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IDENTIFICATION AND INHIBITION OF SPECIFIC PATHWAYS LEADING TO TRANSPLANT OBLITERATIVE BRONCHIOLITIS -AN EXPERIMENTAL APPROACH IN THE RAT

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Academic Dissertation

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ORIGINAL PUBLICATIONS

This thesis is based on the following publications referred to in the text by their Roman numerals:

I Tikkanen JM, Lemström KB, Koskinen PK. Blockade of CD28/B7-2 costimulation inhibits experimental obliterative bronchiolitis in rat tracheal allografts: suppression of helper T cell type1-dominated immune response. Am J Respir Crit Care Med 2002; 165: 724-729.

II Tikkanen JM, Koskinen PK, Lemström KB. Role of endogenous endothelin-1 in transplant obliterative airway disease in the rat. Submitted.

III Tikkanen JM, Kallio EA, Bruggeman C, Koskinen PK, Lemström KB. Prevention of cytomegalovirus infection-enhanced experimental obliterative bronchiolitis by antiviral prophylaxis or immunosuppression in rat tracheal allografts. Am J Respir Crit Care Med 2001; 164: 672-679.

IV Tikkanen JM, Krebs R, Bruggeman C, Lemström KB, Koskinen PK. Platelet-derived growth factor regulates cytomegalovirus infection-enhanced obliterative bronchiolitis in rat tracheal allografts. Transplantation 2003; in press.

ABBREVIATIONS

APC	antigen-presenting cell
AZA	azathioprine
BALF	bronchoalveolar lavage fluid
bFGF	basic fibroblast growth factor
BOS	bronchiolitis obliterans syndrome
BrdU	bromodeoxyuridine
CMV	cytomegalovirus
CTLA	cytotoxic T lymphocyte-associated antigen
CD	cluster of differentiation
CF	cystic fibrosis
COPD	chronic obstructive pulmonary disease
CsA	cyclosporine A
DHPG	ganciclovir (9-(1,3-dihydroxy-2-propoxymethyl)guanine)
DSG	15-deoxyspergualin
EGF	epidermal growth factor
ET-1	endothelin-1
ET-R	endothelin receptor
FEV_1	forced expiratory volume in one second
HIS	hyperimmune serum
HLA	human leukocyte antigen
IFN-γ	interferon-γ
IGF-1	insulin-like growth factor-1
IL	interleukin
IPF	idiopathic pulmonary fibrosis
ISHLT	International Society for Heart and Lung Transplantation
MHC	major histocompatibility complex
MMF	mycophenolate mofetil
NO	nitric oxide
OB	obliterative bronchiolitis
PDGF	platelet-derived growth factor
PDGF-R	platelet-derived growth factor receptor
PFU	plaque-forming unit
RCMV	rat cytomegalovirus
SMC	smooth muscle cell
TBB	transbronchial biopsy
TCR	T cell receptor
TGF-β	transforming growth factor-β
Th cell	T helper cell
TNF-α	tumour necrosis factor-α
VEGF	vascular endothelial growth factor

ABSTRACT

Lung and heart-lung transplantation are the treatments of choice for many end-stage pulmonary diseases. Although short-term survival has increased along with advances in the field of transplantation, the incidence of bronchiolitis obliterans syndrome (BOS) has not decreased and BOS remains the leading cause of late graft loss. The two most important risk factors for the development of BOS are acute rejection and cytomegalovirus (CMV) infection. No specific treatment for BOS is available at the present. This study was set out to identify potential targets of intervention in the development of obliterative bronchiolitis (OB), the pathological manifestation of BOS, using an experimental rat tracheal transplantation model.

In this model, the donor trachea is excised and transplanted into the greater omentum of the recipient. In syngeneic grafts, the epithelium undergoes minor damage but recovers thereafter. The tracheal lumen remains completely open and the trachea is lined with normal, mucus-secreting epithelium 30 days after transplantation. On the other hand, in untreated allografts, the epithelium sustains progressive damage leading to nearly total epithelial necrosis 10 days after transplantation. Allografts develop a strong alloimmune response, which is associated with increased expression of cytokines, chemokines, and growth factors, culminating in the development of a fibroproliferative lesion obliterating the tracheal lumen. This lesion closely resembles obliterative changes seen in small bronchioles in man.

This study underlines the importance of the early intragraft alloimmune response. Inhibition of T cell activation by a single dose of human CTLA4Ig, that blocks CD28/B7-mediated T cell costimulation, resulted in attenuation of alloimmune activation and a shift from the Th1- to Th2-like immune response. The decreased alloimmune response led to marked inhibition of epithelial damage and OB development. The results suggest that interfering in the very proximal steps of alloimmune activation with hCTLA4Ig may have a therapeutic role in clinical lung transplantation (I).

Endothelin-1 (ET-1) ligand and receptor expression was upregulated four-fold after tracheal transplantation in allografts compared to syngeneic grafts. Blockade of ET-1 receptor with bosentan resulted in a decrease in alloimmune activation and epithelial necrosis and led to inhibition of smooth muscle cell proliferation and OB development. The results indicate a

biologically significant role for ET-1 in the development of OB and that bosentan, a drug already in clinical use, could be utilized in treating lung transplant recipients (II).

We modified the tracheal allograft model to investigate the pathogenesis of rat CMV (RCMV) infection-enhanced OB. RCMV infection enhanced the early alloimmune response and accelerated OB development. RCMV infection-enhanced OB was associated with increased Th1-dominated immune activation, epithelial necrosis, platelet-derived growth factor (PDGF) expression, and smooth muscle cell proliferation. These effects were not related to viral load as only few RCMV-positive mononuclear cells could be detected in the allografts at any time point. Antiviral prophylaxis with ganciclovir or hyperimmune serum negated the deleterious effects of RCMV infection but treatment initiated 5 days after infection failed to do so. High dose cyclosporine A treatment resulted in a similar inhibition of RCMV infection-enhanced OB. CGP 53716, a selective PDGF receptor tyrosine kinase inhibitor, also completely abolished OB in tracheal allografts of RCMV-infected recipients. The results indicate that RCMV infection enhances OB development indirectly by inducing proximal alloimmune activation and that CMV prophylaxis is needed to avert the deleterious effects of CMV infection.

The results of this study suggest that rigorous immunosuppression and CMV prophylaxis are called for in the treatment of lung transplant recipients. If BOS develops despite these measures, antiproliferative agents such as bosentan and imatinib, a newer derivative of CGP 53716 already in clinical use in cancer management, may have beneficial effects on lung transplant recipient outcome.

INTRODUCTION

James Hardy with his surgical team performed the first clinical lung transplantation in 1963 (Hardy et al. 1963). Early results were poor with high postoperative mortality due to surgical complications and acute rejection because of lack of efficient immunosuppressants. It was not until the early 1980's that the advent of cyclosporine A (CsA) together with improvement of surgical techniques, postoperative care, and antimicrobial treatment established lung transplantation as the treatment of choice for many end-stage pulmonary diseases (Higenbottam et al. 1990).

Today, short-term survival after lung transplantation is approximately 73% at one year and 45% at five years (Trulock et al. 2003). After the first postoperative year, bronchiolitis obliterans syndrome (BOS) has emerged as the leading cause of death and 30% of all deaths after one year are attributed to BOS. After eight years, half of the surviving lung transplant recipients have developed BOS (Hertz et al. 2002). BOS is defined clinically as lung allograft deterioration secondary to persistent airflow obstruction in the absence of other conditions that may alter graft function, such as acute rejection, infection, disease recurrence, or anastomotic complication (Estenne et al. 2002). There is no specific treatment for BOS, and prevention of this disorder is the leading challenge in lung transplantation.

Pathologically BOS presents as obliterative bronchiolitis (OB). OB is characterized by peribronchial inflammation, epithelial damage, and obliteration of small and medium-sized bronchioli by fibrous plaques (Yousem et al. 1985, 1996). The aim of this study was to investigate and target specific pathways leading to OB using a rat tracheal allograft model.

