J Clin Pathol 1988;**41**:148–154

Histological features of skin and rectal biopsy specimens after autologous and allogeneic bone marrow transplantation

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SUMMARY The histological appearances of skin and rectal biopsy specimens were studied in 31 bone marrow transplant recipients (13 autologous, 18 allogeneic) before transplant, at 28 days, at six months, and as soon as graft versus host disease (GVHD) was clinically suspected. Grades I and II skin changes were commonly seen in patients before transplant and in the autologous group after transplant, as well as in most of the allogeneic recipients with suspected GVHD. Epidermal lymphocytic infiltration was seen only in allogeneic recipients, with clinical GVHD following transplant, but this was not a consistent finding and no other histological features were seen which would distinguish early GVHD from changes caused by cytotoxic agents. Rectal biopsy specimens, however, were normal in patients before transplant and in autologous recipients at 28 days; single cell necrosis of crypt cells was seen only in six of 13 allogeneic recipients studied after transplant with clinical skin GVHD but no gastrointestinal symptoms. Skin changes greater than I and II are required for the histological diagnosis of GVHD. Rectal changes are more specific and may be present despite a lack of intestinal symptoms.

Bone marrow transplantation following supralethal doses of irradiation or chemotherapy is being used with increasing success to treat patients with a variety of malignant and benign disorders.[1,2] One of the major obstacles to the success of bone marrow transplantation is graft versus host disease (GVHD) which occurs in between 20%–80% of patients.[3] GVHD is characterised by skin rash, hepatocellular dysfunction, and diarrhoea. Advanced florid GVHD is easily recognised but carries a poor prognosis,[4] and it was hoped that early diagnosis followed by prompt treatment would improve the outcome, but it is difficult to recognise early GVHD because its signs and symptoms are similar to those of many drug reactions and viral infections.[5]

Histological changes in skin biopsy specimens are often used to confirm a clinical diagnosis of GVHD. Although an abnormal rectal biopsy specimen may support the diagnosis of acute GVHD,[6] its value in detecting early GVHD where there is no clinical intestinal disease is uncertain, and in many centres routine rectal biopsies are not performed.

To establish more clearly the histological criteria required to detect early GVHD we carried out a prospective study of the histological features of simultaneous skin and rectal biopsy specimens from both autologous and allogeneic recipients before and after bone marrow transplantation.

Patients and methods

Between February 1985 and June 1986 34 bone marrow transplants were carried out in Newcastle upon Tyne. Thirty one patients agreed to participate in the biopsy study. Thirteen of the patients received autologous grafts and 18 allogeneic marrow from HLA identical, mixed lymphocyte culture (MLC) non-reactive siblings. The age range of the whole group was 3 to 59 years, and underlying diseases were acute lymphoblastic leukaemia (n = 13), acute myeloid leukaemia (n = 5), chronic myeloid leuk-

Accepted for publication 23 July 1987

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aemia (n = 5), non-Hodgkin's lymphoma (n = 5), neuroblastoma (n = 2) and myelodysplastic syndrome (n = 1).

The preparative regimen in the autologous group was intravenous melphalan with fractionated total body irradiation (three doses of 350 rads) in 11 patients and melphalan alone in two. All the allogeneic recipients were prepared with fractionated total body irradiation (six doses of 200 rads), and 16 of the 18 also received intravenous cyclophosphamide. Two patients received vincristine and melphalan instead of cyclophosphamide. For three days before and up to 100 days after transplant, allogeneic recipients received cyclosporin A (12·5 mg/kg/day) as prophylaxis against GVHD.

All patients were cared for in a protected environment using reversed barrier nursing techniques. Blood products were irradiated and cytomegalovirus (CMV) negative patients received products from CMV negative donors.

BIOPSY SPECIMENS

Skin biopsies were performed with a 4 mm punch biopsy needle. Rectal biopsy specimens were obtained with standard grasp biopsy forceps via a sigmoidoscope. All patients with a neutrophil count of less than $1{\cdot}0 \times 10^9/l$ received intravenous broad spectrum antibiotics for 24 hours after biopsy. There were no complications following these procedures.

Skin and rectal biopsies were performed on 30 of 31 patients before the start of the preparative regimen. All patients were clinically well. After transplant biopsies were performed at four weeks and six months in clinically well patients. In addition, patients were biopsied before specific treatment if skin lesions or diarrhoea thought to be due to GVHD occurred.

