The pathology of heart and heart and lung transplantation—an update

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Introduction

Orthotopic heart transplantation is an established treatment for heart failure refractory to medical treatment due to coronary artery disease or dilated cardiomyopathy.¹ The problems of rejection have, to some extent, been overcome with the introduction of cyclosporin A and right ventricular biopsy to monitor immunosuppression. Nevertheless, rejection and infection, both manifestations of failure to control and monitor adequately immune function, remain the commonest causes of death in the first year after heart transplantation. Long term survival is principally limited by the development of coronary occlusive disease.²

Combined heart and lung transplantation for end stage pulmonary vascular disease and chronic lung disease effectively started in the 1980s, with the introduction of cyclosporin A, an immunosuppressive agent without adverse effects on anastomotic healing. Previous attempts, including some single lung transplantations, produced poor results. Heart and lung transplantation is performed for both primary and secondary pulmonary hypertension³ and end stage parenchymal lung disease.⁴ Some centres offer double lung transplantation for the latter, with the benefit of the patients being able to retain their own heart. Single lung transplantation is suitable for fibrotic lung disease and emphysema, where there is no risk of infection from the remaining contralateral lung.⁵ As with other solid allografts, the main complications in heart and lung transplant recipients are rejection and infection. In combined grafts the lungs reject earlier and more

vigorously than the heart,⁶ and improvement in survival has been achieved by monitoring rejection episodes with the aid of transbronchial lung biopsy.⁷⁻⁸ The lungs are very prone to infections with common and opportunistic pathogens and have a much higher infection rate than other allografts, adding to problems of diagnosis and management.⁹ Obliterative bronchiolitis is the main limiting factor for long term survival.¹⁰ Most of the pathology of heart and lung transplantation is dealt with in the recognised United Kingdom transplantation centres, but with the increasing pool of transplant recipients, some of the biopsy and necropsy pathology may be seen elsewhere.

Rejection in the heart

Billingham devised a simple grading system for rejection in the heart¹¹ in heart transplantation. The interpretation and relevance of the various morphological changes seen had been previously validated in dogs. Other systems introduced subsequently are considerably more complicated, and while they may have provided useful additional histological information in their original institutions, there was a need for a single updated and simplified classification of rejection. This arose partly from the need to carry out trials of new immunosuppressants and partly from the need to pool data from several institutions. Therefore, the International Society of Heart Transplantation sponsored a meeting at Stanford University in July 1990 to propose a new grading system for cardiac allograft biopsy specimens.¹² The essential features are

Table 1 Working formulation for classification and grading of cardiac rejection (heart rejection study group)¹²

Grade	New nomenclature	Old nomenclature
0	No rejection	No rejection
I	A = Focal (perivascular or interstitial infiltrate) B = Diffuse but sparse infiltrate	Mild rejection
II	One focus only with aggressive infiltration or focal myocyte damage	"Focal" moderate rejection
III	A = Multifocal aggressive infiltrates and/or myocyte damage B = Diffuse inflammatory process	"Low" moderate rejection
IV	Diffuse aggressive polymorphous ± oedema ± haemorrhage ± vasculitis Denoted by a lesser grade Denoted by grade 0	"Severe acute" rejection
	Additional information required	"Resolving" rejection "Resolved" rejection
	● Biopsy less than four pieces	
	● Humoral rejection (positive immunofluorescence, vasculitis, or severe oedema in absence cellular infiltrate)	
	● Endocardial lymphoid infiltrate (Quilty effect)	
	A = No myocyte encroachment	
	B = With myocyte encroachment	
	● Ischaemia A = Up to three weeks after transplant	
	B = Late ischaemia	
	● Infection present—biopsy specimen therefore uninterpretable	
	● Lymphoproliferative disorder	
	● Other	

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Figures 1–5 Grades of cardiac rejection (haematoxylin and eosin).

Figure 1 Grade 1A: focal lymphoid infiltrate, in this case perivascular.

Figure 2 Grade 1B: mild diffuse interstitial lymphoid infiltrate.

