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Graft-versus-Host Disease
—Clinical and Pathological analysis of 11 biopsy proven cases—

Kyoung-Mee Kim, Kyoung-Ja Han, Chang-Suck Kang, Sang-In Shim

Catholic University Medical College, Department of Clinical Pathology

Graft-versus-host disease(GVHD) is a life threatening complication that may occur following allogenic bone marrow transplantation(BMT) in the patients with aplastic anemia, leukemia or genetic immunodeficiency. It has been known that GVHD occurs approximately 70% of recipients of BMT in western countries but no definite incidence has been reported in Korea. In our St. Mary's Hospital, GVHD occurs in about 30% of BMT recipients. Histopathologically the acute phase skin shows diffuse lymphocytic infiltrates in the upper dermis with extensive exocytosis. Scattered throughout the epidermis are many degenerated keratinocytes, which are often associated with one or more satellite lymphocytes(satellite cell necrosis). In the chronic phase, acanthosis, eosinophilic keratinocytes resembling colloid bodies and mononuclear cell infiltrates in the upper dermis are noted.

We reviewed 5 cases of acute GVHD and 6 cases of chronic GVHD. All patients received allogenic BMT from Jan. 1, 1992 to July 1, 1993. Ten patients were male and one was female. The mean age was 34(20-70). The pathologic diagnosis was 3 cases of CML, 2 of ALL, 2 of AML(FAB M2), 2 of aplastic anemia, 1 of CLL and 1 of AML(FAB M5). The interval from BMT to GVHD varied from 14 days to 4 years(median 220 days). The skin and GI tract were involved in all eleven cases. Ten cases were histologically proven by skin biopsies, and two cases by salivary gland and colonic biopsies, respectively. The histological findings of the skin, salivary gland and colonic biopsieds were described. Immunohistochemical stain of the skin was done using CD4, CD8, HLA DR and Leu 7 antibodies. The HLA-DR were expressed in the epidermal keratinocytes and CD8 positive lymphocytes were more increased in the epidermis and dermis than CD4 positive lymphocytes in all cases.

Key Words : *GVHD, Biopsies, Clinicopathologic, Immunohistochemical.*

INTRODUCTION

Graft-versus-host disease(GVHD) is the sum expression in a patient of Graft-versus-host reactions(GVHRs) that occur in specific organs. The basic requirements for identifying GVHRs are these : (1) the graft must contain immunocompetent cells, (2) the host must be sufficiently different genetically from the graft to be perceived as antigenically foreign, and (3) the host must be unable to reject the graft effectively(Billignam, 1966). GVHD is most

Address for correspondence : *Chang-Suck Kang, Department of Clinical Pathology, St. Mary's Hospital, #62 Youido-dong, Yongdungpo-gu, Seoul, 150-010, Korea. Tel : (02)7-89-1312.*

commonly recognized as a complication of allogenic bone marrow transplantation(BMT). Less commonly, GVHD occurs following maternofetal cell transfer in immunodeficient children(Morhenn and Maibach, 1974 ; Grogan et al., 1977) or as a result of the transfusion of nonirradiated blood and blood products(immunocompetent cells) into immunodeficient children(Rosen et al., 1978 ; Dismore et al., 1980) or adults(Ford et al., 1976 ; Weiden et al., 1981) In Korea, allogenic BMT has been performed since 1983 and the number of patients who have received BMT at St. Mary's Hospital is about 160 over 10 years. Only a few previous reports of clinical and histopathologic findings of GVHD are reported in the Korean literature(Yi et al., 1989). In these, histopathologic findings of salivary gland and gastrointestinal tract are not described and little attention has been paid to the dynamics of the histopathologic changes after BMT including their relation to symptoms and signs of GVHD. Immunohistochemical studies were performed to identify the proportion of CD4 and CD8 positive cells in the dermis and to observe the distribution of HLA DR and Leu 7 positive cells in the epidermis and dermis.

So we reviewed 5 cases of acute GVHD and 6 cases of chronic GVHD that developed as sequelae of allogenic BMT and were proven by histopathologic examination.