In the autologous group nine of the 13 were biopsied at 28 days. One patient was neutropenic and three were not available for study. Four were biopsied at six months. No patients had symptoms suggestive of GVHD. In the allogeneic group five of 18 patients died in the early period following transplantation of causes other than GVHD. The remaining 13 were biopsied between 16 and 34 days (mean 28 days) after transplant. Nine had suspected GVHD that was manifest by skin rash, but only one had diarrhoea. Eight were biopsied at six months. Two of these had chronic GVHD. The remainder were well.

In patients with suspected GVHD bacteriological and virological investigations were performed, including culture of blood, urine, and sputum, immunofluorescence examination of nasopharyngeal secretions for viruses, and viral cultures of nasopharyngeal secretions, blood, and urine. At the time of biopsy there was no evidence of viral or bacterial infection in any patient. Drug sensitivities were also excluded.

Each biopsy specimen was placed in 10% neutral buffered formalin for paraffin embedding and conventional histological examination after haematoxylin and eosin staining and serial sectioning. All the slides were then examined blind by one pathologist (LS) and 40 randomly chosen slides were also examined blind by a second pathologist (AJM). There was no significant interobserver variation; 98% of the histological features in the skin and 96% of those in the rectum were recorded by both pathologists. Specimens were examined for the presence of the histological features noted in table I[6-8] and in addition, the skin biopsy specimens were graded according to Lerner *et al*[8] (table 2).

The study was passed by the ethical committee of the Area Health Authority and patients or parents of participating children gave their informed consent.

Results

SKIN

Results of examination skin biopsy specimens are shown in table 3.

Before transplant

Only 12 of 30 specimens were considered to be normal. Vacuolar degeneration of basal cells was the most common abnormality in 18 of 30 specimens. Spon-

Table 1 *Histological criteria used in study*

Skin	*Rectum*
Epidermis:	**Epithelium:**
Hyperkeratosis	Single cell necrosis
Acanthosis	Crypt cell degeneration
Spongiosis	Dilated crypts
Pyknosis	Loss of crypts
Presence of binucleate or multinucleate cells	Crypt abscesses
Liquefaction degeneration	Mononuclear cell infiltrate
Mononuclear cell infiltrate	**Lamina propria:**
Basal cell vacuolation	Plasma cells
	Lymphocytes
Dermis	Eosinophils
Oedema	Polymorphs
Sclerosis	Muciphages
Mononuclear cell infiltrate	Ceroid
Pigment deposition	Giant cells
Colloid bodies	Granuloma
Vascular pattern	Oedema
	Vascular pattern

Table 2 *Grading of skin changes seen in GVHD*

Grade I	Vacuolar degeneration of epidermal basal cells and acanthocytes
Grade II	Vacuolar change with spongiosis and dyskeratosis or "eosinophilic bodies"
Grade III	Epidermolysis and bulla formation plus grade II changes
Grade IV	Total denudation of epithelium

Table 3 *Summary of major skin findings*

	Total No of biopsies	*Basal cell vacuolation*	*Spongiosis*	*Eosinophilic bodies*	*Liquefaction degeneration*	*Lymphocyte infiltrate*	
						epidermis	*dermis*
Before transplant:							
Autologous and allogeneic group	30	18	11	4			26
First biopsy after transplant:							
Autologous group	9	7	3	3			7
Allogeneic group	13	13	10	10	2	4	11
Six months after transplant							
Autologous group	4	2					
Allogeneic group	8	7	6	2			6

Table 4 *Skin appearances graded according to Lerner et al*

	Total No of patients	*Normal histology*	*Grade I GVHD*	*Grade II GVHD*	*Grade III GVHD*	*Grade IV GVHD*
Before transplant:						
Autologous and allogeneic group	30	12	14	4		
First biopsy after transplant:						
Autologous group	9	2	4	3		
Allogeneic group	13	0	3	8	2	
Six months after transplant:						
Autologous group	4	2	2			
Allogeneic group	8	1	5	1		