Figure 3 Grade 2: focus of aggressive lymphoid infiltrate. Compared with fig 1 the infiltrate is encroaching on, and by inference damaging, a few myocytes.

shown in table 1 and examples are illustrated in figs 1–7. It is based on haematoxylin and eosin stained sections of formalin fixed, paraffin wax processed material, with a minimum requirement of four biopsy fragments, at least 50% of each fragment being free of fibrosis or biopsy site change. At least three levels are examined with an additional connective tissue stain—for example, Masson's trichrome—on one of these. The difficulty of identifying definite myocyte necrosis as a criterion for a “moderate” grade of rejection has been replaced by the aggressiveness of the infiltrate. This is based on a combination of the intensity and immaturity of the lymphoid infiltrate and the tendency to encroach on and replace myocytes. This latter feature represents circumstantial evidence of myocyte damage without the need to identify clearly fragmenting and irreversibly damaged myocytes. The grades shown represent histological entities; it is not known whether rejection progresses through the various grades or whether focal and diffuse rejection are different processes. Worldwide, treatment thresholds vary. Most centres would treat grade 3 rejection, but it is not clear whether cases showing grade 2 rejection need enhanced immunosuppression. It may be that this grade is more important in the months immediately following transplantation. In the early months grade 2 rejection may represent a developing and potentially clinically important rejection episode; in the later months it may be a manifestation of stable and clinically unimportant rejection. Apart from grading rejection, several other self-explanatory features are recorded. The noting of endocardial infiltrates which encroach underlying myocardium (fig 7) may provide useful information in future about the clinical importance of this lesion. It is not necessarily associated with underlying cellular rejection in the deep myocardium and its association with coronary occlusive disease needs to be elucidated fully as the endocardium could reflect changes in the epicardial coronary arteries.

Rejection in the lung

It was initially thought that in combined heart and lung transplantation lung rejection could be monitored by endomyocardial biopsy. The lungs and heart reject asynchronously, however,¹³ and lung tissue, most easily obtained by transbronchial biopsy, is required for accurate histological diagnosis.^{7,8} Symptoms, signs, pulmonary function and radiography¹⁴ lack specificity for differentiating rejection from other causes of graft dysfunction. The histopathological features of acute pulmonary rejection have been described in dogs,¹⁵ and rats,¹⁶ and human tissue obtained at open or transbronchial lung biopsy shows similar features.¹⁷ Rejection is characterised by perivascular and peribronchiolar mononuclear cell infiltrates which extend to interstitium and ultimately alveolar spaces. With increasing grades of rejection, the infiltrate comprises

Figure 4 Grade 3A: multiple foci of aggressive lymphoid infiltrate.

Figure 5 Grade 3B: diffuse aggressive lymphoid infiltrate. The diffuse nature of the process distinguishes this from grade 3A and in a single field the process may seem similar.

Figure 6 Endocardial lymphoid infiltrate, no encroachment (haematoxylin and eosin).

more activated large lymphoid cells with more conspicuous eosinophils and polymorphs. Infiltration of vessel walls with endothelialitis and of the airway epithelium occurs frequently.

The Lung Rejection Study Group sponsored by the International Society for Heart Transplantation has recently proposed a grading system to incorporate existing classifications and to allow for exchange of data among centres.¹⁸ Grading focuses on histological findings rather than clinical information and excludes biopsy specimens in which there is evidence of infection either in the biopsy specimen itself or in other samples submitted for microbiological examination. Acute rejection is graded 1–4 depending on the frequency, nature, and extent of infiltration by inflammatory cells. Within each grade suffixes (a–d) denote any associated inflammation in airways if these are included in the biopsy fragments. For those biopsy specimens showing lymphocytic bronchitis or bronchiolitis in the absence of perivascular infiltrates, a separate category B is given, to assess the relation of these changes with obliterative bronchiolitis.¹⁹ Chronic airway rejection is manifest by obliterative bronchiolitis which may be total or subtotal and show evidence of active inflammation. This category (C) is distinguished from B by the presence of fibrous scarring. Chronic vascular rejection (D) is associated with fibro-intimal thickening of arteries and veins. Vasculitis that is disproportionate to rejection infiltrates and occasionally seen on open lung biopsy material is designated E. Table 2 summarises this classification and examples are shown in figs 8–17. For example, moderate rejection with bronchiolar injury would be classified A3a, and mild acute rejection superimposed on subtotal small airway scarring without inflammation would be classified as A2a with C1b. At least five parenchymal biopsy specimens from the transplanted lungs should be examined at a minimum of three levels with haematoxylin and eosin, connective tissue stains, and silver stains for fungi and pneumocystis.

