Histopathology of the lung after bone marrow transplantation

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SUMMARY The histopathological changes in the lungs of 32 patients who died after bone marrow transplantation for leukaemia have been studied and compared with those found in 21 patients treated by conventional chemotherapy. The transplanted patients exhibited a higher incidence of interstitial pneumonitis, vascular lesions and viral infections, particularly cytomegalovirus (CMV), although bacterial and fungal diseases were commoner in the non-grafted subjects. The pathogenesis of interstitial pneumonitis is discussed with specific reference to the possible roles of irradiation, chemotherapy, viruses and the immunosuppressive drug cyclosporin A. Ten patients died of a syndrome characterised clinically by fever, skin rash, fluid retention, uraemia, low serum albumin concentrations, low central venous pressure and acute pulmonary oedema. These patients exhibited intra-alveolar haemorrhagic fibrinous exudation with or without interstitial changes. The aetiology of this syndrome is not known but it occurs more frequently in recipients of mismatched grafts and evidence is presented suggesting that viruses may play a significant causative role. No lesion was identified that could be directly attributed to Graft-versus-Host disease.

The use of bone marrow transplantation in the treatment of leukaemia has led to a significant improvement in prognosis.¹ However, it has also introduced a new spectrum of complicated iatrogenic human pathology of which pulmonary complications form a major proportion and account for a significant number of deaths.

The aim of this study was to make a detailed histological appraisal of the various lesions occurring in the lungs of patients who have died after bone marrow transplantation for leukaemia. By comparing these lesions with those seen in conventionally treated patients, it was hoped to identify those pathological processes specifically associated with transplantation. It was also hoped to glean some information about the possible aetiological roles of various factors such as radiation, drugs, infectious agents and Graft-versus-Host (GvH) disease in the production of these lesions by looking for correlations with various other laboratory and clinical data.

At the time of writing allogeneic or syngeneic marrow transplantation has been performed at the

Royal Marsden Hospital on 173 patients, of whom 75 have died. Necropsy material is available on the lungs of 32 of these patients and forms the basis of this study.

Material and methods

Full necropsies were performed on 29 patients; the lungs were weighed, inflated with formol saline and cut into slices 1 cm thick. An average of five blocks per case were taken from any suspicious areas. In the remaining three cases, only post-mortem needle specimens were available.

The lungs from an unselected consecutive series of 21 ungrafted patients who had died of acute myeloid leukaemia were similarly examined for comparison.

Paraffin-embedded sections (3-4 µm) were stained with haematoxylin and eosin (H&E), Gram stain for bacteria, Grocott for fungi and *Pneumocystis carinii*, Perls' for haemosiderin, Picro-Mallory for fibrous tissue and fibrin, and Verhoeff's stain for elastin. Indirect immunocytochemical stains for epithelial membrane antigen (EMA) were also carried out as described previously.² EMA is a large

molecular weight glycoconjugate which can be detected in paraffin-embedded sections by conventional antisera raised to milk-fat globule membranes. It is confined to, but widely distributed in, epithelial cells usually only on the surface membranes. In the lung there is staining of the surface membranes both of type I and type II pneumocytes as well as the luminal membranes of many of the bronchial epithelial cells. All other cell types are negative. The stain is thus of value in distinguishing type II pneumocytes from macrophages, in assessing the thickness of alveolar walls in areas of exudation and, in some cases, assessing the integrity of type I cells where they are too attenuated for resolution by light microscopy (Fig. 1).

