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RAPID COMMUNICATION

Depletion of Bronchus-Associated Lymphoid Tissue Associated with Lung Allograft Rejection

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Pulmonary infections remain one of the major complications of lung transplantation. The bronchus-associated lymphoid tissue (BALT) forms a local immune system that normally protects the lung from infection. The effects of lung transplantation and lung allograft rejection on the BALT were examined using immunoperoxidase techniques. The BALT was evaluated by quantifying the number of immunoglobulin-bearing plasma cells in the lamina propria of sections of trachea and mainstem bronchus. Sections of donor mainstem bronchus from 2 patients with allograft rejection were compared with sections of native trachea from these same patients, and with sections of mainstem bronchus from 2 transplanted lungs without rejection and 20 controls. Lung allografts from the 2 patients with rejection had a marked depletion of submucosal IgA-bearing and IgG-bearing plasma cells. Two sets of transplanted lungs without evidence of rejection showed only a mild reduction of the BALT. The depletion of BALT associated with allograft rejection may contribute to the increased incidence of pulmonary infections seen in these patients. (Am J Pathol 1988, 132: 6–11)

LUNGS DIFFER FROM most transplanted organs in at least 2 ways.[1] First, the lungs must directly interact with the numerous pathogens present in the external environment. Second, the lungs contain a large number of lymphocytes organized into a mucosal immune system. This system, called the bronchus-associated lymphoid tissue (BALT), consists of numerous submucosal lymphoid nodules and plasma cells scattered throughout the tracheobronchial tree.[2–4] These plasma cells synthesize antibodies that are transported to the respiratory mucosa, bathing the entire respiratory tract in secretory immunoglobulins.[5–9]

The BALT, the primary defense mechanism of the lung against environmental pathogens, appears to be a target of lung rejection.[10–12] Prop et al,[10–12] using ^{51}Cr-labeled lymphocytes in experimental lung allograft rejection in the rat, demonstrated that labeled recipient lymphocytes homed into the graft BALT. Once present in the graft BALT, these lymphocytes responded to the graft's lymphocytes as evidenced by their transformation into immunoblasts.

In a previous study[13] of graft-*vs.*-host disease in bone marrow transplantation it has been demonstrated that the gut-associated lymphoid tissue (GALT) can be evaluated by quantifying the number of immunoglobulin-bearing plasma cells in the lamina propria. The authors wished to evaluate the BALT in human lung transplantation and rejection in a similar manner. The relative numbers of submucosal IgA-, IgG-, and IgM-bearing plasma cells were exam-

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ined in sections of trachea and mainstem bronchus. Sections of donor mainstem bronchus from 2 patients with allograft rejection were compared with sections of native trachea from these same patients, and with sections of native trachea and donor mainstem bronchus from 2 transplanted lungs without rejection. Twenty nontransplanted controls were also examined. A deficiency of BALT was found to be associated with lung allograft rejection. This deficiency may contribute to the increased susceptibility to pulmonary infection seen with lung allograft rejection.

Materials and Methods

Between November 1983 and August 1987, 9 patients received a total of 10 combined heart–lung transplants at The Johns Hopkins Hospital. Each of these combined heart–lung transplants has been assigned a unique number (HL-1 to HL-10). Four of these patients have died, including 1 who underwent 2 heart–lung transplants (HL-6 and HL-9). These recipients have been reported elsewhere.[14] Brief clinical summaries are given in Table 1. Lung rejection was defined by histologic evidence of bronchiolitis obliterans occurring in the presence of cough, fever, progressive dyspnea, and diffuse reticulonodular infiltrates on chest radiograph.[15–17] Two sets of lungs were from patients who met these criteria (HL-4, HL-6), and 3 sets were from patients with no signs of rejection (HL-1, HL-3, HL-9). Tissue including native trachea and donor mainstem bronchi was available for study from all except HL-1. Twenty control cases were chosen from consecutive nonneonatal patients who underwent autopsy at The Johns Hopkins Hospital in whom examination of the lung was permitted in the autopsy consent, and in which sections of mainstem bronchus with intact epithelium were available for study.

