fixed in 10% formalin solution and paraffin-embedded.

Two control groups consisting of six patients with oral lichen planus (OLP) and six healthy patients (control group) with no clinical signs of oral mucosal lesions in the study. The biopsy specimens of OLP were taken from buccal mucosa, gingiva and tongue. Those of healthy patients were also taken from buccal mucosa, gingiva, tongue and lip. The sections examined were all taken from one area of the biopsy specimens.

Monoclonal antibodies

The cell infiltrates were studied using monoclonal antibodies against CD4 (helper T cells; Novo, NCL-CD4-1F6), CD8 (cytotoxic T cells; Novo, NCL-CD8-4B11), CD1 (Langerhans cells; Novo, NCL-CD1a-235) and CD68 (macrophages; Novo, NCL-CD68-KP1).

Histopathological observation

About 6 mm sections were cut, mounted and specimens were stained with haematoxylin and eosin (H & E) for histopathological interpretation.

Immunohistochemical observation

Cut and mounted specimens were deparaffinized and rehydrated to distilled water. The sections were incubated with each monoclonal antibody for 24 h at 4°C and were washed in phosphate-buffered saline (PBS). No procedure undertaken to block endogenous peroxidase activity. After washing, the sections were reacted with horseradish peroxidase (HRP)-conjugated anti-mouse IgG. They were incubated in 3-3′-diaminobenzidine tetrahydrochloride dehydrate (DAB; Aldrich, Milwaukee, WI, USA) for 10 min and then rinsed with distilled water. Later they were counterstained with haematoxylin, dehydrated and mounted.

Quantitation of stained cells and statistical analysis

The number of positively stained cells were observed under a light microscopy (Nikon Eclipse, Tokyo, Japan; objective ×400). The counting was carried out in three to four different fields of the basal cell epithelial and subepithelial layers and within the epithelium by three persons.

Statistical analyses of differences between-groups were by an unpaired *t*-test using the estimated mean value of positive cells for each patient (Table 1).

Table 1 Mononuclear cell infiltrations

	Number of cells (range)			
Patient group	CD4[a]	CD8[a]	Langerhans cells (CD1a)[b]	Macrophages (CD68)[a]
cGVHD	163 (50–275) *	215 (80–350) **	21 (14–30) *	18 (16–20) ***
OLP	135 (70–250) *	194 (80–300) **	19 (14–26) *	16 (15–18) ***
Normal oral mucosa	16 (11–20)	32 (20–40)	7 (5–10)	9 (7–11)

[a]Counted in the basal epithelial and subepithelial layers.
[b]Counted within the epithelium.
[c]GVHD, chronic graft-vs.-host disease; OLP, oral lichen planus.
*$P<0.005$; **$P<0.001$; ***$P<0.0001$.

Results

Helper-inducer (CD4) T cells and cytotoxic-suppressor (CD8) T cells

The infiltrations of CD4- and CD8-positive T cells were seen in the basal epithelial and subepithelial layers. The infiltrations of CD4-positive T cells of cGVHD and OLP were significantly larger than those of the normal oral mucosa ($P < 0.005$). A larger number of CD8-positive T cells was infiltrated in cGVHD and OLP compared with the normal oral mucosa ($P < 0.001$). The difference in the number of CD4- and CD8-positive T cells between cGVHD and OLP was not significant (Fig. 1).

Langerhans cells (CD1a)

The infiltrations of Langerhans cells (CD1a) in cGVHD and OLP were significantly larger than those in the normal oral mucosa ($P < 0.005$). The difference in the number of Langerhans cells between cGVHD and OLP was not significant (Fig. 2).

Macrophages (CD68)

CD68-positive macrophages were more frequently seen in cGVHD and OLP than in the normal oral mucosa ($P < 0.0001$). The difference in the number of CD68-positive macrophages between cGVHD and OLP was not significant (Fig. 3).

Discussion

Chronic GVHD is a common and serious complication after BMT in which donor-derived T cells infiltrate and attack host epithelial tissue such as skin and liver, intestine and oral mucosa (2, 6, 7).