Follow up biopsy specimens of treated rejection episodes generally show smaller infiltrates of lymphocytes and haemosiderin-laden macrophages without eosinophils. The differential diagnosis of pulmonary rejection is

Table 2 Working formulation for classification and grading of pulmonary rejection (lung rejection study group)¹⁸

Grade A: Acute rejection
(1) Minimal acute rejection
(2) Mild acute rejection
(3) Moderate acute rejection
(4) Severe acute rejection
(a) With evidence of bronchiolar inflammation
(b) Without evidence of bronchiolar inflammation
(c) With large airway inflammation
(d) No bronchioles are present
Grade B: Active airway damage without scarring
(1) Lymphocytic bronchitis
(2) Lymphocytic bronchiolitis
Grade C: Chronic airway rejection
(1) Bronchiolitis obliterans—subtotal
(2) Bronchiolitis obliterans—total
(a) Active
(b) Inactive
Grade D: Chronic vascular rejection
Grade E: Vasculitis

Figure 7 Endocardial lymphoid infiltrate with encroachment on underlying myocardium (haematoxylin and eosin).

Figures 8–11 Grades of pulmonary rejection (haematoxylin and eosin).

Figure 8 Grade A1: scattered small foci of perivascular lymphoid infiltration. No lymphoid infiltration of bronchioles included in these specimens, the complete grade is therefore A1b.

Figure 9 Grade A2: more frequent larger foci of perivascular lymphoid infiltration than grade A1. Bronchioles included in these specimens showed lymphoid infiltration, the complete grade is therefore A2a.

broad.¹⁸ The perivascular, peribronchiolar, and interstitial mononuclear infiltrates are not specific for rejection. Differential diagnoses include cytomegalovirus (CMV) pneumonitis, pneumocystis, Epstein-Barr virus associated lymphoproliferations, normal bronchial associated lymphoid tissue, previous biopsy sites, recurrent primary disease and ischaemia. The submucosal scarring in airways may also be related to infection, aspiration, or ischaemia. Organising pneumonia can be differentiated from the chronic bronchiolar injury of rejection by the presence of granulation tissue in distal alveolar ducts or air spaces. The biopsy assessment of lung rejection is complicated when there is concomitant infection. It may only be possible to favour one diagnosis over another and suggest follow up repeat biopsies.

Problems of infection

Heart and lung transplantations have a higher rate of opportunistic infections than heart transplantations.²⁰ Reasons for this include decreased mucociliary clearance, absent cough reflex, and more frequent rejection episodes that require additional immunosuppression. The bronchus-associated lymphoid tissue has been shown to be depleted in heart and lung transplant recipients, further lowering defences.²¹

MYCOBACTERIAL DISEASE

Both typical and atypical mycobacterial infections occur in heart and lung transplant recipients²² and can be diagnosed by trans-bronchial biopsy and lavage with culture. Patients with obliterative bronchiolitis are most at risk.

CYTOMEGALOVIRUS

CMV infection is a major clinical problem in both types of transplant recipient,²³ causing both morbidity and mortality and probably modulating rejection.