Immunoperoxidase Technique

Representative sections of native trachea and of donor mainstem bronchi were used. Tissue was fixed in neutral buffered 4% formaldehyde solution, processed, and embedded in paraffin. Six-micron sections were washed briefly with phosphate-buffered-saline (PBS) containing bovine serum albumin (0.2%), and incubated with peroxidase conjugated goat anti-human IgA (1:20), IgM (1:30), or IgG (1:40) (all from Tago, Inc., Burlingame, CA). The cells were identified with diaminobenzidine (0.6%) and hydrogen peroxidase (0.01%) and the stain intensified with copper sulfate.[13] The slides were counterstained with Giemsa stain, dehydrated, and coverslipped. The sections of

Table 1—Clinical Features of Heart–Lung Transplant Recipients

Transplant number	Age	Sex	Primary lung diseases	Immunosuppression	Posttransplant symptoms and physical exam	Posttransplant chest radiograph	Posttransplant pulmonary function tests	Pulmonary infections	Length of graft survival	Pulmonary pathology
HL-3	38	M	Eisenmenger's syndrome secondary to aortico-pulmonary window	CsA AZA Steroids	Fever	Diffuse pulmonary infiltrates	Moderate restrictive defect	*Staph. epidermidis* and *Streptococcus viridans*	19 months	Broncho-pneumonia
HL-4	26	F	Eisenmenger's syndrome secondary to ventricular septal defect	CsA AZA Steroids	Respiratory failure	Diffuse pulmonary infiltrates	Moderate obstructive defect	*Pneumocystis carinii*, and *cytomegalovirus*	6 months	BO, perivascular and peribronchial mononuclear infiltrates
HL-6	40	F	Primary pulmonary hypertension	CsA AZA Steroids	Fever	Diffuse pulmonary infiltrates	Severe obstructive and ventilatory defects	*Pneumocystis carinii*, *Candida albicans*, and disseminated *cytomegalovirus*	14 months	BO, interstitial pneumonitis and fibrosis, severe bronchiectasis, broncho-pneumonia
HL-9	40	F	Bronchiolitis obliterans	AZA Steroids	Hypotension	Diffuse pulmonary edema	None	*Pseudomonas aeruginosa*	12 hours	Donor aspiration pneumonia

CsA, Cyclosporine.
AZA, Azathioprine.
Steroids, methylprednisolone.
BO, bronchiolitis obliterans.

mainstem bronchus had an average diameter of 1.0 cm.

Data Collection

Sections of mainstem bronchus and native trachea were independently ranked by 2 authors (RHH and WEB) with respect to density of IgA-, IgM-, and IgG-containing plasma cells in the submucosa. The ranking was performed without knowledge of the origin of the section. One section was evaluated from each of the 20 controls, 2 native tracheas, 2 allografts with rejection, and 2 allografts without rejection. These 26 sections were ranked from 1 to 26 (least to greatest). A high degree of correlation was found between the 2 observers (IgA, r = 0.85, $P < 0.001$; IgG, r = 0.87, $P < 0.001$; IgM, r = 0.87, $P < 0.001$), supporting the validity of this semiquantitative method in assessing plasma cell density.

While the ranking of cases was useful in identifying those cases in which the plasma cells were depleted, the density of plasma cell staining in the different cases was not a continuum, but in fact the cases tended to group themselves into distinct grades. Therefore, each ranked series was subdivided into 5 grades, 0 to 4+, (corresponding to absence to very high density of plasma cells), based on similarities between numbers of each grade. Each grade did not necessarily contain equal numbers of cases. For example, 7 sections were given a grade of 4+ for IgA staining, while only 1 section was given a grade of 4+ for IgM staining. The grade of the median number of each group is presented (Table 2).