The age of the transplanted patients ranged from 10–46 yr (mean 28 yr). The primary diagnosis was acute myeloid leukaemia in 26 cases, chronic granulocytic leukaemia in three and acute lymphoblastic leukaemia in three. The time interval between transplantation and death varied between 2 and 120 wk (mean 27 wk). Pretransplant conditioning was achieved using cyclophosphamide ($60 \text{ mg/kg} \times 2$) and total body irradiation (TBI) in all patients. Irradiation was given as a single fraction with a maximum lung dose of 9.10–12.95 Gy (mean 10.13). The dose rate was 0.028 Gy/min in 30 patients and 0.08 Gy/min in two. Four patients also received a second graft from their original donors following relapse and were conditioned with cyclophosphamide (300 mg/m^2) and melphalan ($180\text{--}240 \text{ mg/m}^2$). Twenty-eight patients received cyclosporin A; in two of these, the drug was given therapeutically to treat established GvH disease but

in the remainder, it was given prophylactically, generally at a dose of 12.5 mg/kg/day starting one day before grafting. All patients received marrow from family member donors. In 20 (including one identical twin), the graft was matched on HLA typing and mixed lymphocyte reaction (MLR). In the other 12 mismatched grafts there was a varying degree of HLA and/or MLR incompatibility.

Results

Details of the transplanted patients are summarised in Table 1.

PATHOLOGICAL FEATURES

The lung lesions fell into seven major categories, namely interstitial pneumonitis, acute haemorrhagic pulmonary oedema, pneumonitis due to bacteria, fungi or pneumocystis, leukaemic infiltration and vascular abnormalities. The incidence of these lesions in the transplant recipients is compared with that in the 21 non-transplanted patients in Table 2.

INTERSTITIAL PNEUMONITIS

This was seen in 16 (50%) of the 32 transplant patients and was characterised by thickening of the alveolar walls by oedema, fibrosis and mononuclear cell infiltration usually of minor to moderate severity. The alveoli in the affected zones were lined by hyaline membranes and many contained haemorrhagic fibrinous exudate (Fig. 2). The hyaline membranes showed very variable and sometimes negative staining for fibrin. They often contained finely granular EMA-positive material and this together

Fig. 1 Lung exhibiting interstitial pneumonitis stained for EMA. Note positive type II pneumocytes and negative macrophages. The alveolar walls are thickened (largely by fibrosis) and there is a lack of continuity of the epithelium at the bottom right of the picture associated with intra-alveolar shedding and phagocytosis of granular EMA-positive material (arrow). Haemalum counterstain $\times 500$

Table 1 Summary of clinical and pathological details of 32 patients who have died after bone marrow transplantation

Patient number Sex Age (yr)	Diagnosis and status on grafting	TBI		Histocompatibility	Survival from first (second) graft (wk)
		Max lung dose (Gy)	Dose rate (Gy/min)		
5-M-21	AML rel	9.50	0.028	M	24
8-M-10	ALL 2nd CR	9.50	0.028	Syn	12
13-M-21	ALL 2nd CR	9.50	0.028	M	41
23-M-10	ALL 3rd CR	9.28	0.028	M	13
28-M-21	AML 2nd CR	9.10	0.028	M	7
32-F-33	AML 1st CR	9.75	0.028	M	5
34-M-34	AML 1st CR	9.44	0.028	M	39
52-M-46	AML 1st CR	9.44	0.028	M	11
35-F-27	AML 1st CR	9.05	0.028	M	58
48-F-36	AML 2nd CR	9.33	0.028	M	28
59-M-45	AML rel	9.55	0.028	MM	7
64-F-21	AML 2nd CR	9.37	0.028	MM	4
50-M-37	AML 1st CR	9.65	0.028	MM	42
74-M-21	AML 1st CR	10.37	0.028	MM	2
51-M-29	AML 1st CR	9.52	0.028	M	63 (8)
89-M-37	AML 1st CR	10.44	0.028	M	7
100-F-28	CGL	10.41	0.028	M	6
79-M-31	CGL	10.12	0.028	M	31
61-M-17	AML 1st CR	9.38	0.028	M	79 (16)
43-F-13	AML rel	10.00	0.028	M	120 (53)
109-F-34	AML 1st CR	10.29	0.028	M	14
114-F-34	AML 2nd CR	12.95	0.028	MM	9
127-F-44	AML rel	10.20	0.028	MM	4
132-M-19	AML 2nd CR	10.37	0.028	MM	3
134-M-33	AML 1st CR	11.31	0.028	MM	2
118-F-20	AML 2nd CR	10.60	0.028	MM	24
108-F-27	AML 1st CR	10.71	0.028	M	13
117-F-15	AML 1st CR	11.88	0.028	MM	10
121-M-44	AML 1st CR	10.78	0.08	MM	22
137-F-27	CGL	10.59	0.028	M	6
83-F-20	AML 2nd CR	10.28	0.028	MM	79 (3)
82-M-30	AML 1st CR	9.95	0.08	M	73