Primary (donor transmitted)

Among Papworth transplant recipients this is mainly confined to those with heart transplantation as those with heart and lung transplantations are screened and donor matched for CMV antibody response as in most other centres. Nevertheless, mismatches can happen and seronegative recipients may also acquire primary CMV from sources other than the donor organ—for example, a blood transfusion. Primary CMV infection may cause a fatal pneumonitis with evidence of a generalised systemic disease and accompanying splenomegaly. The gastrointestinal tract may be affected,²⁴ as may the heart, and the resultant myocarditis may be difficult to distinguish from rejection in the absence of classic CMV inclusions.

Reactivation

CMV reactivation is a common and indolent problem in both sets of patients.²³ These patients often shed CMV in a variety of body fluids including saliva and urine and may show

Figure 10 Grade A3: changes of A2 with extension into surrounding interstitium. Bronchioles included in these specimens showed lymphoid infiltration, the complete grade is therefore A3a.

Figure 11 Grade A4: extensive perivascular, interstitial, and intra-alveolar lymphoid infiltrates with intra-alveolar fibrin. Bronchial and bronchiolar lymphoid infiltration was also present, the complete grade is therefore A4a,c.

Figure 12 Grade A2a rejection with conspicuous endothelialitis (haematoxylin and eosin).

changing antibody titres. These events may occur in the absence of clinically important diseases and the essential feature for diagnosis of disease as opposed to infection is organ damage and associated inflammation.²⁵ Diagnosis of CMV disease in the lung requires the demonstration of histological pneumonitis in association with CMV inclusions (fig 18).¹⁷ The presence of inclusions alone may simply represent infection and be clinically unimportant, rendering redundant special techniques that increase sensitivity of detection.²⁶ CMV pneumonitis can be distinguished from rejection by diffuse alveolitis, perivascular oedema, and neutrophilic microabscesses, even when inclusions are not conspicuous.¹⁷ Difficulties may be experienced when both conditions coexist. In patients already being given ganciclovir viral inclusions may consist of globules of degenerate eosinophilic material without the formation of typical “owls’ eyes”. The lung remains the principal centre of disease in CMV reactivation, and this raises important immunological questions about antigen presentation and cell killing in donor organs, given that these are not usually HLA matched to the recipient. Other organs may also be affected.

HERPES SIMPLEX VIRUS (HSV)

Herpes simplex virus occurs principally in those seropositive before transplantation and may be avoided by prophylactic acyclovir given during increased immunosuppression.²⁷ Tracheobronchitis, pneumonia, and generalised dissemination from mucocutaneous lesions may be seen. The presence of necrosis, often centred on bronchioles, and the lack of cytomegaly distinguishes herpes simplex virus from CMV pneumonia. The viral inclusions are distinctive but previous treatment or prophylaxis with acyclovir or ganciclovir may change their morphology. Immunohistochemical methods and nucleic acid probes are sometimes helpful in further differentiation, but herpes simplex virus and CMV can occur concomitantly.^{26 28}

ASPERGILLUS

There is a much higher incidence of Aspergillus infection in heart and lung transplant recipients than in heart transplant recipients, and this ranges from colonisation of bronchiectatic airways and bronchocentric granulomatous mycosis²⁹ to cavitating pneumonia and disseminated invasive disease (fig 19). It is common in chronic lung rejection. Paired samples of transbronchial biopsy and lavage specimens are useful in determining the clinical importance and extent of infection.³⁰ Prompt diagnosis can result in eradication using antifungal treatment.

PNEUMOCYSTIS

This is a sizeable problem in both types of transplant recipient,³¹ though in heart and lung transplant recipients usually only if standard prophylaxis with co-trimoxazole has not been taken. In specimens from heart transplant recipients intra-alveolar foam is usually

Figure 13 Lymphocytic bronchiolitis in this case in the absence of perivascular infiltrates, the grade is therefore B1 (haematoxylin and eosin).

Figure 14 Severe lymphocytic bronchiolitis as part of A4a,c rejection (same case as figure 11) (haematoxylin and eosin).