Table 2—Relative Number of Submucosal Immunoglobulin-Bearing Plasma Cells

Case	IgA	IgG	IgM
Transplanted lungs with rejection			
HL-4	1+ (1.5)	1+ (7)	2+ (11.5)
HL-6	1+ (3.5)	1+ (1)	0+ (2.5)
Transplanted lungs without rejection			
HL-3	3+ (14.5)	3+ (15)	2+ (16.5)
HL-9	3+ (11.5)	3+ (17.5)	1+ (11)
Native tracheas			
HL-4	NA	NA	NA
HL-6 and HL-9	2+ (8.5)	4+ (20.5)	3+ (20.0)
HL-3	3+ (17)	3+ (14.5)	3+ (20.0)
Controls			
Mean of 20	3+ (14.7)	2+ (13.6)	2+ (13.5)

0–4+, median quantitative grade (least to greatest).
( ), average of two independent rankings 1–26 (least to highest).
NA, not available for quantification (because of severe epithelial ulceration).

Results

Two of the 5 lungs were from patients who met the criteria for lung rejection (HL-4 and HL-6) and 3 were from patients who had no clinical or histologic evidence of lung rejection (HL-1, HL-3, HL-9). The mainstem bronchi were not available for study from HL-1, therefore this case will not be considered in the remainder of the study. Bronchial immunity could not be evaluated in the native trachea in HL-4 because of severe epithelial ulceration secondary to long-term mechanical ventilation. For purposes of this study the lungs from HL-9 are considered transplanted lungs without rejection, however, it should be noted that this patient died 12 hours after surgery and may not be representative of longer surviving allografts.

Primary diseases among the twenty control patients included: leukemia (5 cases), carcinoma (5 cases), cardiomyopathy (2 cases), acquired immune deficiency syndrome (AIDS, 2 cases), and 1 case each of myocardial infarction, lymphoma, plasmacytoma, ulcerative colitis, aortic aneurysm, and trauma. One of the 5 carcinoma cases was a lung primary.

Morphology

The histologic appearance of the 2 lungs with rejection (HL-4 and HL-6) was similar. The distal lung parenchyma was remarkable for striking bronchiolitis obliterans and perivascular and peribronchial chronic inflammation. Interstitial fibrosis and striking bronchiectasis were also present in HL-6. The mainstem bronchi from the 2 lungs showed focal ulceration and squamous metaplasia of the epithelium, marked submucosal gland dropout, and a diffuse depletion of submucosal plasma cells. Epithelial ulceration second to long-term mechanical ventilation was present in the native trachea of HL-4, while the native trachea of HL-6 was histologically unremarkable.

Bronchiolitis obliterans was not identified in the 2 transplanted lungs without rejection (HL-3 and HL-9). Instead, these lungs were remarkable for extensive bronchopneumonia. The mainstem bronchi from these 2 lungs showed an unremarkable epithelium and numerous plasma cells in the submucosa.

Semiquantitative Assessment of Bronchial Immunity

The relative density of IgA-, IgG-, and IgM-bearing plasma cells in the submucosa was estimated by ranking the sections from 1 to 26 (Table 2). No decrease in plasma cells was seen in the 2 transplanted sets of lungs without evidence of rejection. The 2 patients with lung rejection showed a marked decrease in the relative number of IgA-, and IgG-bearing plasma cells (Figure 1A) relative to the native trachea (Figure 1B) and controls. One of the 2 patients with lung rejection (HL-6) also showed a decrease in the number of IgM-bearing plasma cells. The 2 patients with AIDS had average rankings (15 and 23).