MATERIALS AND METHODS

The files of the clinical pathology department of St. Mary's Hospital were searched for all cases indexed as GVHD. 5 cases of acute GVHD and 6 cases of chronic GVHD were identified from Jan. 1, 1992 to Dec. 31, 1993. Fresh, 4 um thick sections were cut from original frozen blocks for immunohistochemical staining. All histologic specimens were reviewed ; these had been roughly fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin(H&E). The age and sex of the patients, the diagnoses of the patients, the clinical GVHD occurred organ, the interval between BMT and GVHD, the histopathologic findings of biopsied material were recorded. The salivary gland biopsy material was graded according to the scale suggested by Chisholm and Mason(Chisholm, 1968). In summary, in the 4mm salivary gland tissue sections that were examined, grade 0 denotes absence of lymphocytes, grade 1 denotes slight lymphocytic infiltration, grade 2 denotes moderate infiltrate or less than one focus(where a focus designates an aggregate of 50 or more lymphocytes), grade 3 denotes one focus per 4mm and grade 4 more than one focus. In case 1, 2 and 8, immunohistochemical studies were performed by avidin-biotin-peroxidase method as previously described(Hsu et al., 1981), using CD4, CD8, HLA DR and Leu 7 antibodies Controls included pre-transplant skin biopsies and skin from normal healthy volunteers.

RESULTS

Clinical Features

The clinical features are detailed in Table 1. Ten patients were male and one was female. The mean age was 34(20-70). The pathologic diagnosis was 3 cases of CML, 2 of ALL, 2 of AML(FAB M2), 2 of aplastic anemia, 1 of CLL and 1 of AML(FAB M5). The interval between BMT and GVHD varied from 14 days to 4 years(mean 220days). The skin and gastrointestinal tract were involved in all eleven cases and in case 9 and 11, sicca syndrome was also developed. The cutaneous manifestations were slightly painful or pruritic, multiple discrete erythematous maculopapules or scaly patches on the whole

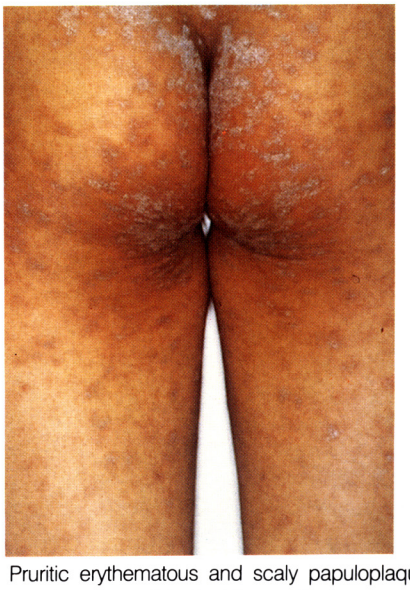

Fig. 1. Pruritic erythematous and scaly papuloplaques in the lower extremity and buttock(case 7).

Table 1. The clinical characteristics of 11 pathologically proven GVHD patients.

CASE	1	2	3	4	5	6	7	8	9	10	11
Sex	M	M	M	M	M	M	F	M	M	M	M
Age	37	25	22	20	22	41	70	32	38	32	30
Dx	CML	AML5	AML2	ALL	AA	ALL	CML	AA	CML	CLL	AML2
Post BMT	14d	17d	27d	37d	86d	137d	144d	204d	207d	270d	4y
Clinical GVHD	skin GI sicca	skin GI	skin GI sicca	skin GI	skin GI	skin GI	skin GI	skin GI	skin GI sicca	skin GI	skin GI sicca
Biopsy site	skin sal.gl.	skin	sal.gl.	skin	skin	skin gut	skin	gut	skin	skin	skin

AML ; acute myelogenous leukemia, CML ; chronic myelogenous leukemia, AA ; aplastic anemia, ALL ; acute lymphoblastic leukemia, d ; days, GI ; gastrointestinal, sal. gl. ; salivary gland, sicca ; sicca syndrome.

body(Fig. 1). The gastrointestinal manifestation of gut GVHD was mainly diarrhea in all cases. Ten cases of GVHD were histologically diagnosed by skin biopsies and two cases were diagnosed by minor salivary gland and colonic biopsies, respectively.