Figure 15 Subtotal active obliterative bronchiolitis, grade C1a. Elsewhere there was A2a rejection (elastic van Gieson).

obvious in haematoxylin and eosin stained sections of transbronchial biopsy specimens, with numerous organisms in a Grocott methenamine silver stain. The associated inflammation may be non-specific or granulomatous.³² In heart and lung transplantation foam may be less obvious, making a carefully screened Grocott methenamine silver stained section mandatory. A granulomatous reaction is common, a feature less often seen in other immunosuppressed groups, and organisms are scanty.³³ Pneumocysts have not been seen in any extrapulmonary organs.

TOXOPLASMOSIS

There is a high risk of serious or fatal primary toxoplasmosis if seronegative patients receiving grafts from seropositive donors are not given adequate prophylaxis with pyrimethamine.³⁴ Features include necrotising encephalitis, pneumonitis, and myocarditis, and the latter may be difficult to distinguish from cellular rejection, particularly as the intramyocyte toxoplasma cysts tend to be seen away from the areas of inflammation (fig 20). Recrudescence may occur in those who have recovered from a primary graft acquired infection a few years earlier.

Broncho-alveolar lavage (BAL)

This is useful for the diagnosis of infections in immunosuppressed hosts,³⁵ particularly when paired with transbronchial biopsy. In a study of 522 paired samples of heart and lung transplantations from Papworth³⁰ viral and pneumocystis infections were more frequently identified in transbronchial biopsy specimens and fungal infection in the BAL. Both samples were complementary in the diagnosis of opportunistic infection. In heart and lung transplantation transbronchial lung biopsy is necessary for the accurate diagnosis and grading of rejection. Lavage cytology has been used to investigate rejection, but the changes in cell numbers and profiles have been disappointingly non-specific and do not correlate with biopsy specimen grade.³⁶ CD8 positive T cells increase during rejection episodes³⁷ but these are the commonest intraepithelial lymphocytes in the lung³⁸ and the increase is again not specific. In other centres cytolytic activity of lavage fluid against donor spleen cells and spontaneous proliferation have been used to monitor rejection but without a biopsy gold standard.³⁹

Graft versus host disease (GvHD)

Transplanted lung includes a considerable proportion of lymphoid tissue which can cause GvHD in the early postoperative months when peripheral blood and BAL lymphocyte chimaerism is demonstrable (Wood H, Higenbottam TW, Joysey V, Wallwork J, personal communication). In Papworth patients skin rashes, diarrhoea, bone marrow failure and increased susceptibility to infection have been seen, the former with consistent histology. Colonic perforation in two patients may have been related to GvHD but lack of specific histological findings for this condition and

Figure 16 Total inactive obliterative bronchiolitis, grade C2b. No acute rejection changes present (elastic van Gieson).

Figure 17 Small pulmonary artery intimal proliferation as a manifestation of chronic pulmonary vascular rejection, grade D. Elsewhere there was evidence of A1b and C2b (elastic van Gieson).

Figure 18 CMV pneumonitis with numerous large cells showing both nuclear and cytoplasmic inclusions (haematoxylin and eosin).

concomitant CMV infection render precise diagnosis difficult.

Long term complications

CORONARY OCCLUSIVE DISEASE

This is the main cause of death after the first year following cardiac allograft transplantation.² The aetiology is controversial with repeated episodes of rejection, allograft injury due to suboptimal preservation, and viral infections being possible factors.^{40 41} Proximal disease in major epicardial coronary arteries is indistinguishable from conventional atherosclerosis. The concentric obliteration of smaller epicardial (fig 21) and intramyocardial arteries, however, is a form of coronary vascular disease that is restricted to cardiac allografts, with failure of collaterals to develop an important predisposing factor for ischaemia.

Long term survival after heart and lung transplantation is at present mainly limited by obliterative bronchiolitis¹⁰ which develops earlier than clinically important coronary occlusive disease. Two Papworth patients with end stage obliterative bronchiolitis have died of myocardial infarction due to coronary occlusive disease. As strategies are developed to limit obliterative bronchiolitis, coronary occlusive disease may become more important. There are no data available on the relative prevalence of coronary occlusive disease in heart and lung transplantation compared with heart transplantation but comparison of potential factors in its development, such as graft preservation, level of immunosuppression, and frequency of rejection and viral infection may provide useful information about pathogenesis.