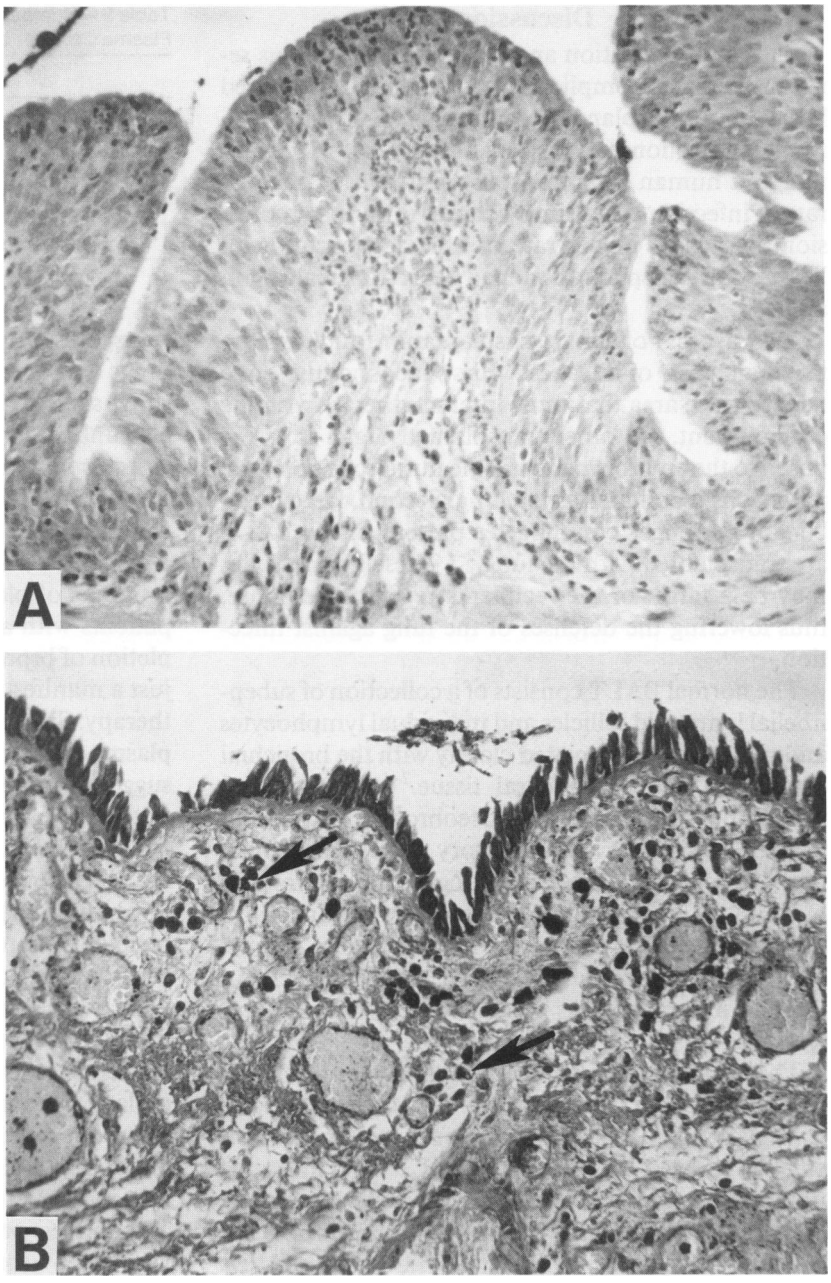

Figure 1—HL-6. Immunoperoxidase staining for IgA demonstrates a marked reduction in the number of IgA-bearing plasma cells (arrows) in the donor mainstem bronchus (**A**, ×225) relative to the native trachea (**B**, ×225). Plasma cells are virtually absence in the donor mainstem bronchus, while all of the darkly staining submucosal cells (arrows) in the native trachea are plasma cells. Note also the associated depletion in secretory immunoglobulins as manifested by reduced staining for IgA in the surface epithelium.

Quantification of Submucosal Plasma Cells

The total number of plasma cells and the number of plasma cells bearing IgA, IgG, and IgM were determined in 2 cases (HL-3 and HL-6). Lung rejection (HL-6) was associated with a marked reduction in all plasma cells in the transplanted mainstem bronchus compared to the native trachea (Table 3). In contrast, equal numbers of submucosal plasma cells were present in the donor mainstem bronchus and native trachea of the transplanted patient without rejection (HL-3).