Microscopic and Immunohistochemical Features

The microscopic findings of the skin revealed typical findings of acute and/or chronic GVHD. In acute phase GVHD, the dermis shows diffuse lymphocytic infiltrate in the upper dermis with extensive exocytosis. Some degenerated keratinocytes with pyknotic nuclei and eosinophilic, hyalinized cytoplasm were often associated with one or more satellite lymphocytes, an association referred to as satellite cell necrosis. Bullae were not found in any of the cases. In the chronic cases, there were acanthosis and eosinophilic keratinocytes resembling colloid or Civatte bodies in the epidermis and a mononuclear cell infiltrate in the dermis immediately below the epidermis(Fig. 2). In the epidermis of acute GVHD there is a decrease in the number of HLA DR positive dendritic cells and the basal keratinocytes are HLA DR positive. The lymphocytic infiltrate is composed of CD4 and CD8 positive cells. The CD8 positive cells predominate in the epidermis, dermoepidermal junction and upper dermis, while CD4 positive cells are present in greatest numbers around blood vessels in the mid to lower dermis (Fig. 3). Only a few Leu 7 positive cells were identified. The salivary gland biopsy material in case 11

Fig. 2. Skin biopsy material(case 11) showing individual keratinocytes necrosis, lymphoid cell exocytosis and perivascular mononuclear cell infiltration(H&E, ×200).

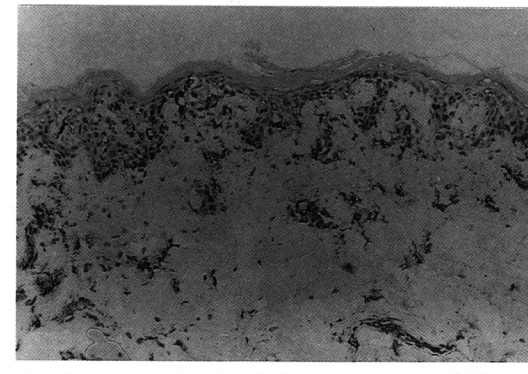

Fig. 3. Immunohistochemical staining using CD8 antibodies in case 2. CD8(+) cells are more prevalent than CD4(+) cells in the epidermis and dermis(×200).

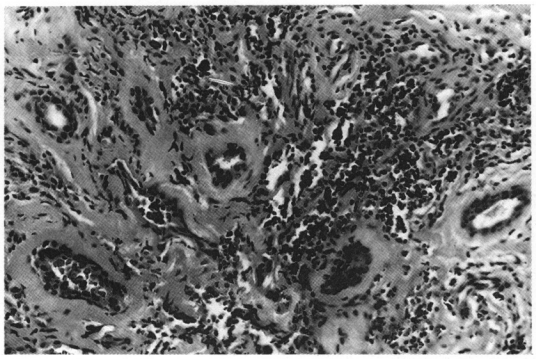

Fig. 4. The salivary gland biopsy material(case 9) showing marked periductal lymphocytic infiltration with fibrosis. But individual necrosis(apoptosis) of the epithelium was not present(H&E, ×200).

showed slight periductal lymphocytic infiltration (Grade 1). In case 9 the biopsy material revealed marked periductal lymphocytic infiltration(Grade 4)(Fig. 4) with fibrosis indistinguishable from what is described in Sjogren's syndrome patients. The correlation between marked histopathological changes and symptoms or signs of sicca syndrome was not present. Individual cell necrosis(apoptosis) of the epithelium was not found in neither case. The colonic biopsy material(case 6 & 8) showed individual crypt epithelial cell degeneration with crypt abscess formation and interstital mononuclear cell infiltration (Fig. 5, 6).

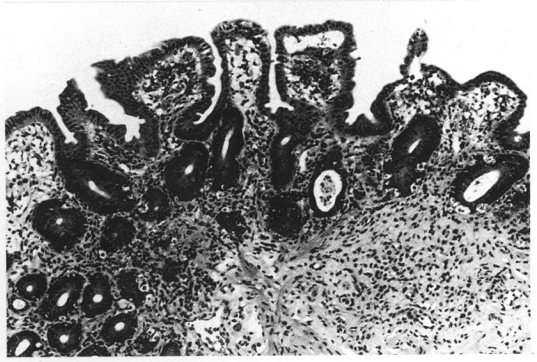

Fig. 5. Colonic biopsy material(case 4) showing individual crypt epithelial cell necrosis and interstitial mononuclear cell infiltration(H&E, ×200).

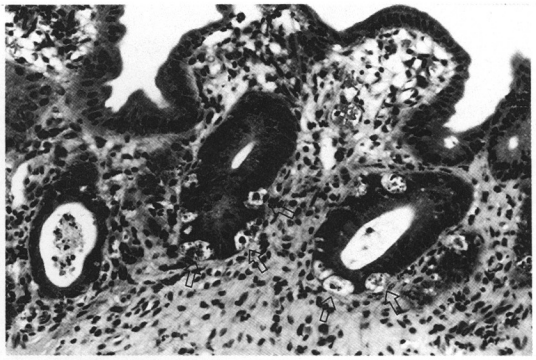

Fig. 6. Colonic biopsy material(case 5) showing crypt abscess formation(H&E, ×200).