REVIEW OF THE LITERATURE

1. Clinical lung transplantation

1.1. Indications

The number of lung transplantations performed per year has reached a plateau because of worldwide shortage of suitable donors and today approximately 1600 transplantations are reported annually (Trulock et al. 2003). Due to shortage of organs, careful allocation of lung grafts is essential. For patients who are considered for lung or heart-lung transplantation, end-stage lung disease despite optimal medical and other organ preserving therapy is mandatory. Also, recipient candidates are evaluated for contraindications for lung transplantation, such as lifetime-limiting multisystemic disorders, active malignancies, significant coronary artery disease or ventricular dysfunction, active extrapulmonary infection, active connective tissue diseases, complicated diabetes mellitus, end-stage renal disease, and smoking during the last 6 months (Harringer and Haverich 2002). Indications for lung transplantation are listed below (Table 1).

Table 1. Indications for lung and heart-lung transplantation

Diagnosis	Single lung	Bilateral lung	Heart-lung
COPD	54.0%	22.0%	4.0%
Idiopathic pulmonary fibrosis	24.0%	9.0%	2.7%
Cystic fibrosis	1.0%	32.0%	15.6%
α1-antitrypsin deficiency	8.6%	9.7%	2.6%
Primary pulmonary hypertension	1.3%	8.0%	24.0%
Sarcoidosis	2.5%	2.5%	1.2%
Bronchiectasis	0.2%	4.3%	0.6%
Congenital heart disease	0.2%	2.2%	32.2%
Lymphangiomyomatosis	0.9%	1.3%	-
Retransplantation	1.6%	1.8%	2.5%
Connective tissue disorder	0.4%	0.5%	-
Cancer	0.1%	0.6%	-
Histiocytosis X	0.2%	0.2%	-
Acquired heart disease	-	-	4.2%
Other	4.6%	4.2%	10.0%

Data from the Registry of the International Society for Heart and Lung Transplantation (ISHLT) (Trulock et al. 2003). COPD, chronic obstructive pulmonary disease

1.2. Survival

Actuarial survival after lung transplantation between 1990 and 2000 is shown in Figure 1. In general, patients with primary pulmonary hypertension, idiopathic pulmonary fibrosis (IPF), and sarcoidosis as their pre-transplant diagnosis show an increase in perioperative mortality. In the long run, recipients with IPF have the poorest outcome while recipients with cystic fibrosis fare better (Trulock et al. 2003). Survival after bilateral transplantation for COPD or α1-antitrypsin deficiency, but not IPF, is increased in comparison to single lung transplantation (Hertz et al. 2002).

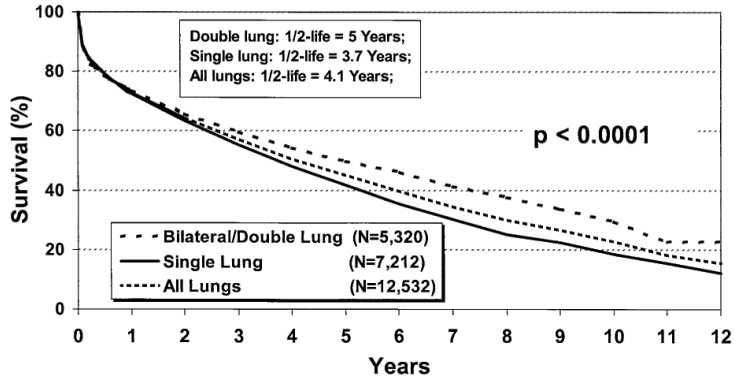

Figure 1. Actuarial survival after lung transplantation. Data from the ISHLT Registry (Trulock et al. 2003).

1.3. Complications and comorbidity

The leading cause of death during the first month after transplantation is non-cytomegalovirus (CMV) infection followed by graft failure. Infection remains the leading cause of death during the first postoperative year but after one year BOS emerges as the most prominent reason for death (Trulock et al. 2003). Problems related to immunosuppression are also important and are summarized in Table 2. Despite all the burdens lung transplant recipients must endure, >80% of survivors at 1, 3, or 5 years have no activity limitations (Trulock et al. 2003).

Table 2. Prevalence of comorbidity in lung transplant recipients at 1 and 5 years

Outcome	Within 1 year	Within 5 years
Hypertension	49.4%	86.5%
Renal dysfunction	25.0%	38.3%
Hyperlipidemia	15.2%	43.4%
Diabetes	18.6%	27.8%
Malignancy	3.9%	13.1%

Data from the ISHLT registry report 2003 (Trulock et al. 2003).

1.4. Immunosuppressive treatment

Triple drug immunosuppression with a calcineurin inhibitor, purine synthesis inhibitor, and corticosteroids forms the cornerstone of immunosuppressive therapy after lung transplantation (Hertz et al. 2002). In addition, half of the patients receive induction therapy before transplantation in the form of anti-lymphocyte/thymocyte globulin or IL-2 receptor antibodies. Table 3 summarizes the most commonly used immunosuppressive drugs and their mechanisms of action.

Table 3. Immunosuppressive drugs used in clinical lung transplantation

Drug	Action	Clinical use
Calcineurin inhibitors:		
Cyclosporine A (CsA)	Inhibition of IL-2 transcription and T cell activation and proliferation	Used together with AZA or MMF in 40% of lung transplant recipients
Tacrolimus (FK506)	Inhibition of IL-2 transcription and T cell activation and proliferation	Used together with AZA or MMF in 40% of lung transplant recipients
Sirolimus	Inhibition of IL-2 receptor signalling and T cell activation and proliferation	Used mainly as rescue therapy if CsA or FK506 treatment fails (5% use)
Purine synthesis inhibitors		
Azathioprine (AZA)	Inhibition of cell proliferation	Used with CsA or FK506 in 40% of lung transplant recipients
Mycophenolate mofetil (MMF)	Inhibition of T and B cell proliferation	Used with CsA or FK506 in 40% of lung transplant recipients
Corticosteroids	A wide range of anti-inflammatory effects by inhibition of RNA, DNA, and protein synthesis	Used by nearly all of lung transplant recipients (only 5% are steroid free at 5 years)
Induction therapies		36% of lung transplant recipients receive any induction therapy
Anti-lymphocyte and anti-thymocyte globulin	Depletion of lymphocytes	13% of lung transplant recipients
IL-2 receptor antibody	Inhibition of T cell activation, T cell depletion	23% of lung transplant recipients

Data modified from the ISHLT registry report 2003 (Trulock et al. 2003).

2. Bronchiolitis obliterans syndrome

2.1. Clinical manifestation and definition

The development of BOS is insidious and it is often diagnosed during routine surveillance. The first symptom of BOS is usually shortness of breath due to narrowing of airways. BOS is defined as lung allograft deterioration secondary to persistent airflow obstruction in the absence of other conditions that may alter graft function. It can be diagnosed without histologic evidence of OB on purely clinical grounds as the patchy distribution of OB makes transbronchial biopsy (TBB) rather insensitive for diagnosis of OB (Kramer et al. 1993). After transplantation, spirometric measurements are performed to assess a baseline that later values can be compared against. The baseline value is the average of the two highest measurements obtained at least three weeks apart. The diagnosis of BOS can be made if there is a persistent >20% decrease in forced expiratory volume in 1 second (FEV_1) without other explaining factors (Cooper et al. 1993). The refined classification for BOS is shown below (Table 4). If histological proof of OB is available, the letter b should be added to the classification (i.e. BOS1a, no histological evidence; BOS1b, biopsy proven OB) (Estenne et al. 2002).