Pulmonary Infections

While all of the heart–lung transplant recipients had pulmonary infections, the 2 with evidence of rejection (HL-4, HL-6) had pulmonary infections usually associated with an immunocompromised state. *Pneumocystis carinii* and Cytomegalovirus were both isolated from HL-4 and HL-6, and HL-6 also had a *Candida albicans* pulmonary infection. The bacterial pneumonia in 2 of the 3 (HL-1 and HL-9) transplanted lungs without evidence of rejection were secondary to donor aspiration before transplantation.

Discussion

Pulmonary rejection and pulmonary infections remain the major complications of lung and combined heart–lung transplants. Recurrent and unusual pulmonary infections have been a problem with both animal and human lung transplants.[15,16,18–22] This high rate of infection is not simply due to immunosuppression, because a higher rate of infection occurs with heart–lung transplantation than heart transplantation alone.[22]

Two aspects of lung transplantation may explain this higher rate of infection.[1] First, the lungs present a large surface area that must interact directly with the environment. No other transplanted organ is as exposed to the environment and the numerous potential pathogens it contains as the lung. Second, large numbers of passenger lymphocytes present in the BALT are transplanted with the lung.[2–9] These lymphocytes may be a target of the recipients rejection response, thus lowering the defenses of the lung against infection.

The normal BALT consists of a collection of subepithelial lymphoid follicles and individual lymphocytes and plasma cells associated closely with the bronchial epithelium and submucosal tissue.[2–4] It is present throughout most of the tracheobronchial tree and provides resistance to respiratory infection through the local production of immunoglobulins, primarily IgA and IgG.[5–9] Locally produced IgA and IgG have been shown to be important in the defense against both viral and bacterial pathogens, and deficiencies in these immunoglobulins predispose to pulmonary infections.[23,24]

The BALT, because of its immunogenicity, is a target in lung allograft rejection. In a rat model of lung transplantation, it has been demonstrated that recipient lymphocytes infiltrate the BALT early in rejection, resulting in the histologic equivalent of a mixed lymphocyte reaction.[10–12] This infiltration of the BALT by recipient lymphocytes leads to the destruction of the donor lymphocytes in the BALT. HLA typing of lymphocytes obtained by bronchoalveolar lavage has demonstrated that donor lymphocytes are gradually eliminated from the donor lung.[22,25,26]

The purpose of this study was to evaluate the BALT in human lung transplantation and rejection. Mucosal immunity was evaluated using morphologic and immunoperoxidase techniques have been applied successfully to the study of intestinal mucosal immunity.[13] The study found that the cellularity of the BALT was markedly decreased in the mainstem bronchi of lungs from patients with allograft rejection as compared with the native tracheas of these patients and mainstem bronchi from controls. This deficiency in the BALT was not simply a manifestation of the transplantation procedure itself, as the 2 transplanted lungs without evidence of allograft rejection did not show such a dramatic deficiency of the BALT. It is also unlikely that the deficiency in the BALT was due to transplant ischemia, as the 2 lungs that showed a loss of BALT had shorter ischemic times (67 and 45 minutes) than the 2 that did not show a deficiency of BALT (90 and 269 minutes). The finding of normal numbers of plasma cells in the native tracheas from patients with allograft rejection suggests that the depletion of bronchial immunity in these patients is not just a manifestation of high dose immunosuppressive therapy. Similarly, the presence of normal numbers of plasma cells in the allograft without rejection (HL-3) suggests that the depletion of bronchial immunity is not simply a chronic sequela of transplantation.