Several studies of skin GVHD reported a predominantly CD4-positive T-cell infiltration (8, 9). On the contrary, some studies of oral cGVHD showed CD8-positive T-cell infiltration (10, 11). In cGVHD, focal infiltration of the basal layers of the epidermis by CD8-positive T cells, sometimes accompanied by keratinocyte apoptosis, indicates activity and progression of the disease. These focal epidermotropic lymphocytic infiltrates usually consist of CD8-positive T cells (12). In addition, Van Epps reported that transforming growth factor (TGF)-β-dependent CD103 expression by CD8-positive T cells promote selective destruction of the host intestinal epithelium during GVHD (7). Moreover, T-cell receptor (TCR) ligation triggers the secretion of chemokines that draw other damaging cells, such as tumour necrosis factor (TNF)-producing macrophages. However, the detailed process of oral lichenoid lesions of cGVHD is still unknown.

In the present study, the infiltration of CD4- and CD8-positive T cells was seen under the basal epithelial and subepithelial layers. A larger number of CD4- and CD8-positive T cells in the specimen from cGVHD and OLP groups was found than those from healthy control groups. However, Mattsson et al. reported that a similar ratio of CD4:CD8 cells was

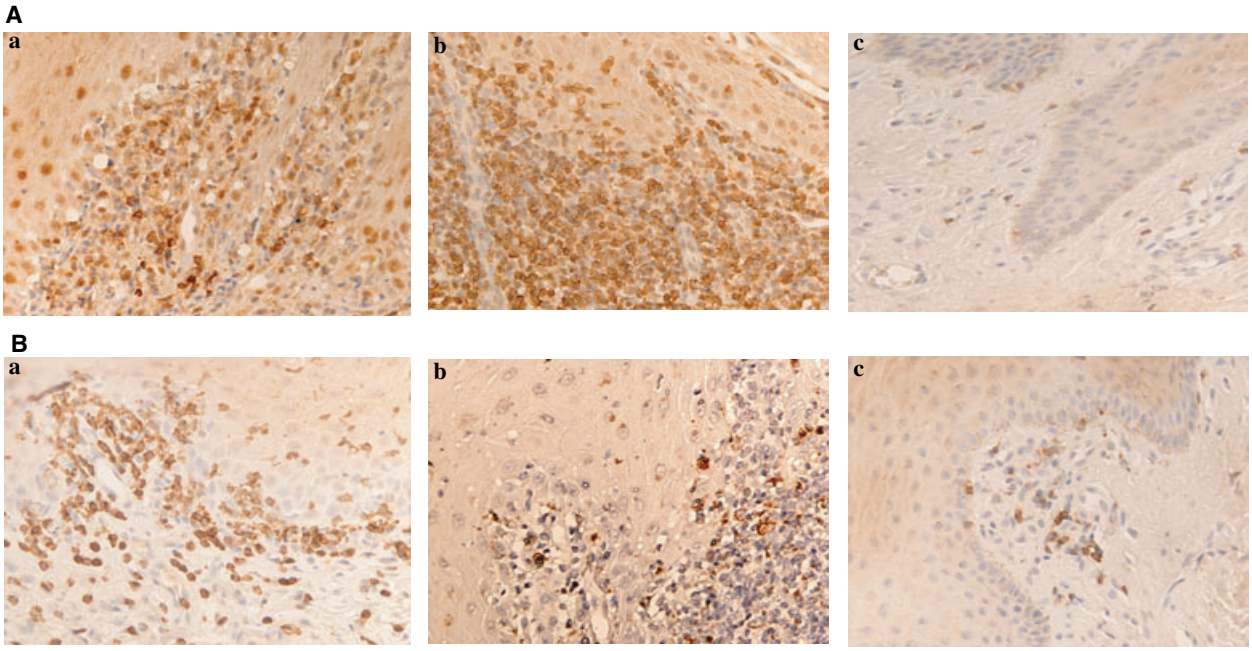

Figure 1 Infiltrations of (A) CD4- and (B) CD8-positive T cells: the infiltrations of CD4- and CD8-positive T cells in oral biopsy specimens from patients with cGVHD, chronic graft-vs.-host disease (a); OLP, oral lichen planus (b) and normal oral mucosa (c). CD4- and CD8-positive T cells were found in the basal epithelial and subepithelial layers (×400).