CARDIOMEGALY

This is a conspicuous feature of long term cardiac allograft recipients and is a potential contributory factor in sudden death, particularly in association with coronary occlusive disease. Cardiac muscle hypertrophy seems to develop before clinically important coronary occlusive disease, suggesting a true myopathic process rather than a hypertrophic response to ischaemia.⁴²

OBLITERATIVE BRONCHIOLITIS

Chronic pulmonary rejection is manifest mainly by obliterative bronchiolitis (figs 15 and 16) which causes progressive loss of pulmonary function.⁴³ Obliterative bronchiolitis is not specific to pulmonary grafts and has many other causes⁴⁴ but there is evidence to link it with severe and frequent episodes of acute rejection.¹⁰ It is associated with proximal airway damage with bronchiectasis, ulceration, and squamous metaplasia.⁴⁵ There may also be evidence of obstructive pneumonitis in trans-bronchial lung biopsy specimens. Obliterative bronchiolitis is occasionally seen in active CMV pneumonitis,⁴⁶ a risk factor in its development.¹⁰ Heart and lung transplantation recipients often have active panbronchiolitis in their biopsy specimens that is not associated with perivascular infiltrates of rejection; these may be of infective aetiology.⁴⁷ Recurrent episodes of bronchiolitis of whatever cause may

Figure 19 Invasive aspergillosis in the lung characterised by foci of necrosis in which hyphal fragments are identifiable (haematoxylin and eosin with Grocott methenamine silver, inset).

Figure 20 *Toxoplasma* cyst in routine follow up endomyocardial biopsy specimen at seven years. In other areas there were changes indistinguishable from grade 3A rejection, unlikely this long after transplant. This man had developed and recovered from primary toxoplasmosis soon after transplantation as a result of mismatching (haematoxylin and eosin).

result in scarring and obliteration. Other potential causes include bronchial arterial and lymphatic interruption, denervation, and aspiration.

Occlusive pulmonary vascular disease (fig 17) analogous to coronary occlusive disease is commonly seen in allografts with obliterative bronchiolitis but is not always present. A similar discordance exists between pulmonary and coronary artery occlusive disease in patients with combined grafts despite the significant incidence of acute cardiac rejection.⁴⁸

MALIGNANCY

There is an increased incidence of malignancy in recipients of solid organ transplant that is

associated with necessary immunosuppressive treatment.⁴⁹ Squamous cell carcinomas and non-Hodgkin's lymphomas are the most frequent malignancies seen in heart transplantation.⁵⁰ Heart and lung transplantation recipients seem to be particularly prone to lymphoproliferative disorders,⁵¹ mainly of B cell type and thought to be related to Epstein-Barr virus, which probably reflects the relatively high level of immunosuppression compared with other organ grafts. There have been five cases of lymphoproliferative disorder in 94 heart and lung and single lung transplant recipients at Papworth. These were diagnosed by a combination of nodular infiltrates on chest x ray picture, computed tomogram, and a sheet-like lymphoid infiltrate in transbronchial lung biopsy specimens (fig 22), features reflecting the tendency of this disease to affect the grafted organ. In four cases the condition resolved clinically and radiologically after a reduction in immunosuppression and ganciclovir treatment and in a further case the process was progressive and caused death.

RECURRENT DISEASE

The possibility of the primary disease recurring in the graft should be considered, the most frequent example of this in Papworth patients being the recurrence of sarcoidosis in the lungs of three heart and lung transplantation recipients.

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Figure 21 Coronary occlusive disease in small epicardial coronary artery. Coronary arterial tree formalin fixed at physiological pressure (haematoxylin and eosin).

Figure 22 Lymphoproliferative disease in heart and lung transplantation, diagnosed by transbronchial lung biopsy. Sheet-like infiltrate of mixed lymphoid population (haematoxylin and eosin).

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