DISCUSSION

GVHD is a major cause of morbidity among patients surviving allogenic bone marrow transplantation. Within 100 days of BMT, up to 50% of the patients develop skin rash, diarrhea and hepatic abnormalities denoted as acute GVHD, whereas the symptoms and signs developing later than 100 days after BMT, named chronic GVHD, are seen in approximately 45% of patients in the western world(Lindahl et al., 1988). But no definite incidence has been reported in Korea. In our St. Mary's Hospital, BMT has been performed since 1983. The number of patients who have received BMT are about 160 and GVHD which occurs in about 30% of BMT recipients.

Human GVHD is composed of two distinct clinical entities. Acute GVHD and chronic GVHD, which have different histogeneses. Acute GVHD is produced by the attack of donor immunocompetent T or null lymphocytes against recipient histocompatibility antigens. The null lymphocytes may attack antigens shared by the donor and recipient and are autocytotoxic lymphocytes which can produce acute GVHD occurs when suppressor lymphocytes appear in the recipient's peripheral circulation. Chronic GVHD is produced by immunocompetent lymphocytes that differentiate in the recipient(Parkman and Rappaport, 1980).

Histopathologically, the skin biopsies in the acute phase show diffuse lymphocytic infiltrate in the upper dermis with extensive exocytosis. Scattered throughout the epidermis are many degenerated keratinocytes, which are often associated with one

or more satellite lymphocytes. In the chronic phase, acanthosis, eosinophilic keratinocytes resembling colloid bodies and mononuclear cell infiltrates in the upper dermis are noted. The late phase of chronic GVHD is characterized by an atrophic epidermis without other cellular changes, dense fibrosis of the dermis, thin walled, dilated blood vessels, dermal macrophages and absence of inflammation(Shulman et al., 1978). In our cases, the skin biopsy findings were compatible with previously described acute and chronic GVHD findings including individual cell necrosis, lymphoid cell exocytosis and perivascular lymphocytic infiltrate. The changing pattern of cell population at different levels in the skin were studied by the ABC method using the following monoclonal antibodies : CD3, 4, 6, 8, anti HLA DR and Leu 7(Harper et al., 1984). In our cases, the immunohistochemical staining results were correlated with previously performed studies(Harper et al., 1984 ; Yi et al., 1989) except HLA DR. In our cases, the HLA DR positive dendritic cells were decreased in number and basal keratinocytes were HLA DR positive. After BMT there is early regeneration of circulating CD8 cells with a reversal of the normal CD4/CD8 ratio. Proposed targets in cutaneous GVHD include Langerhans cells and keratinocytes expressing either minor histocompatibility antigens or induced class II antigens. Langerhans cells are depleted from human very shortly after transplantation and are now thought to be sensitive to the preparatory regimens. Thus these cells are not available during GVHD(Beschorner et al., 1987). In our cases, the HLA DR positive Langerhans cells were absent in the epidermis and HLA DR positive keratinocytes were observed in all cases.

Sale et al.(1979) and Epstein et al.,(1980) have utilized rectal biopsy in the evaluation of GVHD in humans. The rectal biopsied specimens show crypt epithelial degenerative changes and occasional crypt abscesses. The changes proved to be a good indication of GVHD when the biopsy was performed after resolution of radiation or chemotherapeutic effects(Whitehead, 1985). In Korea the gastrointestinal findings of GVHD have not been reported. In our cases, the colonic biopsies revealed individual epithelial cell degeneration with crypt abscess formation and interstital mononuclear cell infiltration in both cases. These findings are compatible with acute GVHD involving the gastrointestinal tract. The histologic and immunohistochemical findings of salivary glands in GVHD have been reported in the western literature(Lindahl et al., 1988). In our cases, the salivary gland biopsy material in case 3 showed diffuse lymphocytic infiltration in the periductal area associated with fibrosis although in case 1, very mild lymphocytic infiltration only was found. These findings are indistinguishable in histologic background from Sjogren's syndrome without clinical information. So the histologic diagnosis of GVHD is mainly based on clinical information and the effect of chemotherapy and radiation therapy should be ruled out completely.

In our experience, it is possible that histologic diagnosis of GVHD can be done in the biopsied specimen including skin, salivary gland, GI tract and liver. So after diagnostic biopsy in the early stage of GVHD, appropriate and effective combination therapy would be possible and the patient's chances of survival would be improved.

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