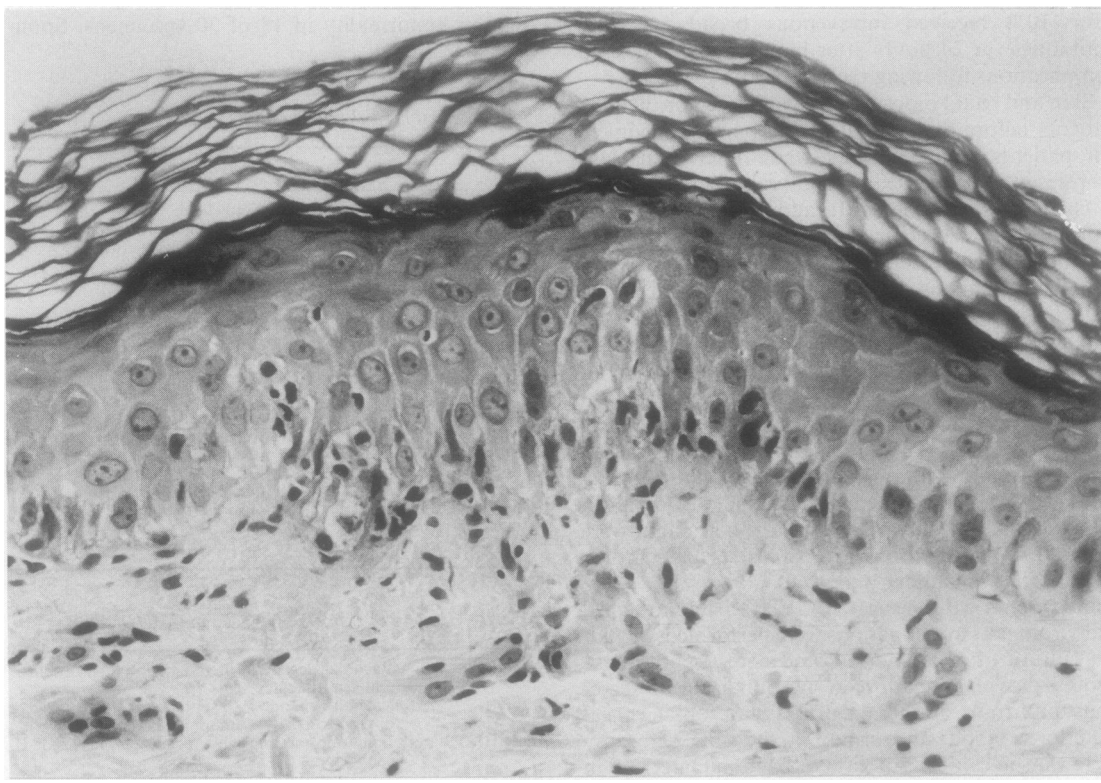

Fig 1 *Grade II GVHD showing vacuolar degeneration of basal cells, spongiosis, dyskeratotic bodies and lymphocytic infiltrate. (Haematoxylin and eosin.)*