Table 4. Classification of BOS

BOS 0 (no BOS)	FEV_1>90% and FEF_{25-75}>75% of baseline
BOS 0-p (potential BOS)	FEV_1 81-90% and/or FEF_{25-75}<75% of baseline
BOS 1	FEV_1 66-80% of baseline
BOS 2	FEV_1 51-65% of baseline
BOS3	FEV_1 ≤50% of baseline

FEV_1, forced expiratory volume in 1 second; FEF_{25-75}, mid-expiratory flow rate, data from BOS update (Estenne et al. 2002)

In addition to the classification above, surrogate markers for BOS have been studied. Bronchoalveolar lavage fluid (BALF) neutrophilia (DiGiovine et al. 1996, Riise et al. 1999), exhaled nitric oxide (NO) (Fisher et al. 1998), and air trapping on expiratory computed tomography (Worthy et al. 1997, Leung et al. 1998, Lee et al. 2000, Bankier et al. 2001) have been proposed as markers for BOS. Bronchial hyperresponsiveness may precede the onset of BOS (Stanbrook and Kesten 1999). However, these surrogate markers have not been validated well enough to be used as general guidelines for the assessment of BOS and may be too unspecific for BOS (Estenne et al. 2002).

2.2. Risk factors

Very few prospective studies investigating risk factors for BOS have been published and data available result from retrospective analyses or the ISHLT registry. In Table 5, suggested risk factors for BOS are listed.

Table 5. Risk factors for BOS

Risk factor	Comments	References
Immunologic		
Acute rejection	Repeatedly shown to be an independent and strong risk factor for BOS	Scott et al. 1991, Yousem et al. 1991, Bando et al. 1995, Keller et al. 1995, Baudet et al. 1996, Reichenspurner et al. 1996, Kroshus et al. 1997
Histoincompatibility	HLA-mismatches correlate with BOS development	Sundaresan et al. 1998, Chalermskulrat et al. 2003
Panel reactive antibodies	Development of anti-HLA antibodies is associated with BOS	Smith et al. 1998, Jaramillo et al. 1999, Palmer et al. 2002
Non-immunologic		
CMV infection	CMV pneumonia is a risk factor for BOS, CMV seropositivity correlates with BOS, absence of ganciclovir prophylaxis is also a risk factor	Keenan et al. 1991, Bando et al. 1995, 1995b, Keller et al. 1995, Baudet et al. 1996, Reichenspurner et al. 1996, Soghikian et al. 1996, Kroshus et al. 1997, Valantine et al. 2001, Westall et al. 2003
Other infection	Bacterial and fungal infections increase acute rejection and thereby BOS	Duncan et al. 1991, Reichenspurner et al. 1996
Ischemic time	Linear correlation with BOS development	Hosenpud et al. 2001
Recipient weight	Linear correlation with body mass index and BOS development	Hosenpud et al. 2001
Donor age	Linear correlation with BOS development	Hosenpud et al. 2000
Retransplantation	Associated with accelerated development of BOS	Hosenpud et al. 2001

Acute rejection is the best-documented risk factor for the development of BOS. Especially patients with multiple severe acute rejection episodes are prone to develop BOS (Bando et al. 1995, Kroshus et al. 1997, Kanasky et al. 2002). CMV pneumonia is regarded as a risk factor for BOS (Bando et al. 1995, Kroshus et al. 1997) but the role of CMV infection is more controversial. Although there are numerous studies arguing for CMV infection as a risk factor for BOS, reports not linking CMV and BOS also exist (Scott et al. 1991, Ettinger et al. 1993). On the other hand, ganciclovir treatment has been associated with decreased incidence of BOS (Duncan et al. 1994, Soghikian et al. 1996, Speich et al. 1999) and combined ganciclovir and CMV hyperimmune globulin prophylaxis reduced BOS development (Valantine et al.

2001). Another probable risk factor for OB development is lack of medication compliance. Non-compliance has not been studied in lung transplant patients, but it is a major reason for graft loss due to acute rejection after kidney, heart, and liver transplantation and may therefore also be regarded a probable risk factor for BOS in lung transplant recipients (Schweizer et al. 1990). There is considerable variance between different studies concentrating on risk factors of BOS. For instance, the 2001 ISHLT registry identified older donor age and graft ischemia as risk factors for BOS but another study found no association between BOS and donor age or ischemic time (Heng et al. 1998, Hosenpud et al. 2001). Therefore, more studies addressing the subject are required in the future to identify true risk factors of BOS.

2.3. Pathology

Obliterative bronchiolitis is the histopathological manifestation of BOS. The diagnosis of OB requires histological proof by lung biopsy. OB is restricted to membranous and respiratory bronchioles and refers to dense eosinophilic hyaline fibrous plaques in the submucosa of the small airways that partially or totally occlude the airway lumen. The scar tissue may be concentric or eccentric, may be associated with fragmentation and destruction of the smooth muscle wall, and may extend to the peribronchiolar interstitium (Yousem et al. 1996). OB is classified as either active or inactive according to the intensity of mononuclear cell infiltrates in the obliterative lesion. In addition to fibrosis, active OB is associated with epithelial damage and intrabronchiolar and/or peribronchiolar submucosal mononuclear cell infiltrates, while inactive OB is characterized by dense fibrous scarring without cellular infiltrates. The fibrosis of larger airways seems to be an unspecific finding and does not by itself warrant the diagnosis of OB (Yousem et al. 1996) (Figure 2).

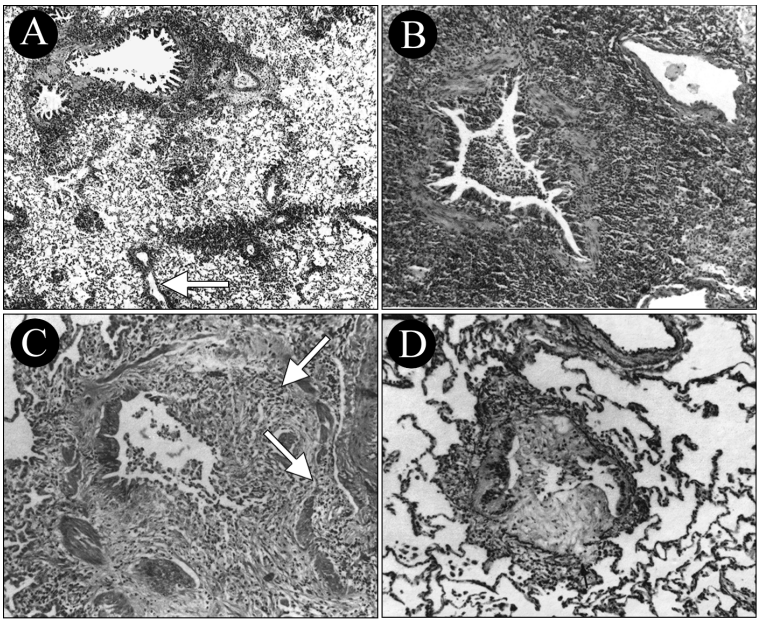

Figure 2. Histological findings seen in acute rejection and OB. (A) Acute lung rejection. A marked inflammatory infiltrate cuffs the pulmonary veins and interlobular septa (arrow). (B) Later in acute rejection, the intense infiltrate envelops bronchioles and arteries, and spills into the alveolar septa resulting in diffuse alveolar damage with necrosis of pneumocytes and their shedding, along with macrophages, into air spaces. (C, D) OB. After epithelial necrosis, fibroblasts, histiocytes, and endothelial cells infiltrate the lumenal debris and form an intralumenal plug of granulation tissue along with disruption of bronchiolar elastica (arrows). Photomicrographs from The transplantation and replacement of thoracic organs by Cooper DKC and Novitzky D, Kluwer Publishing, 1990. Permission for reproduction was received from both publisher and authors.

3. Pathogenesis of OB

3.1. Lung allograft injury and fibroproliferative response

Although the obliterative lesion develops in the time span of months and years, the mechanisms behind the fibroproliferative lesion are initiated already before transplantation. In the donor, brain death leads to increased cytokine expression and the allograft loses its blood supply depriving the lung from oxygen and nutrients (Bittner et al. 1995, Kusaka et al. 2000, Wilhelm et al. 2000). After transplantation, ischemia-reperfusion injury and lytic induction therapy induce release of a variety of proinflammatory cytokines such as TNF-α, IFN-γ, IL-2, IL-8, IL-10, IL-12, and IL-18 both in lung allografts and peripheral blood and cause injury to the epithelial and endothelial structures of the transplanted lung (Serrick et al. 1994, Moore et al. 1995, DiGiovine et al. 1996, Mal et al. 1998, de Perrot et al. 2002, de Perrot et al. 2003).