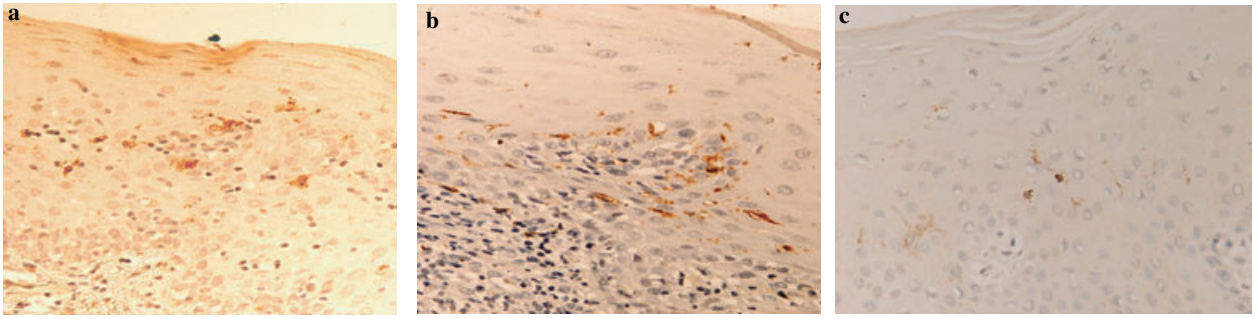

Figure 2 Langerhans cells (CD1a): the infiltrations of Langerhans cells (CD1a) in oral biopsies from patients with chronic graft-vs.-host disease (cGVHD; a), oral lichen planus (OLP; b) and normal oral mucosa (c). Langerhans cells (CD1a) were seen within the epithelium (×400).

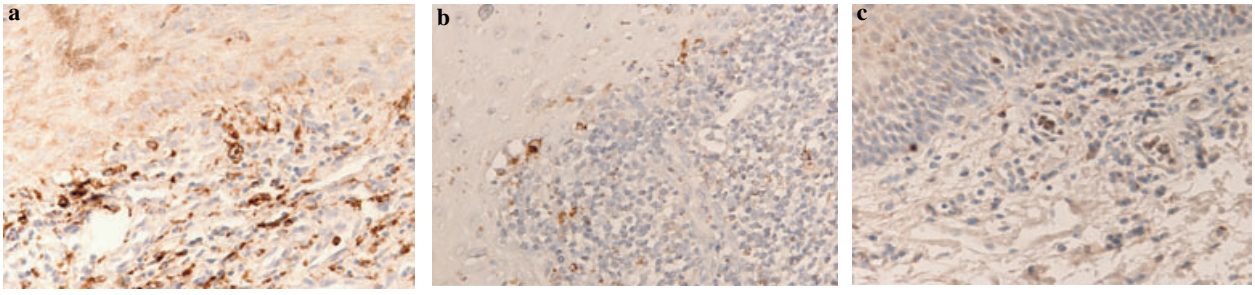

Figure 3 Macrophages (CD68): the infiltrations of CD68-positive macrophages in oral biopsies from patients with chronic graft-vs.-host disease (cGVHD; a), oral lichen planus (OLP; b) and normal oral mucosa (c). CD68-positive macrophages were mainly seen in the subepithelial layer (×400).

found in the specimens as in the healthy control group (11). On the contrary, Fujii et al. also found an increased number of CD8 cells in the oral mucosa of patients with cGVHD (2). Our results are agree with those of Fujii et al. (2).

In addition, the difference in the number of CD4- and CD8-positive T cells between cGVHD and OLP was not significant in this study. De Panfilis and Rowden reported that CD8-positive T cells was increasing according to the several stages (light, moderate, severe) of OLP (13).

In addition, CD68-positive macrophages were more frequently seen in cGVHD and OLP than in the normal oral mucosa. However, the number of CD68-positive macrophages between cGVHD and OLP was not significant in this study.