Diagnosis and status on grafting:

AML = acute myeloid leukaemia; ALL = acute lymphoid leukaemia; CGL = granulocytic leukaemia; rel = leukaemic relapse; CR = complete remission.

Histocompatibility

M = matched graft; Syn = syngeneic graft; MM = mismatched graft.

Cyclosporin A

N = cyclosporin A not given. T = cyclosporin A given therapeutically only. P = cyclosporin A given prophylactically.

Figures in second column = mean of serum concentrations on days 8, 15 and 29 after grafting in ng/ml.

Table 2 Incidence of pulmonary lesions in leukaemic patients treated by bone marrow transplantation and conventional chemotherapy

Lesion	32 patients receiving transplants	21 patients treated by chemotherapy alone
Interstitial pneumonitis (total)	16 (50%)	2 (10%)
—with CMV inclusions	8 (25%)	0
—with pneumocyte atypia alone	5 (16%)	0
—with normal or hyperplastic pneumocytes	3 (9%)	2 (10%)
Acute haemorrhagic pulmonary oedema	5 (16%)	0
Vascular abnormalities	16 (50%)	3 (14%)
Giant cell pneumonitis (measles type)	0	1 (5%)
Pneumocystis pneumonitis	2 (6%)	0
Bacterial pneumonitis	9 (28%)	11 (52%)
Fungal pneumonitis	1 (3%)	5 (24%)
Leukaemic infiltration	2 (6%)	7 (33%)
Normal	2 (6%)	0

with the lack of EMA staining of the alveolar walls underlying the membranes indicated that they were, at least in part, derived from degenerate epithelial cells.

Zones of bacterial bronchopneumonia coexisted with interstitial pneumonitis in three cases and leukaemic infiltration in one. Here the histological assessment was made well away from these areas. In

Histological GvH disease	Cyclosporin A	Lung histology	Vascular lesions	Acute pulmonary oedema syndrome
Yes	N —	Bact Pn*	ES	No
No	N —	IPn with PA*	None	No
Yes	N —	Fungal Pn, <i>P. carinii</i> Pn*	None	No
No	N —	IPn with CMV	ES + T	No
Yes	T —	IPn	None	No
Yes	T —	IPn	ES + T	No
Yes	P —	IPn with CMV, * Bact Pn*	T	No
Yes	P 1162	IPn with CMV, * Bact Pn*	T	No
Yes	P —	IPn with PA, LI	None	No
No	P —	<i>P. carinii</i> Pn	T	No
Yes	P —	IPn with CMV*	None	Yes
No	P 889	IPn with PA*	T	Yes
Yes	P 703	Bact Pn*	None	No
Yes	P —	AHPO*	ES + T	Yes
Yes	P 1197	Bact Pn*	T	No
Yes	P 92	IPn with CMV	T	No
Yes	P 888	Normal	None	No
Yes	P 249	Bact Pn, LI	None	No
Yes	P 455	Normal	None	No
No	P —	Bact Pn	None	No
No	P 1294	IPn with CMV*	T	No
Yes	P 1596	IPn with CMV*	None	No
No	P 1427	Bact Pn*	None	No
Yes	P 1066	AHPO*	None	Yes
No	P 1234	AHPO*	ES + T	Yes
Yes	P 1190	AHPO*	None	Yes
Yes	P 1861	IPn with PA*	T	Yes
No	P 1526	IPn with CMV*	T	Yes
Yes	P 2796	IPn with PA*	None	Yes
No	P 2000	IPn, * Bact Pn*	T	No
No	P 1006	Haemorrhage	None	No
Yes	P 282	AHPO*	T	Yes