Prolonged allograft ischemia may therefore play an important role in the development of BOS (Fiser et al. 2002, Hertz et al. 2002). However, experimental studies have shown that OB does not generally develop in the absence of alloimmune activation, suggesting that alloimmune-mediated injury is needed for the development of OB (Koskinen et al. 1997, Neuringer et al. 2002).

Alloimmune activation together with non-alloimmune factors cause epithelial and endothelial cell damage and increase infiltration of the lung allograft by inflammatory cells (Boehler and Estenne 2003). Acute lung injury is followed by a desperate attempt to repair the damaged lung. This reparative process is characterized by expression of factors promoting smooth muscle cell (SMC) growth, mesenchymal cell proliferation, and tissue deposition of extracellular matrix (Martinet et al. 1987, Myers and Katzenstein 1988, Snyder et al. 1991, Aubert et al. 1997). Epithelial damage seems to be the key initiating event in the development of OB as few obliterative changes are observed in experimental models of OB when the allograft lumen is lined with epithelium (Ikonen et al. 2000, King et al. 2002, Neuringer et al. 2002). After the epithelium has succumbed due to alloimmune injury, rapid proliferation and expansion of the fibroproliferative lesion is observed (Neuringer et al. 2002).

3.2. Allograft recognition

After transplantation, immune cells of the recipient recognize donor major histocompatibility complex (MHC) antigens. MHC class I antigens are expressed by most nucleated cells, while MHC class II antigens are expressed mainly by antigen-presenting cells (APC) (Daar et al. 1984, 1984b). Allorecognition is traditionally divided into direct and indirect pathways. In direct allorecognition, recipient T cells recognize foreign MHC antigens on the surface of donor APC (Lechler et al. 1990). Direct allorecognition induces an early strong alloimmune response and is an important mediator of early acute rejection (Gould and Auchincloss 1999). Later on, however, its importance is reduced as donor-derived APC are depleted and direct allorecognition may even inhibit rejection in some cases (Markmann et al. 1992, Campos et al. 1995). In indirect allorecognition, donor antigen is taken up by recipient APC and presented in normal fashion to recipient CD4+ T cells and largely accounts for the alloimmune activation leading to OB. In the transplant setting, indirect allorecognition predominantly leads to a Th1-like cellular immune response that is characterized by IL-2 and

IFN-γ expression in contrast to the Th2-like humoral response associated with IL-4 and IL-10 expression (Boehler et al. 1999).

3.3. T cell activation

Naïve T cells require three distinct signals for activation. The first signal is provided by interaction of the T cell receptor (TCR) with the MHC molecule/antigen complex on the APC. In addition, a second costimulatory signal is required for full T cell activation. Signalling through the TCR leads to prolonged state of T cell anergy in the absence of costimulatory stimulus (Schwartz 1992). The most important costimulatory stimulus results from engagement of CD28 on the T cell surface to its ligands B7-1 and B7-2 on APC (Lesslauer et al. 1986, June et al. 1987, Linsley and Ledbetter 1993). Binding of CD28 to its ligands is blocked by human CTLA4Ig (hCTLA4Ig), a recombinant fusion protein that contains the extracellular domain of hCTLA4 (a gene highly homologous to CD28) fused to a human IgG1 heavy chain (Linsley and Ledbetter 1993, Sayegh et al. 1995). CD40/CD40L interaction forms another means of costimulation and may additionally enhance the expression of B7-1 and B7-2 on APC thus strengthening CD28/B7-mediated costimulation (Barrett et al. 1991, Sayegh and Turka 1998). It has been suggested that CD40/CD40L interaction predominantly induces humoral responses while engagement of CD28 and B7 leads to primarily cellular responses (McArthur and Raulet 1993, Sayegh et al. 1995, Niimi et al. 1998), although opposite findings also exist (Corry et al. 1994). The third signal is mediated by proinflammatory cytokines that stimulate proliferation of immune cells in an autocrine and paracrine fashion and is reviewed in the following chapter (Suthanthiran and Strom 1994).

3.4. Alloimmune response

T cell activation leads to the production of IL-2, a cytokine central for the development of allograft rejection (Kirkman et al. 1985, Diamantstein and Osawa 1986, Kupiec-Weglinski et al. 1987, Sakagami et al. 1989). IL-2 induces T cell growth and differentiation and is required for the differentiation of CD4+ T cells to either Th1- or Th2-type cytokine production (Mosmann et al. 1986, McDyer et al. 2002). Although Th2-mediated humoral responses have been linked to accelerated and vascular rejection (Magro et al. 2002), and overexpression of

IL-10 can cause chronic allograft rejection in the absence of Th1 responses (Furukawa et al. 1999), Th1-like alloimmune activation is thought to play the major role in the pathogenesis of BOS (Kallio et al. 1997, Okada et al. 1998). Th1 cells activate CD8+ T cells to become cytotoxic T lymphocytes that can induce lysis and apoptosis of target cells (Mason 1988). More importantly, Th1 cells secrete TNF-α and IL-12 that activate macrophages. Macrophages are capable of eliminating foreign antigens by phagocytosis and can act as APC (Rosen et al. 1995). In addition, macrophages produce proinflammatory cytokines and chemoattractants such as IL-1, TNF-α, IL-8, and other inflammatory mediators, including leukotrienes, prostaglandins, and free radicals, thus mediating allograft injury (Farver et al. 2000). Macrophages are also the primary source of growth factors promoting SMC proliferation and fibrosis (Mosmann et al. 1986, Martinet et al. 1987).

4. Treatment of OB/BOS

In spite of experimental studies showing that immunosuppression with CsA, tacrolimus or rapamycin successfully inhibits OB development (Fahrni et al. 1997, Koskinen et al. 1997, Adams et al. 2000, Hashimoto et al. 2000), there is no specific treatment for the prevention or inhibition of clinical BOS. Enhanced immunosuppression resulted mainly in slowing down the development of BOS and only in some patients (Iacono et al. 1996, Kesten et al. 1997, Speich et al. 1997). Additionally, anti-lymphocyte antibodies (Date et al. 1998), total body irradiation (Diamond et al. 1998), aerosolised cyclosporine (Iacono et al. 1996), and methotrexate (Dusmet et al. 1996) have been tried with modest success. The latest results of the ISHLT show a slight reduction in the incidence of BOS, which is probably due to improved perioperative care and better immunosuppressive drugs (Hertz et al. 2002). Also, the introduction of newer antiproliferative agents, such as sirolimus, everolimus, and mycophenolate mofetil (MMF) may inhibit OB development (Eisen et al. 2003). However, BOS remains the leading challenge in the field of lung transplantation. Uncovering the pathobiology of OB and BOS is needed for the invention of new specific drugs aiming at the prevention and treatment of BOS.

5. Regulatory molecules in OB

During OB development, a variety of cytokines and growth factors are secreted by epithelial, endothelial, inflammatory, and smooth muscle cells. Data from clinical and experimental

studies facilitate the understanding of the mechanisms surrounding the pathogenesis of OB. Some of the molecular factors associated with OB/BOS are shown in Table 6.