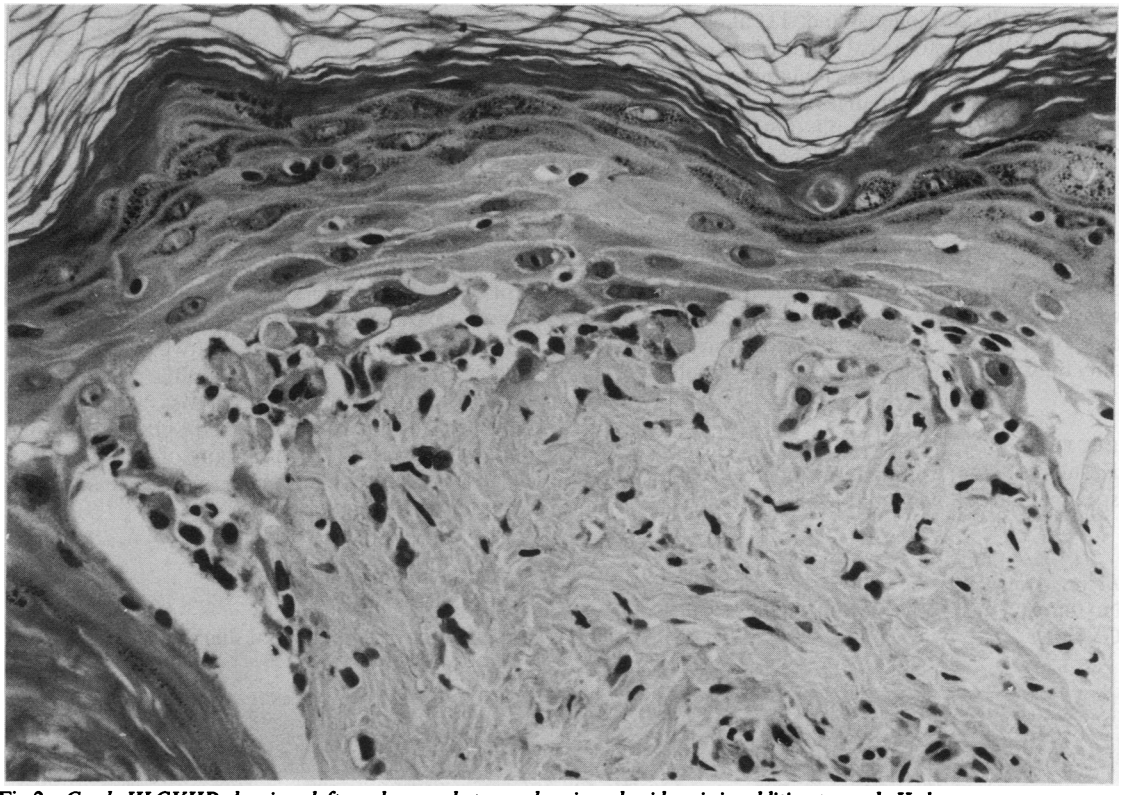

Fig 2 *Grade III GVHD showing clefts and spaces between dermis and epidermis in addition to grade II changes. (Haemotoxylin and eosin.)*

Table 5 *Summary of major rectal findings*

	Total No of biopsies	*Single cell necrosis*	*Degenerate crypts*	*Loss of crypts*	*Crypt abscesses*
Before transplant:					
Autologous and allogeneic group	30				
First biopsy after transplant:					
Autologous group	9				
Allogeneic group	13	6	4	3	2
Six months after transplant:					
Autologous group	4				
Allogeneic group	8	2	2		

giosis was also noticed in many and eosinophilic bodies were found in four patients. A mild perivascular lymphocytic infiltrate of the dermis occurred in most biopsy specimens. Eighteen showed histological features consistent with grade I or II GVHD (table 4).

After transplant

Vacuolar degeneration was again the most common abnormality present in seven of 10 autologous and in 13 allogeneic recipients, about four weeks after transplantation. Spongiosis and eosinophilic bodies were detected in both groups. In only two allogeneic recipients were liquefaction degeneration and focal epidermodermal separation found. A lymphocytic infiltrate of epidermis was seen only in four allogeneic recipients; this was mild and showed no correlation with severity of epidermal damage but was associated with an increased lymphocytic infiltrate of the upper dermis. A mild perivascular infiltrate, however, was present in both autologous and allogeneic patients.

Seven of 10 autologous and 11 of 13 allogeneic recipients had histological features of grade I or II GVHD (table 4) (fig 1). The remaining two allogeneic patients had grade III changes (fig 2). Six months after transplant most patients still showed grade I or II GVHD changes. Only one patient had the characteristic histological features of early chronic GVHD.[9]

An increased number of binucleate cells in the epidermis were seen in both groups after transplant, and melanin incontinence in the dermis was present in a similar number before and after transplant. There was no clinically important hyperkeratosis, acanthosis, or sclerosis and a few colloid bodies were seen only in two allogeneic recipients at six months.

RECTUM

Results of the examination of the rectal biopsy specimens are shown in table 5.

Before transplant

No biopsy specimen showed changes suggestive of GVHD.

After transplant

Apart from a reduced number of lymphocytes in the lamina propria, all rectal biopsy specimens from the autologous group remained unchanged after transplant. In the allogeneic group abnormalities were seen four weeks after transplant, with single cell necrosis of epithelial cells being the most common feature. Degeneration of, loss of, and abscesses in crypts were seen only in patients with single cell necrosis (fig 3). The affected cells were generally located at the base of crypts. Six months after transplant two recipients still showed occasional single cell necrosis.

It was difficult to assess the relative proportions of plasma cells, lymphocytes, eosinophils and polymorphs without the use of morphometrics, which was not attempted in this study. Ceroid, giant cells, and granulomas were not found in any of the specimens. The vascular pattern showed no difference before or after transplant.

CLINICAL FEATURES

Before transplant

No patient had a skin rash or diarrhoea.