Histology

IPn = interstitial pneumonitis. IPn with PA = interstitial pneumonitis with pneumocyte atypia. IPn with CMV = interstitial pneumonitis with cytomegalovirus inclusions. Bact Pn = bacterial pneumonitis, Fungal Pn = fungal pneumonitis. *P. carinii* Pn = pneumonitis due to pneumocystis. AHPO = acute haemorrhagic pulmonary oedema. LI = leukaemic infiltration.

*Asterisk indicates that pulmonary pathology was considered to have contributed to patient's death.

Vascular lesions

ES = endothelial swelling; T = thrombi.

Patients are listed in chronological order of death.

the remaining 12 uncomplicated cases the changes were randomly distributed throughout the lung; they were patchy but extensive, with lung weights varying from 630 to 1260 g (mean 926 g). A small clear pleural effusion (up to 250 ml) was present in three of the cases. Apart from CMV inclusions as described below, no organisms could be demonstrated within the zones of interstitial pneumonitis. Three histological types of interstitial pneumonitis could be recognised on the basis of the appearance of the pneumocytes.

(a) *Interstitial pneumonitis with pneumocyte atypia and cytomegalovirus (CMV) inclusions* was seen in eight (25%) of the 32 transplanted patients but in none of the ungrafted subjects. The pneumocytes (presumably type II cells) were atypical in appearance with marked swelling and pleomorphism and a proportion of them contained CMV inclusions

(Fig. 3a). The CMV-containing cells were identified as epithelial from their positive EMA staining.

(b) *Interstitial pneumonitis with pneumocyte atypia alone* was also restricted to the transplanted group and found in five patients (16%). The appearance of pneumocytes was essentially similar to that in group (a) but viral inclusions could not be identified (Fig. 3b).

(c) *Interstitial pneumonitis with normal or hyperplastic pneumocytes* was seen in three (9%) of the transplanted and in two (10%) of the ungrafted patients. Although the pneumocytes were usually hyperplastic, the atypical features present in groups (a) and (b) were absent. The two non-grafted patients had both received unusually large doses of chemotherapy. In one, acute myeloid leukaemia (treated by conventional chemotherapy) developed six months after successful chemotherapy for Hodg-

Fig. 2 *Interstitial pneumonitis showing mononuclear cell infiltration of the alveolar wall, hyaline membranes and intra-alveolar exudation of fibrin, erythrocytes and macrophages. Haematoxylin and eosin $\times 315$*

Fig. 3 (a) *A pneumocyte containing a classical CMV inclusion is seen in the centre of the field; (b) interstitial pneumonitis with pneumocyte atypia showing an atypical pneumocyte which resembles that seen in (a) except for the presence of the intranuclear inclusion. Haematoxylin and eosin $\times 500$*

kin's disease. In the other, high dose melphalan had been given.³

Acute haemorrhagic pulmonary oedema

This was seen in five (16%) of the transplant cases and in none of the ungrafted group. It was characterised by intra-alveolar and, to a lesser extent, interstitial exudation of oedema fluid, fibrin and erythrocytes. Hyaline membranes, pneumocyte changes and interstitial mononuclear cell infiltration and fibrosis were absent. In one case, occasional tiny foci of interstitial pneumonitis with hyperplastic pneumocytes were also present but changes were not extensive enough to warrant inclusion in the interstitial pneumonitis group. No organisms could be demonstrated in any of these cases.

Lung weights were not always very high and varied from 600 to 1250 g (mean 873 g). In one case (an adult male of average size) the lungs weighed only 580 g and 600 g and yet all patients in this group died of an acute lung death as described below.