Table 6. Molecular factors associated with OB

Molecule	Observations	References
Cytokines		
IL-1α	IL-1α upregulated during OB, IL-1 inhibition decreases OB	*Smith et al. 2001*, Belperio et al. 2002
IL-2	Blockade of action decreases OB and administration increases OB	*Koskinen et al. 1997, Gu et al. 2000, Neuringer et al. 2000*
IL-4	Slightly upregulated during OB development	*Neuringer et al. 2000*
IL-6	IL-6 is increased in BALF of patients with OB	Yoshida et al. 1993, Scholma et al. 2000
IL-8	IL-8 is increased in BALF of patients with OB	DiGiovine et al. 1996, Zheng et al. 2000, Elssner and Vogelmeier 2001
IL-10	Decreases or increases OB development	*Boehler et al. 1998, Naidu et al. 2002*
IFN-γ	Gene polymorphism associates with OB, inhibition had no effect on OB development	*Smith et al. 2001*, Lu et al. 2002
TNF-α	Inhibition decreases OB	*Smith et al. 2001*
Chemokines		
MCP-1	Expressed in OB, inhibition decreases OB	Belperio et al. 2001
RANTES	Expressed in OB, inhibition decreases OB	*Suga et al. 2000*
CXCR3	Increased in BALF during OB	Belperio et al. 2002b
Growth factors		
PDGF	Upregulated during OB, inhibition prevents OB	Hertz et al. 1992, al-Dossari et al. 1995, *Kallio et al. 1999*
TGF-β	Upregulated during OB	Magnan et al. 1996, El-Gamel et al. 1999, Elssner et al. 2000
bFGF	Upregulated during OB	al-Dossari et al. 1995, *Aris et al. 2002*
ET-1	Upregulated during OB	*Aris et al. 2002*
VEGF	Upregulated during OB, over-expression accelerates and inhibition slows OB development	*Tikkanen et al. 2003*
IGF-1	Upregulated during OB	Charpin et al. 2000, *Aris et al. 2002*
iNOS	Either inhibits or enhances OB development	*Kallio et al. 1997, Minamoto and Pinsky 2002*
Complement	Blocking complement receptor-1 prevented OB	*Kallio et al. 2000*

Abbreviations: RANTES, regulated on activation, normally T cell-expressed and -secreted; CXCR3, chemokine receptor-3. Experimental studies are in italics.

5.1. Endothelin-1

Endothelin was first identified in 1988 by Yanasigawa and coworkers and was quickly recognized as an important factor in the pathophysiology of lung and other tissues (Yanagisawa et al. 1988, Boscoe et al. 2000). The family of endothelins is divided into ET-1, -2, and –3 of which ET-1 is the most common and the only one produced by endothelial cells.

In the human lung, ET-1 immunoreactivity is detectable from several cell types, including airway epithelial cells, submucosal glands, endothelial cells, and type II pneumocytes (Barnes 1994). In the rat lung, ET-1 immunoreactivity is localized mainly to the epithelium with intense staining of goblet and Clara cells (Rozengurt et al. 1990). In man, the effects of ET-1 are relayed through two different receptor subtypes, the ET-A and ET-B receptors (ET-RA, ET-RB). The action and molecular biology of ET-1 in the lung are illustrated in Figure 3.

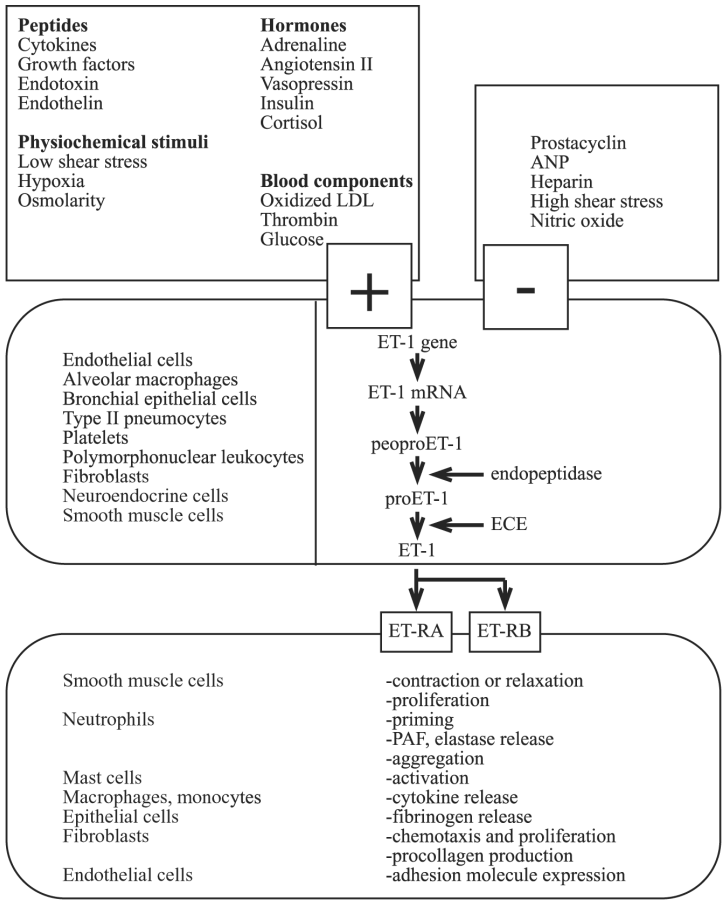

Figure 3. Schematic representation of regulators of endothelin-1 (ET-1) production, cellular source, and biological effects. LDL, low-density lipoprotein; ANP, atrial natriuretic peptide; ECE, endothelin-converting enzyme; PAF, platelet-activating factor. Data from Teder and Noble 2000. The figure is reproduced with permission form publisher and author.

After transplantation, upregulation of ET-1 expression is induced by allograft ischemia (Schersten et al. 1994, Aarnio et al. 1996, Jeppsson et al. 1998). Acute rejection also increases ET-1 levels in bronchoalveloar lavage fluid of pig lung allograft recipients (Aarnio et al. 1998). Furthermore, ET-1 mRNA expression is increased during the development of

experimental OB in the rat (Aris et al. 2002). However, very little is known of the biological role and significance of ET-1 and its receptors in OB development.

5.2. Platelet-derived growth factor

PDGF is a polypeptide growth factor originally purified from platelets (Antoniades et al. 1979). Four different PDGF genes have been identified so far: the PDGF-A, -B, -C, and -D. PDGF-A and -B form disulfide-bonded hetero- or homodimers (PDGF-AA, -AB, and –BB) while PDGF-C and -D genes form only homodimers PDGF-CC and –DD (Heldin et al. 2002). PDGF ligands act through two protein tyrosine kinase receptors, the PDGFR-α (PDGF-A, -B, -C) and -Rβ (PDGF-B and -D) (Heldin et al. 2002). The action and molecular biology of PDGF in the lung is summarized in Figure 4.

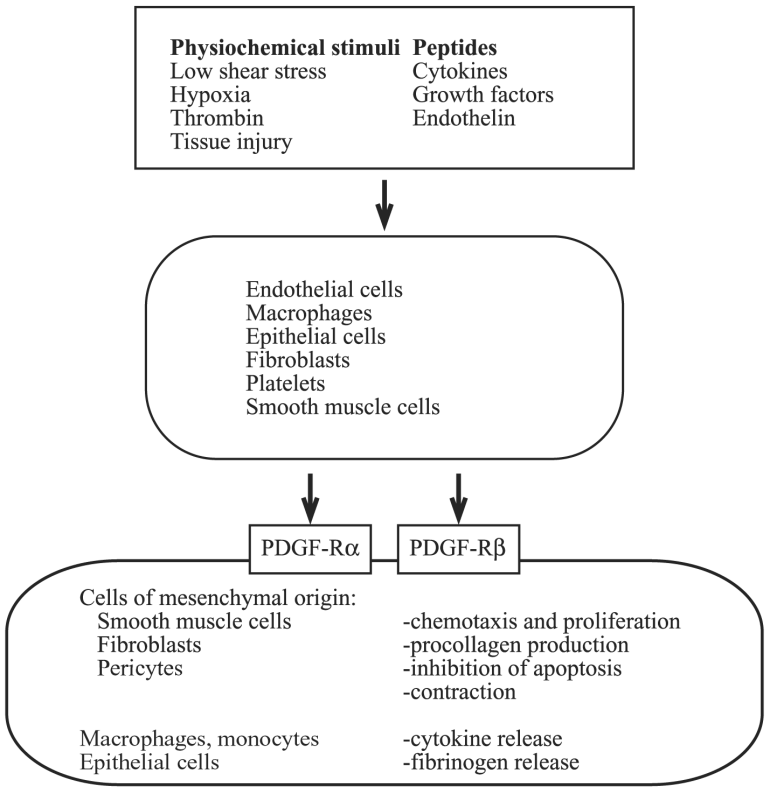

Figure 4. Schematic representation of regulators of PDGF ligand production, cellular source, and biological effects in the lung. Data based on Heldin and Westermark 1999, Heldin et al. 2002.