Later Duffer et al. reported that host-derived dendritic cells are critical in priming donor CD4- and CD8-positive T cells to cause GVHD, and selective targeting of host dendritic cells may be a promising strategy to prevent GVHD (14). In addition, Zhang et al. indicated that host dendritic cells prime donor T cells before their disappearance and play a critical role triggering donor CD8-positive T-cell mediated GVHD, although host dendritic cells disappear rapidly after allogenic BMT (15). Some studies of skin GVHD in mice models reported that host Langerhans cells that persist in the skin following BMT are responsible for the induction of GVHD (4, 5). In this study, the number of Langerhans cells in the specimens from cGVHD and OLP was significantly larger than those found in specimen from the normal oral mucosa. The number of Langerhans cells infiltrates was found in oral lichenoid lesions of cGVHD as well as in OLP. These results suggested that Langerhans cells are also responsible for the induction of oral lichenoid lesions of cGVHD as well as previous reports (4, 5, 11, 14–16).

In conclusion, it is suggested that Langerhans cells and CD8-positive T cell may play a major role in the pathogenesis of the oral lichenoid lesions of cGVHD. Furthermore, it is suspected that the immune response was inducted in OLP as well as the oral lichenoid lesion of cGVHD in this study.

References

1. Sulliva KM, Shulman HM, Atkinson K, Thomas ED. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. *Blood* 1981; **57**: 267–76.
2. Fujii H, Ohashi M, Nagura H. Immunohistochemical analysis of oral lichen planus-like eruption in graft-versus-host disease after allogenic bone marrow transplantation. *Am J Clin Pathol* 1988; **89**: 177–86.
3. Saurat JH, Gluckman E. Lichen planus-like eruption following bone marrow transplantation: a manifestation of the graft-versus-host disease. *Clin Exp Dermatol* 1977; **2**: 335–44.
4. Emerson SG. Tanning before transplant: lancing the Langerhans cell. *Nat Med* 2004; **5**: 451–2.
5. Merad M, Hoffmann P, Ranheim E, et al. Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. *Nat Med* 2004; **5**: 510–7.
6. Silverman S Jr, Eversole LR, Truelove EL. *Essential of oral medicine: immunopathologic mucosal lesions*. Hamilton: BC Decker Inc., 2001; 82–3.
7. Van Epps HL. Gut-grabbing T-cells trigger GVHD. *Exp Hematol* 2005; **16**: 1521.
8. Lampert IA, Suitters AJ, Janossy G, Thomas JA, Palmer S, Gordon-Smith E. Lymphoid infiltrates in skin in graft-versus-host disease (Letter). *Lancet* 1981; **2**: 1352.
9. Lever R, Turbitt M, Mackie R, et al. Prospective study of the histological changes in the skin in patients receiving bone marrow transplants. *Br J Dermatol* 1986; **114**: 161–70.
10. Kasten-Sportes C, Masset M, Varrin F, Devergie A, Gluckman E. Phenotype and function of T-lymphocytes infiltrating the skin during graft-versus-host disease following allogenic bone marrow transplantation. *Transplantation* 1989; **4**: 621–4.
11. Mattsson T, Sundqvist KG, Heimdahl A, Dahllof G, Ljungman P. A comparative immunological analysis of the oral mucosa in chronic graft-versus-host disease and oral lichen planus. *Arch Oral Biol* 1992; **37**: 539–47.
12. Heymer B. *Clinical and diagnostic pathology of graft-versus-host disease*. Berlin, Germany: Springer-Verlag, 2002; 103–21.
13. De Panfilis G, Rowden G. Quantitative studies on nonlymphoid mononuclear cell subpopulations in cutaneous infiltrates: I. Stage-related changes of dendritic nonlymphoid mononuclear cells, Langerhans cells, and macrophages in lichen planus lesions. *Am J Dermatopathol* 1986; **8**: 44–52.
14. Duffer UA, Maeda Y, Cooke KR, et al. Host dendritic cells alone are sufficient to initiate acute graft-versus-host disease. *J Immunol* 2004; **172**: 7393–8.
15. Zhang Y, Louboutin J-P, Zhu J, Rivera AJ, Emerson SG. Preterminal host dendritic cells in irradiated mice prime CD8+ T cell-mediated acute graft-versus-host disease. *J Clin Invest* 2002; **109**: 1335–44.
16. Atkinson K, Munro V, Vasak E, Biggs J. Mononuclear cell populations in the skin defined by monoclonal antibodies after HLA-identical sibling marrow transplantation. *Br J Dermatol* 1986; **114**: 145–60.