Vascular abnormalities

These took the form of endothelial swelling and thrombi (Fig. 4). The latter were usually confined to arterioles, capillaries and venules although they were occasionally seen in larger vessels. They were never extensive with usually only a few lesions per section. They were found in 16 (50%) of the transplanted patients and in three (14%) of the ungrafted group. Of the transplant patients, the lesions were found in 10/16 (63%) patients with interstitial

pneumonitis, 3/5 patients with acute haemorrhagic pulmonary oedema (60%) and in 3/11 remaining cases (27%). In the three ungrafted cases, thrombi were seen in one case of interstitial pneumonitis and in two cases of bacterial bronchopneumonia.

Pneumocystis pneumonia

This was seen in two (6%) of the transplant patients and in none of the ungrafted group. In one case there was also *Aspergillus* sp but in the other *Pneumocystis carinii* was the only infectious agent.

Bacterial pneumonia

Bacterial pneumonia occurred in 9 (28%) of the grafted patients and in 11 (52%) of those not receiving a transplant. There was coexistent interstitial pneumonitis in three and leukaemic infiltration in one. The appearances were usually typical of bacterial bronchopneumonia seen in non-immunosuppressed patients except that the degree of neutrophil infiltration was often reduced. Gram staining revealed a variety of organisms.

Fungal pneumonia

This was seen in only one (3%) of the grafted patients and in five (24%) of those treated by chemotherapy alone. Three cases were due to *Candida* sp, two to *Aspergillus* sp and one to *Trichosporon* sp.

Leukaemic infiltration

This was seen more commonly in patients treated with chemotherapy alone (see Table 2).

CLINICOPATHOLOGICAL CORRELATIONS IN TRANSPLANTED PATIENTS

There was no difference in mean age in any of the pathological groups. The time interval between grafting and death ranged from 4–58 wk (mean 15 wk) for patients with interstitial pneumonitis, 2–73 wk (mean 21 wk) for patients with acute haemorrhagic pulmonary oedema, and 4–120 wk (mean 47 wk) for the remainder. Although the degree of fibrosis and mononuclear cell infiltration of the alveolar wall varied markedly in cases of interstitial pneumonitis, this was not related to the time after transplantation. The mean dose of radiation to the lung was 10–13 Gy for patients with interstitial pneumonitis, 10–52 Gy with those with acute haemorrhagic pulmonary oedema and 9–81 Gy for the remainder. These differences are not significant. However, both patients in this study who had received the higher dose rate of 0.08 Gy/min died of acute respiratory failure as described below; one of them had interstitial pneumonitis (with pneumocyte atypia) and one acute haemorrhagic pulmonary

Fig. 4 Pulmonary blood vessel showing endothelial hyperplasia and thrombosis. Haematoxylin and eosin $\times 126$

oedema.

The incidence of previous GvH disease was 63% in the interstitial pneumonitis group, 80% in the acute haemorrhagic pulmonary oedema group and 64% in the remainder. The difference between the acute haemorrhagic pulmonary oedema cases and the remainder does not reach statistical significance. Thirty-one per cent of patients with interstitial pneumonitis received mismatched grafts, all with atypical pneumocytes with or without CMV inclusions. This compares with 80% for patients with acute haemorrhagic pulmonary oedema and 27% for the remainder. The number of patients in the acute haemorrhagic pulmonary oedema group is too small to achieve statistical significance. Five of the 16 patients with histological interstitial pneumonitis died of acute pulmonary oedema as described below and four of these five received mismatched grafts. Of the remaining 11 patients, interstitial pneumonitis was considered to have contributed to death in six.

Serum concentrations of cyclosporin A were determined by radioimmunoassay at two hours after the dose on days 8, 15, and 29 after grafting in 20 patients. The mean concentration for nine patients with interstitial pneumonitis was 1468 ng/ml compared to 881 ng/ml for the remainder. This is a significant difference ($p < 0.05$).