PDGF is one of the most important mitogens for mesenchymal cells such as SMC and fibroblasts (Ross et al. 1986). It has been linked to a variety of fibroproliferative disorders of the lung, such as idiopathic pulmonary fibrosis, adult respiratory distress syndrome, and bronchiolitis obliterans organizing pneumonia (Antoniades et al. 1990, Snyder et al. 1991, Aubert et al. 1997). Increased levels of PDGF have been reported in BAL fluid of lung transplant recipients with OB (Hertz et al. 1992). Our previous observations in rat tracheal allografts show that PDGF ligand and receptor expression are upregulated in allografts in comparison to syngeneic grafts, and that inhibition of PDGF action by CGP 53716, a selective inhibitor of PDGF receptor tyrosine kinase (Buchdunger et al. 1995), effectively prevented the development of experimental OB (Kallio et al. 1999).

6. CMV infection in lung transplantion

6.1. Human CMV

CMV is the largest member of the human herpes virus family. It has a linear double-stranded DNA genome of 250 kb encoding over 200 proteins. CMV is widely prevalent in the general population with 50-100% of the adult population being CMV seropositive (Sissons et al. 2002). Upon infection of permissive cells, immediate-early (IE) gene expression is activated followed by early (E) and late (L) genes, which ultimately leads to virus assembly and release. CMV may turn latent and reactivate at a later time point. Current evidence suggests that human CMV is carried in myeloid lineage progenitor cells in the bone marrow and maintained in the cells as they divide down the myeloid lineage into peripheral blood mononuclear cells (Sissons et al. 2002). According to a recent report, CMV uses the EGF receptor to enter permissive cells (Wang et al. 2003).

Following lung transplantation, the incidence of CMV infection in the era of ganciclovir is reported as 35-86% with an associated mortality of 2-12% (Fishman and Rubin 1998). The wide variance in reported incidence and mortality is mainly due to differences in the definition of CMV infection and disease (Zamora 2002). Currently, CMV infection is defined by isolation of virus or demonstration of its presence by immunologic or molecular techniques or demonstration of characteristic intracellular inclusion bodies. Currently available techniques include the rapid shell vial assay, pp65 antigenemia, polymerase chain reaction or hybrid capture assays for CMV DNAemia. CMV pneumonia is defined by the

presence of signs and/or symptoms of pulmonary disease combined with the detection of CMV in BALF or lung tissue samples. Detection of CMV should be performed by virus isolation, histopathologic testing, immunohistochemical analysis, or in situ hybridisation. Detection of CMV by PCR alone may be too sensitive for the diagnosis of CMV pneumonia (Ljungman et al. 2002). In other organs, CMV disease is defined by the presence of compatible symptoms and documentation of CMV by biopsy with other relevant causes excluded (Ljungman et al. 2002). Symptoms and clinical manifestations caused by CMV disease include fever, leukopenia, thrombosytopenia, pneumonia, hepatitis, encephalitis, retinitis, myocarditis, colitis, and gastroenteritis (Rubin 2001). After transplantation, CMV infection may occur as a primary infection in seronegative recipients that receive organs from seropositive donors. In seropositive recipients, reactivation of latent CMV is common and reinfection by a different CMV strain is also possible.

6.2. Rat CMV

The length of the RCMV genome is 230 kb and the whole sequence of its genome is known (Vink et al. 2000). The genome of RCMV shares many characteristics with human CMV and the pathogenesis of RCMV infection is similar to that of its human counterpart making RCMV an attractive model for studying the role of CMV infection in context of transplantation (Bruggeman et al. 1985, Vink et al. 2000). When native rats are exposed to 10^5 PFU of RCMV, acute infection with systemic virus dissemination occurs within 3 to 7 days. Shortly thereafter, the infection progresses to chronic phase, and the virus can be recovered only from the salivary glands of the host. Approximately 3-4 months after inoculation of the virus, infectious virions are no longer present in any tissue or organ, and the rat is considered latently infected (Bruggeman et al. 1985, Lemstrom et al. 1994).

6.3. Role of CMV infection in alloimmune activation

The role of CMV on host immune responses is complex. CMV possesses some anti-inflammatory properties to evade the host immune response but these are mainly direct effects on infected cells. In the transplantation setting, the early alloimmune activation together with

cytokines released during donor death, ischemia-reperfusion, and as a result of lymphocyte-depleting induction therapy may activate latent CMV. On the other hand, CMV infection promotes alloimmune activation by inducing the production of a variety of inflammatory mediators. These observations suggest a bi-directional relationship between CMV infection and alloimmune activation where one can enhance/activate the other and vice versa (Lemstrom et al. 1994, Koskinen et al. 1999, Rubin 2001). The immunomodulatory properties of CMV are summarized in Table 7.

Table 7. Immunomodulatory properties of CMV

Effect of CMV	Comment	Reference
Proinflammatory effects:		
MHC class I and II	Increases expression either directly or indirectly	*Waldman et al. 1993, van Dorp et al. 1993*
ICAM-1	Increased expression on CMV-infected endothelial cells, increased macrophage chemotaxis	*Sedmak et al. 1994, Steinhoff et al. 1995*
IL-2	HCMV-IE plasmid construct upregulated expression	*Geist et al. 1991*
TNF-α	Increased production by CMV-infected macrophages	Smith et al. 1992
IFN-γ	Increased expression in infected macrophage cell culture, upregulated expression in CMV-infected rats	*Yamaguchi et al. 1988, Zhou et al. 1999*
IL-6	NF-κB-mediated expression in CMV-infected lung fibroblasts	*Carlquist et al. 1999*
RANTES	HCMV-IE induces expression	*Michelson et al. 1997*
IP-10	CMV increases expression in heart allografts	*Streblow et al. 2003*
Anti-inflammatory effects:		
MHC class I and II	Direct degradation in infected cells	*Chevalier and Johnson 2003*
IL-10	CMV genome encodes viral homologue	*Kotenko et al. 2000*
Other effects:		
PDGF	Upregulates PDGF ligand and receptor expression in tracheal, aortic, and heart allografts	*Lemstrom et al. 1994, Koskinen et al. 1997b, Zhou et al. 1999b*
p53 tumour suppressor gene	Inhibits action in SMC	Speir et al. 1994
eNOS	CMV infection impairs eNOS function	*Valantine 2003*
TGF-β	Increases expression in CMV-infected splenocytes	*Haagmans et al. 1997, Inkinen et al. 2003*

Abbreviation: IP-10, IL-8, interferon-γ-induced protein-10, HCMV-IE, human cytomegalovirus-immediate early gene; eNOS, endothelial nitric oxide synthase. Experimental studies are in italics.

The rate of CMV infection also varies according to type of the transplanted organ. Interestingly, it seems that CMV infection is most prevalent in patients receiving transplants that are highly immunogenic, such as bone marrow and lung (Roitt et al. 1985, Appelbaum 2003). The correlation between immunogenicity of the transplant and the incidence of CMV disease is shown in Table 8.

Table 8. Incidence of CMV disease and immunogenicity of the transplanted tissue

Tissue	Immunogenicity	Incidence of CMV disease	Incidence of chronic rejection at five years
Lung	high	50%	50-60%
Heart	moderate	25%	40-50%
Kidney	moderate	8%	20-30%
Liver	low	29%	5-10%

Data modified from Bowden et al. 1998 and Roitt et al. 1985.