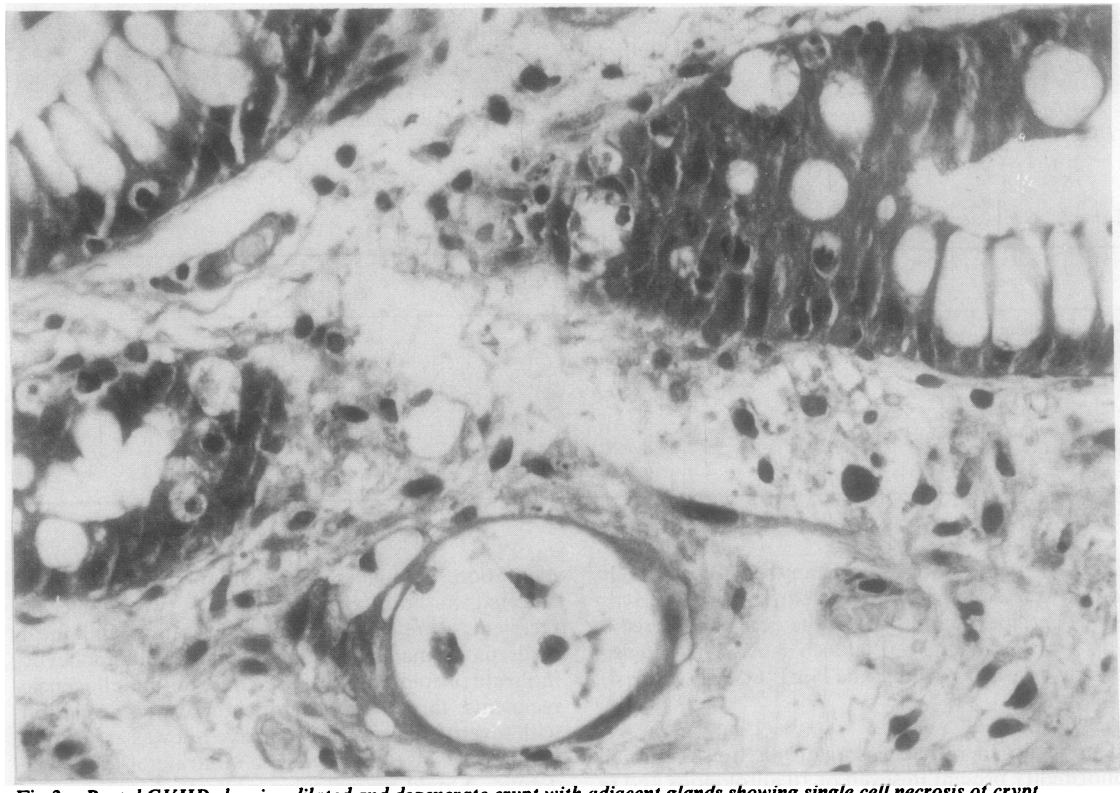

Fig 3 *Rectal GVHD showing dilated and degenerate crypt with adjacent glands showing single cell necrosis of crypt epithelium. (Haematoxylin and eosin.)*

Table 6 *Clinical and histological correlation*

Allogeneic recipients after transplant (mean 28 days)	*Skin*					*Rectum*
	Total No of patients	*Grade I*	*Grade II*	*Grade III*	*Lymphocytic infiltration of epidermis*	*Single cell necrosis*
No skin rash or diarrhoea	4	1	3	0	0	0
Skin rash, no diarrhoea	8	1	5	2	4	6
Skin rash and mild diarrhoea	1	1	0	0	0	0

After transplant

No autologous recipient developed a rash suggestive of GVHD or clinically important diarrhoea. Skin rashes consistent with GVHD developed in nine allogeneic recipients at four weeks. In all of these patients the rash was maculopapular, affecting upper trunk, palms, and soles. It varied in severity but was extensive only in one patient at the time of biopsy. No patients had liver disease manifest by raised hepatic enzyme activity and only one had mild diarrhoea (500 ml/day). Table 6 shows the correlation between these first symptoms occurring after transplant and the abnormal histological findings on biopsy. Patients thought to have GVHD were treated with a course of steroids and they all showed improvement, though three patients required prolonged treatment.

The association between lymphocytic infiltrate of epidermis and single cell necrosis in these patients thought to have GVHD is shown in table 6.

Only one patient had diarrhoea, but pronounced single cell necrosis of the rectum was present in six of the allogeneic recipients, all of whom had a skin rash. The two patients with more severe skin changes, both clinically and histologically, also had rectal changes, but one patient with very mild skin lesions at the time of biopsy and no diarrhoea showed quite extensive damage to the rectal glandular epithelium. This patient developed an extensive skin rash a few days later and he responded well to treatment for GVHD but he never developed diarrhoea.