Ten patients died with a syndrome characterised by fever, skin rash, fluid retention, uraemia, low serum albumin concentrations, low central venous pressure and pulmonary oedema^{4,5} (Table 1). All patients had interstitial pneumonitis associated with extensive intra-alveolar exudation, or acute haemorrhagic pulmonary oedema. There was no significant difference between this group and the other patients in mean cyclosporin concentration as defined above, dose of TBI to the lung, time after grafting, or in the incidence of GvH disease. However, the proportion of patients who received mismatched grafts was 8/10 (80%) compared with 4/22 (18%) of the remaining patients who did not die with this syndrome. This difference is significant ($p < 0.001$). Only one out of the 11 patients with interstitial pneumonitis who did not die of acute pulmonary oedema received a mismatched graft.

Discussion

This study demonstrates that patients dying after bone marrow transplantation for acute leukaemia are more liable to have interstitial pneumonitis, acute pulmonary oedema of the permeability type, viral infections and vascular lesions in the lung than those dying after treatment with conventional chemotherapy. The incidence of bacterial and fungal

pneumonitis was higher in non-grafted patients, probably because they are more likely to suffer from prolonged and severe neutropenia due to chemotherapy or leukaemic relapse.

Clinically manifest interstitial pneumonitis is a well recognised complication of bone marrow transplantation which occurs in up to 40% of patients in some centres and may carry a high mortality. Some cases are associated with CMV inclusions, whereas in the remainder, labelled idiopathic, organisms cannot be found.

Previous studies from our unit have reported a low clinical incidence of interstitial pneumonitis of 10% in 107 patients.⁶ This difference between clinical and necropsy incidences is explained partly by patient selection. However, a few patients with histological evidence of interstitial pneumonitis at necropsy were not diagnosed in life because the changes were of insufficient severity to be detected by conventional clinical methods. Recent lung function studies, however, have shown an invariable deterioration in function after grafting⁷ but a lack of biopsy data prevents us from assessing the histological changes in these patients. It is thus clearly important to distinguish interstitial pneumonitis as diagnosed clinically and histologically.

The pathogenesis of interstitial pneumonitis appears to be multifactorial and irradiation almost certainly plays a role. Interstitial thickening, thrombi, desquamation, hyperplasia and atypia of alveolar epithelial cells together with intra-alveolar fibrinous exudation and hyaline membrane formation may all occur after radiotherapy to the lung.⁸

The Seattle group has shown that TBI increases the risk of interstitial pneumonitis in patients receiving transplants for aplastic anaemia⁹ and others have demonstrated a relation between interstitial pneumonitis and the dose rate of TBI.^{10,11} The observation that both patients in the present study who received the higher dose rate of 0.08 Gy/min died with acute pulmonary oedema clearly warrants further study.

However, there is much evidence to indicate that irradiation is by no means the only factor involved. In most histological studies, changes have been described in lungs which have been irradiated in the course of treating malignant tumours where much higher, fractionated doses are given. Interstitial pneumonitis has, however, been described in a significant number of patients receiving a single dose of 6–10 Gy to the upper body only but the dose rate has been much higher (0.4–4.0 Gy/min) than is used in bone marrow recipients.¹² Dose rate has been shown experimentally to be a crucial factor in the genesis of pulmonary lesions.¹³ In short, the precise effect of a single dose of irradiation, uncomplicated

by other factors, on the human lung at the level and dose rate given to the patients in this study is not known.