6.4. Treatment of CMV infection

Strategies to prevent CMV infection after lung transplantation include using leukocyte-depleted blood products (Ettinger et al. 1993) and antiviral therapy. The advent of newer and CMV-specific antiviral drugs has reduced the importance of CMV seromatching before transplantation (Sissons et al. 2002). Because of the considerable variance between antiviral treatment protocols in different transplant centres, no universal guidelines for CMV prophylaxis and treatment are available. However, ganciclovir has emerged as the drug of choice for the treatment of CMV infection in solid organ transplant recipients. Ganciclovir is a synthetic analogue of 2'-deoxy-guanosine that must first be phosphorylated to a deoxyguanosine triphosphate (dGTP) analogue. The resulting dGTP analogue competitively inhibits the incorporation of dGTP by viral DNA polymerase resulting in termination of viral DNA elongation. The phosphorylation of ganciclovir is initiated by a kinase coded by the UL97 gene and completed by cellular kinases (Faulds and Heel 1990). Ganciclovir is 10-50 times more potent against CMV than acyclovir. The mechanisms of action of antiviral drugs used against CMV are summarized in Table 9.

Table 9. Anti-CMV drugs and their mechanism of action

Drug	Target	Clinical use in transplantation
(Val)acyclovir	UL97 protein kinase, UL54 DNA polymerase	used for herpes simplex virus-prophylaxis, also used as CMV prophylaxis
(Val)ganciclovir	UL97 protein kinase, UL54 DNA polymerase	mainstay for CMV treatment and prophylaxis, valganciclovir emerging as bioactive oral form
Foscarnet	UL54 DNA polymerase	mainly used if ganciclovir treatment fails to control CMV infection
Cidofovir	UL54 DNA polymerase	mainly used if ganciclovir treatment fails to control CMV infection
Fomivirsen	UL122 transactivation	used locally in CMV retinitis
CMV hyperimmune globulin	antibody-mediated inhibition of viral replication and immuno-modulation	used either alone or in combination with ganciclovir for prophylaxis and treatment of CMV infection
AG1478	blocks EGFR-mediated internalization of CMV into permissive cells	experimental drug, no data on clinical applicability

Data based on the 2001 Garrod Lecture by P.D.Griffiths (Griffiths 2002) and Wang et al. 2003. EGFR, epidermal growth factor receptor.

A host of studies report ganciclovir prophylaxis to prevent early episodes of CMV infection and disease but in most studies this effect seems to be limited to the time of prophylaxis and CMV activation occurs after discontinuing antiviral prophylaxis. Thus, ganciclovir monotherapy seems to delay but not prevent the onset of CMV infection (Bailey et al. 1992, Maurer et al. 1993, Kelly et al. 1995, Soghikian et al. 1996, Hertz et al. 1998). However, high-risk patients, such as seronegative recipients receiving seropositive allografts benefit from CMV prophylaxis with ganciclovir and CMV hyperimmune globulin and this effect persists for at least three years (Valantine et al. 2001). Another approach to treat CMV infection is pre-emptive therapy based on the detection of CMV antigenemia or DNAemia from routinely collected blood samples. Two studies suggested that pre-emptive therapy is equally effective compared to ganciclovir prophylaxis in preventing CMV disease but the onset of CMV disease was earlier in the pre-emptively treated groups, and in some cases antigenemia monitoring failed to identify CMV activation resulting in CMV disease (Egan et al. 1998, Kelly et al. 2000). The new orally bioactive form of ganciclovir, valganciclovir, has been shown to be effective in preventing CMV disease (Akalin et al. 2003). Prospective studies to address the need for and the length of CMV prophylaxis are needed (Zamora 2002).

7. Heterotopic tracheal transplantation as a model for OB

When OB became a widely acknowledged problem in the 1980's, one lacked simple and reproducible models for investigation of OB. Most studies concentrated on surgical and operative issues and treatment of acute rejection. Orthotopic rat lung transplantation is a technically demanding procedure with great variation in histopathological findings, and lesions characteristic to OB are not always observed (Uyama et al. 1992, Hirt et al. 1999, 1999b).

In 1993, Hertz and coworkers described the heterotopic mouse tracheal allograft model, where the donor trachea is inserted into a subcutaneous pouch in the recipient (Hertz et al. 1993). These tracheal allografts developed similar histological changes to those seen in OB in man. This model was then introduced to the rat by our group and later by Dr. Morris' group (Koskinen et al. 1995, Fahrni et al. 1997, Koskinen et al. 1997). The picture of OB development is very similar in all tracheal allograft models: in syngeneic grafts, epithelium undergoes minor damage but recovers thereafter. The tracheal lumen remains completely open and the trachea is lined with normal, mucus-secreting epithelium 28 days after transplantation. In untreated allografts, epithelium undergoes progressive damage leading to near total necrosis 10 days after transplantation. By 28 days, allografts develop a fibroproliferative lesion obliterating the tracheal lumen that closely resembles obliterative changes detected in small bronchioles in man. The lack of obliteration in syngeneic grafts indicates that OB development is an alloimmune-driven phenomenon in this model. Lately, more and more publications utilising this model have been published and the heterotopic tracheal allograft model is a well-accepted model for studying OB development (Hele et al. 2001).

AIMS OF THE STUDY

The aim of this study was to investigate and target specific pathways leading to OB using the rat tracheal allograft model. A special emphasis was placed on the role of CMV infection.

The specific aims of the study were:

1) to dissect the roles of CD28/B7-1 and CD28/B7-2 costimulatory pathways in the pathogenesis of OB and prevention of OB development by interfering with CD28/B7-costimulation
2) to characterize the kinetics and the functional role of ET-1 in the development of OB
3) to develop a modified tracheal allograft model for the investigation of the pathophysiology of RCMV infection-enhanced OB
4) to investigate the efficacy of antiviral treatment and prophylaxis and immunosuppression in RCMV infection-enhanced OB
5) to characterize the role of PDGF in RCMV infection-enhanced OB

METHODS

1. Heterotopic tracheal transplantations

Specific pathogen-free inbred male DA (AG-B4, $RT1^a$) and WF (AG-B2, $RT1^u$) rats weighing 200-300 g and of 2-3 mo of age (Harlan, The Netherlands) were used. Permission for animal experimentation was obtained from the Provincial State Office of Southern Finland. Rats received care in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Pub. No. 80-23, revised 1978).

A 3 cm long segment of the donor trachea was excised just above the bifurcation, perfused with PBS containing 10 000 IU/ml penicillin and 1 000 μg/ml streptomycin, and stored in the same solution at +4ºC until transplantation. In the recipient, the trachea was wrapped into the greater omentum and the abdomen was closed using absorbable continuous 3-0 sutures. Both donor and recipient operations were performed under ether anaesthesia. The recipients received buprenorphine 0.25 mg/kg s.c. for postoperative pain relief. Syngeneic transplantations were performed from DA to DA rats and allogeneic transplantations from DA to WF rats. Nontransplanted DA trachea were used as normal controls. At sacrifice, the grafted trachea were excised, embedded in Tissue-Tek (Miles Inc., Elkhart, IN), snap-frozen in liquid nitrogen, and stored at -70 °C until used.

2. Rat CMV infection

Stocks of Maastricht strain rat cytomegalovirus (RCMV) salivary gland extracts kindly provided by Professor Cathrien Bruggeman were used. Acute RCMV infection was initiated intraperitoneally 3 h after transplantation with 5×10^5 plaque-forming units (PFU) of RCMV in saline. Chronic RCMV infection was established by inoculating RCMV 8 weeks before transplantation.