Table 3—Number of Submucosal Immunoglobulin-Bearing Plasma Cells per cm of Epithelium

	Total	IgA	IgG	IgM
Transplanted lung with rejection (HL-6)				
Native trachea	267	73	146	48
Donor mainstem bronchus	17	13	3	1
Transplanted lung without rejection (HL-3)				
Native trachea	334	149	120	65
Donor mainstem bronchus	305	121	137	47

The deficiency of BALT in lung allograft rejection may be the result of a combination of rejection of donor lymphocytes by the recipient and an inability of recipient lymphocytes to establish themselves in the environment of the donor lung. As discussed previously, the rejection of donor lymphocytes in the BALT has been demonstrated in rat transplantation models.[10–12] The inability of recipient lymphocytes to replace the donor lymphocytes in the BALT may result from an inadequate microenvironment. For example, epithelial injury could interfere with proper homing. Alternately, persistent donor macrophages and antigen-presenting cells could establish an unfavorable environment for incompatible recipient lymphocytes. HLA typing of bronchoalveolar lavage cells has demonstrated a persistence of donor macrophages relative to lymphocytes following lung transplantation.[25,26]

A deficiency of intestinal immunity has been demonstrated in bone marrow recipients with graft-*vs.*host disease.[13] This deficiency of intestinal immunity is believed to predispose the patients with graft-*vs.*-host disease to infection from intestinal organisms, and therefore account for the increased incidence of intestinal infections seen in these patients.[27] By analogy, the authors believe that the deficiency in mucosal immunity in lung allograft rejection may account for the increased incidence of pulmonary infections seen in these patients.

Theoretically, these patients could have graft-*vs.*-host disease, due to either engraftment of the BALT, blood transfusions, or autoimmune phenomenon.[28] No evidence for this was found, however, in the sections of skin, tongue, liver, and gut examined from these patients.

Finally, the finding of a deficiency in the BALT in lung allograft rejection suggests a potential new mode of infection prophylaxis. Immunoglobulin prophylaxis reduces the number of infections in patients with IgA and IgG deficiency.[8,23] One might speculate that immunoglobulin prophylaxis may reduce the incidence of pulmonary infections in lung allograft recipients with a deficiency of the mucosal immune system secondary to rejection.