Several of our observations are against radiation being the sole cause. There appears to be no relation between the extent of lung damage, the degree of fibrosis or the amount of mononuclear cell infiltration and the time after grafting. There was no relation between interstitial pneumonitis and dose of TBI, and bronchial epithelial changes such as focal necrosis and squamous metaplasia which have been seen in radiation pneumonitis were not observed. Furthermore, Depledge *et al* have shown that there is no correlation between the extent of deterioration in lung function after transplantation and the dose of TBI.⁷

Cytotoxic drugs must also be implicated as lung changes similar to those induced by irradiation have been seen after administration of a wide range of agents including busulphan,¹⁴ bleomycin,¹⁵ melphalan,¹⁶ and cyclophosphamide;¹⁷ the last of these is, of course, particularly relevant to this study. Furthermore, it has been shown that chemotherapy (including cyclophosphamide) may enhance lung damage after thoracic irradiation.¹⁸

We found significantly higher serum concentrations of cyclosporin A in those patients who were found to have interstitial pneumonitis at necropsy. Previous authors have observed that this drug may affect small blood vessels producing angio-oedema,¹⁹ or intravascular thrombosis.^{20, 21} Increased alveolar capillary permeability and thrombosis induced by cyclosporin A may be significant factors in the genesis of interstitial pneumonitis.

Although the transplanted patients in the present study showed no increased tendency to die with bacterial or fungal pneumonia, there was a striking increase in the incidence of pulmonary viral infections. The predominant organism in this study, as well as in those from other centres, was CMV. Whether this represents a specific susceptibility of the transplant recipient to this particular virus or whether CMV is merely easier to detect in histological sections is not clear. Appelbaum *et al*²² studied 100 recipients of marrow from identical twins and found no evidence of CMV infection and a markedly reduced incidence of interstitial pneumonitis even though all patients had received cyclophosphamide and TBI prior to grafting. This suggests that CMV is causally related to interstitial pneumonitis; it also demonstrates an important relation between the histocompatibility of the graft and the incidence of viral infections. Appelbaum *et al* suggested that this may be due to GvH disease destroying host immune cells and necessitating the administration of post-transplant immunosuppression. Indeed, Meyers *et*

*al*⁹ have reported that moderate to severe GvH disease is associated with an increased risk of CMV associated interstitial pneumonitis. In the present study, there was an increased incidence of GvH disease in patients with interstitial pneumonitis but this was not related specifically to those associated with CMV.

It has been shown recently that CMV infection in bone marrow transplant recipients is associated with low or absent CMV specific cytotoxic lymphocyte activity before the onset of infection and that survival depends on being able to mount cytotoxic responses.²³ Some of these cytotoxic cells are T cells with HLA-restricted activity; that is, they lyse HLA-matched target cells better than mismatched ones. This may be another significant factor in the increased susceptibility to virus infections of recipients of allogeneic transplants compared to those receiving syngeneic grafts.

In our study there was a high incidence of mismatched grafts in those who died of acute pulmonary oedema. Some of these patients had interstitial pneumonitis whereas in others the alveolar walls appeared normal, at least at the light microscope level. All patients though, had extensive intra-alveolar exudation of fibrinous haemorrhagic material. Although endothelial damage may contribute significantly to the pathogenesis of interstitial pneumonitis, current evidence indicates that alveolar epithelial cells form the major barrier between the blood and the alveolar lumen.²⁴ The demonstration, by EMA staining, that CMV is within the epithelial cells, the association of acute pulmonary oedema with the transplantation of mismatched marrow and the associated clinical features of skin rash and fever suggest that viral infections of pneumocytes may play a significant role in producing high molecular weight intra-alveolar leaks in transplanted patients. Although definite CMV inclusions were seen in only 3/11 patients, conventional histopathology is a most insensitive method for detecting viruses. Further studies using electronmicroscopy, immunohistological techniques with antibodies to viral components and in situ nuclei acid hybridisation methods would be of value and are being investigated at present.

Although GvH disease has occasionally been reported in the lung, the appearance is that of a lymphocytic bronchitis²⁵ which was not observed in any of the patients in this study. None of the lesions we have described was related to the occurrence of GvH disease in the skin or other sites and none has been reported as a graft versus host reaction in controlled studies on experimental animals.²⁶

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