Six months after transplant two of the allogeneic recipients had the clinical skin changes of chronic GVHD but only one showed confirmatory skin biopsy features. No patients had any gut symptoms six months after transplant.

Discussion

Although severe GVHD causes substantial mortality in most bone marrow transplantation patients, it developed only rarely in patients in this series. The belief that early diagnosis followed by immediate treatment might help prevent severe GVHD stimulated us to investigate the relative values of taking skin and rectal biopsy specimens in the diagnosis of early GVHD. As there is a parallel programme of both autologous and allogeneic bone marrow transplantation in Newcastle, albeit with a somewhat different preparative regimen, the autologous recipients acted as a good control group for the allogeneic transplants when examining the biopsy specimens before and after transplant.

The results of this study highlight the fact that skin biopsy is of little value in diagnosing early GVHD. Grade I or II changes often occurred both before transplant and in the autologous group after transplant. Such changes were probably induced by chemotherapy: cytotoxic agents have a cytopathic effect related to dose on epithelial surfaces.[5 10] These changes were still present in the skin four weeks, and to a lesser extent, six months after transplant. Grade III skin changes, however, were found only in allogeneic recipients with clinical GVHD, and this may reflect active GVHD. A recent study[11] has shown that a mononuclear cell infiltrate in the epidermis is seen only in GVHD and not as a result of the administration of cytotoxic drugs. Our results agree with these conclusions: epidermal infiltrate was seen only in patients with clinical GVHD. It was not a consistent finding, however, and of limited diagnostic value when performing a skin biopsy in early lesions of GVHD.

The two different stimuli of early GVHD and cytotoxic agents seem to produce similar damage, though probably via separate mechanisms, and as Sale *et al*[5] suggested, in allografted patients with GVHD the effects of both are probably cumulative, as the more severe changes were seen only in this group.

A mild perivascular lymphocytic infiltrate was seen both in the autologous and allogeneic recipients. Although lymphocytic exocytosis into the epidermis was seen only in allogeneic patients after transplant, satillosis[12] was not a feature. This finding, togther with those of recent publications[13 14] calls into question the importance of an "aggressor" lymphocyte which produces direct tissue damage in GVHD.

Changes seen in the rectal mucosa were more helpful, but it is important to note that all the biopsies were performed at a mean of 28 days after transplant. A previous study[6] showed that rectal biopsies performed before 20 days after transplant may be difficult to interpret as mucosal changes caused by the preparative regimen may still be present. In our study

all the rectal biopsy specimens in the autologous recipients were normal after transplant, confirming that any mucosal damage induced by cytotoxic drugs or irradiation had disappeared four weeks later. This contrasts with the findings in skin biopsy specimens where abnormalities were detected both before transplant and up to six months afterwards.

Focal clusters of glands showing single cell necrosis of epithelial cells were seen only in rectal biopsy specimens from allogeneic patients after transplant and were associated with a skin rash thought to be due to GVHD in all cases. None of the patients with an abnormal rectal biopsy specimen had any gastrointestinal symptoms, including diarrhoea. This finding differs from those of two previous reports[6 7] in which single cell necrosis was found only in patients with secretory diarrhoea as part of suspected GVHD.

No previous studies have performed simultaneous skin and rectal biopsies as soon as early GVHD is clinically suspected, which is normally when a skin rash develops. By doing this we have been able to show that an abnormal rectal biopsy specimen supports the diagnosis of GVHD, when the histology of the skin biopsy is non-specific and even when the patient has no diarrhoea when the biopsy is performed.

All patients in our series were vigorously screened for virus infections and all were negative. This precluded any assessment of skin and rectal changes in viral infections. Other authors have described single cell necrosis of rectal glands in CMV proctitis[15] and therefore before GVHD is definitively diagnosed on a rectal biopsy specimen viral studies should also be performed.

In conclusion, grade I and II histological changes in skin are non-specific and of no diagnostic value in detecting GVHD. Examination of a rectal mucosal biopsy specimen offers a simple alternative approach in the diagnosis of early GVHD. It may provide the only reliable histological indication of early or mild GVHD in patients with haematological malignant disease who receive an HLA identical, MLC non-reactive sibling marrow transplant.

Part of this paper was presented at the XIIIth annual meeting of the European Cooperative Group for Bone Marrow Transplantation in Interlaken, Switzerland, March, 1987.

We thank Dr M M Reid for a critical review of the paper and Mrs Paula McEwen for typing the manuscript.

The study was supported by a grant from the North of England Cancer Research Campaign.

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