References

1. Burke CM, Glanville AR, Theodore J, Robin ED: Lung immunogenicity, rejection, and obliterative bronchiolitis. Chest 1987, 92:547–549
2. Kaltreider HB: Expression of immune mechanisms in the lung. Am Rev Resp Dis 1976, 113:347–379
3. Bienenstock J, Johnston N, Perey DYE: Bronchial lymphoid tissue. I. Morphologic characteristics. Lab Invest 1973, 28:686–692
4. Bienenstock J, Johnston N, Perey DYE: Bronchial lymphoid tissue. II. Functional characteristics. Lab Invest 1973, 28:693–698
5. Tomasi TB Jr, DeCoteau E: Mucosal antibodies in respiratory and gastrointestinal disease. Adv Int Med 1970, 16:401–425
6. DeCoteau WE: The role of secretory IgA in defense of the distal lung. Ann NY Acad Sci 1974, 211:214–219
7. Green GM, Jakab GJ, Low RB, Davis GS: Defense mechanisms of the respiratory membrane. Am Rev Resp Dis 1977, 115:479–514
8. Bienenstock J, Befus AD: Mucosal immunology. Immunology 1980, 41:249–270
9. Reynolds HY: Lung immunology and its contribution to the immunopathogenesis of certain respiratory diseases. J Allergy Clin Immunol 1986, 78:833–847
10. Prop J, Wildevuur CRH, Nieuwenhuis P: Lung allograft rejection in the rat. II. Specific immunological properties of lung grafts. Transplantation 1985, 40: 126–131
11. Prop J, Wildevuur CRH, Nieuwenhuis P: Lung allograft rejection in the rat. III. Corresponding morphological rejection phases in various rat strain combinations. Transplantation 1985, 40:132–136
12. Prop J, Tazelaar HD, Billingham ME: Rejection of combined heart–lung transplants in rats: Function and pathology. Am J Pathol 1987, 127:97–105
13. Beschorner WE, Yardley JH, Tutschka PJ, Santos GW: Deficiency of intestinal immunity with graft-vs.-host disease in humans. J Infect Dis 1981, 144:38–46
14. Hruban RH, Beschorner WE, Gupta PK, Baumgartner WA, Achuff SC, Traill TA, Reitz BA, Hutchins GM: Diagnosis of rejection of lung allografts. N Engl J Med, 1988, 318:1129
15. Burke CM, Theodore J, Dawkins KD, Yousem SA, Blank N, Billingham ME, Van Kessel A, Jamieson SW, Oyer PE, Baldwin JC, Stinson EB, Shumway NE, Robin ED: Post-transplant obliterative bronchiolitis and other late lung sequelae in human heart–lung transplantation. Chest 1984, 86:824–829
16. McGregor CGA, Jamieson SW, Baldwin JC, Burke CM, Dawkins KD, Stinson EB, Oyer PE, Billingham ME, Zusman DR, Reitz BA, Morris A, Yousem S, Hunt SA, Shumway NE: Combined heart–lung transplantation for end-stage Eisenmenger's syndrome. J Thoracic Cardiovasc Surg 1986, 91:443–450
17. Glanville AR, Baldwin JC, Burke CM, Theodore J, Robin ED: Obliterative bronchiolitis after heart–lung transplantation: Apparent arrest by augmented immunosuppression. Ann Intern Med 1987, 107:300–304
18. Wildevuur CRH, Benfield JR: A review of 23 human lung transplantations by 20 surgeons. Ann Thorac Surg 1970, 9:489–515
19. Byers JM III, Sabanayagam P, Baker RR, Hutchins GM: Pathologic changes in baboon lung allografts: Comparison of two immunosuppression regimes. Ann Surg 1973, 178:754–760
20. Baker RR, Sabanayagam P, Zarins CK, James AE, Hutchins GM, Byers JM III, Natarajan TK, Lee JM: Functional and morphologic changes after lung allografting in baboons. Surgery Gynecol Obstet 1973, 137: 650–654
21. Jamieson SW: Recent developments in heart and heart–lung transplantation. Transplant Proc 1985, 17: 199–203
22. Griffith BP, Hardesty RL, Trento A, Paradis IL, Duquesnoy RJ, Zeevi A, Dauber JH, Dummer JS, Thompson ME, Gryzan S, Bahnson HT: Heart–lung transplantation: Lessons learned and future hopes. Ann Thorac Surg 1987, 43:6–16
23. Bjorkander J, Bake B, Oxelius V-A, Hanson LA: Impaired lung function in patients with IgA deficiency and low levels of IgG2 or Ig3. N Engl J Med 1985, 313:720–724
24. Umetsu DT, Ambrosino DM, Quinti I, Siber GR, Geha RS: Recurrent sinopulmonary infection and impaired antibody response to bacterial capsular polysaccharide antigen in children with selective IgG-subclass deficiency. N Engl J Med 1985, 313:1247–1251
25. Fung JJ, Zeevi A, Kaufman C, Paradis IL, Dauber JH, Hardesty RL, Griffith B, Duquesnoy RJ: Interactions between bronchoalveolar lymphocytes and macrophages in heart–lung transplant recipients. Hum Immunol 1985, 14:287–294
26. Paradis IL, Marrari M, Zeevi A, Duquesnoy RJ, Griffith BP, Hardesty RL, Dauber JH: HLA phenotype of lung lavage cells following heart–lung transplantation. Heart Transplant 1985, 4:422–425
27. Yolken RH, Bishop CA, Townsend TR, Bolyard EA, Bartlett J, Santos GW, Saral R: Infectious gastroenteritis in bone-marrow transplant recipients. N Engl J Med 1982, 306:1009–1012
28. Sliman GA, Beschorner WE, Baughman K, Hutchins GM, Borkon MA, Baumgartner WA: Graft-versus-host disease in a heart allograft recipient: A possible autoimmune phenomenon. Transplantation 1988, 45:253–256

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