At graft removal, tissue biopsies from salivary glands, liver, and spleen were obtained aseptically in modified Eagle's medium supplemented with 200mM L-glutamine, penicillin-streptomycin, and 2% FCS. The specimens were stored in –70ºC until used. For plaque assays, the specimens were homogenized in a tissue grinder and suspended in BME supplemented with 2% NCS, L-glutamine, and penicillin-streptomycin in Potter's tube, and 10-fold dilutions of 10% homogenates (wt/vol) were inoculated on confluent rat embryo fibroblast monolayers. After an incubation period of 7 days, the number of plaques was counted microscopically. The expression of the major immediate early DNA of RCMV from samples of tracheal allografts was determined by a sensitive, single-tube, nested PCR reaction (Beisser et al. 1998).

3. Drug regimens

Cyclosporine A. CsA was used for base immunosuppression. CsA (Novartis, Basle, Switzerland) was dissolved in Intralipid (KabiVitrum, Stockholm, Sweden) to a final concentration of 1-2 mg/ml and administered daily at doses of 1-2 mg/kg s.c. from the day of transplantation until sacrifice. Whole blood CsA 24-hour trough levels were measured weekly using radioimmunoassay (Sandimmun-Kit; Novartis) to ensure equal CsA immunosuppression between different treatment groups.

Ganciclovir. Ganciclovir (DHPG, Cymevene, Roche, Palo Alto, CA) was diluted into saline and given 20 mg/kg/d i.p. in two doses. Treatment was initiated 12 h before transplantation or 5 days after transplantation and continued until sacrifice. Controls received an equal volume saline.

Hyperimmune serum. HIS with a neutralization titer of 160 was diluted at 1:4 in PBS and 1 ml of this solution was administered i.v. as a single dose 12 h before transplantation or 5 days after transplantation. Controls received an equal volume of control serum.

Costimulatory blockade. Both human and murine CTLA4Ig, its mutant counterpart CTLA4IgY100F, and control IgG were generously provided by Dr. Robert Peach (Bristol-Myers Squibb, Seattle, WA). CTLA4Ig effectively inhibits both CD28/B7-1 and CD28/B7-2 costimulatory signalling. Either human or murine CTLA4Ig was administered 2 d after

transplantation as a single dose of 0.5 mg i.p. Controls received an equal amount of respective IgG. CTLA4IgY100F is a mutant form of CTLA4Ig and blocks only CD28/B7-1 interaction, but not CD28/B7-1 interaction, and was given as CTLA4Ig.

Bosentan. Bosentan, a nonselective ET-1 receptor antagonist (a kind gift from Dr. Martine Clozel, Actelion Ltd, Allschwil, Switzerland), was used to inhibit ET-1 activity. Bosentan was suspended in 5 % gummi arabicum to a concentration of 25 mg/ml and administered at the dose of 100 mg/kg/d by oral gavage. Vehicle solution consisted of 5 % gummi arabicum.

CGP 53716. CGP 53716 (Novartis Pharma, Basle, Switzerland) is a potent protein tyrosine kinase inhibitor both *in vitro* and *in vivo* (Buchdunger et al. 1995). The compound is also a selective inhibitor of PDGF-mediated events such as PDGF-R autophosphorylation, cellular tyrosine phosphorylation, and *c-fos* mRNA induction in response to PDGF stimulation of intact cells. In contrast, ligand-induced autophosphorylation of epidermal growth factor receptor (EGF), insulin receptors, and the insulin-like growth factor-1 (IGF-1) receptor, as well as *c*-fos mRNA expression induced by EGF, basic fibroblast growth factor (bFGF), and phorbol ester are insensitive to inhibition by CGP 53716 (Buchdunger et al. 1995). CGP 53716 was dissolved in dimethylsulfoxide to a concentration of 200 mg/kg, diluted at 1:20 with 1% Tween in 0.9% NaCl, and sonicated. Rats received CGP 53716 or vehicle solution as a daily dose of 50 mg/kg i.p.

4. Histological and immunohistological evaluation

Morphometry. For histological evaluation, frozen sections were stained with Mayer's hematoxylin and eosin. Luminal occlusion was evaluated by determining the reduction in luminal area using the public domain NIH Image program version 1.59 (National Technical Information Service, Springfield, VI). Epithelial necrosis was evaluated as percentage of the tracheal circumference not lined by epithelium.

Immunohistochemistry. Immunohistochemistry was performed using the standard Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Briefly, frozen tracheal sections (4-6 μm) were air-dried on silane-coated slides, and fixed in acetone for 20 min. After incubation with appropriate 1.5% nonimmune serum, frozen sections were incubated with

mouse, rabbit, or goat mono- or polyclonal antibodies at room temperature for 30 min or at +4°C for 12 h depending on the staining in question. With intervening washes in Tris-buffered saline, the following steps were performed: appropriate biotinylated antibodies at room temperature for 30 min; avidin-biotinylated horse-radish complex in PBS at room temperature for 30 min; the reaction was revealed by 3-amino-9-ethylcarbazole (AEC) containing 0.1% hydrogen peroxidase, yielding a brown-red reaction product. The specimens were counterstained with hematoxylin and cover slips were aquamounted (Aquamount; BDH Ltd., Poole, UK). Specificity controls were performed using the same immunoglobulin concentration of species- and isotype-matched antibodies. The antibodies used in the study are listed in Table 10.

Table 10. Antibodies used for immunohistochemistry

Clone/code	Specificity	Source	Species	Dilution
M 744	BrdU	DAKO, Glostrup, Denmark	mouse monoclonal	15 μg/ml
W3/25	CD4	Sera Lab, Sussex, UK	mouse monoclonal	30 μg/ml
OX8	CD8	Sera Lab	mouse monoclonal	29 μg/ml
sc-1624	CD28	Santa Cruz Biotechnology, Santa Cruz, CA	goat polyclonal	1 μg/ml
22661D	CD80	BD Pharmingen, San Diego, CA	mouse monoclonal	5 μg/ml
22671D	CD86	BD Pharmingen	mouse monoclonal	5 μg/ml
MCA 341	ED1	Sera Lab	mouse monoclonal	5 μg/ml
IHC 6901	ET-1	Peninsula Laboratories Inc, San Carlos, CA	rabbit polyclonal	5 μg/ml
E 3100	ET-RA	US Biological, Swampscott, MA	rabbit polyclonal	10 μg/ml
E 3110	ET-RB	US Biological	rabbit polyclonal	10 μg/ml
sc-9344	IFN-γ	Santa Cruz	goat polyclonal	2 μg/ml
sc-1252	IL-1β	Genzyme Diagnostics, Cambridge, MA	goat polyclonal	2 μg/ml
sc-17896	IL-2	Santa Cruz Biotechnology	rabbit polyclonal	2 μg/ml
MCA 1200	IL-4	Serotec	mouse monoclonal	10 μg/ml
24072D	IL-10	BD Pharmingen	mouse monoclonal	5 μg/ml
OX6	MHC class II	Sera Lab	mouse monoclonal	6 μg/ml
sc-7958	PDGF-A	Santa Cruz	rabbit polyclonal	1 μg/ml
sc-7878	PDGF-B	Santa Cruz	rabbit polyclonal	1 μg/ml
sc-338	PDGF-Rα	Santa Cruz	rabbit polyclonal	1 μg/ml
sc-339	PDGF-Rβ	Santa Cruz	rabbit polyclonal	1 μg/ml
CY-051	TNF-α	Innogenetics, Zwijndrecht, Belgium	goat polyclonal	5 μg/ml

Quantification of immunoreactivity. Immunohistochemical analyses were performed in blinded review by two independent observers. The intensity of staining was scored from 0 to 3: 0, no visible expression; 1, few cells with faint expression; 2, moderate intensity with multifocal expression; and 3, intense expression throughout the tracheal allografts or as positive staining cells / cross section, depending on the staining in question.

***In vivo* labelling for cell proliferation.** Tracheal allograft recipients were given 300 µl of a concentrated solution of bromodeoxyuridine (BrdU; 5-bromo-2'-deoxyuridine 3 mg/ml and 5-fluoro-2'deoxyuridine 0.3 mg/ml) 4 hours before sacrifice. Cell proliferation from frozen sections was revealed by the Vectastain Elite ABC kit and quantitated by counting positive staining cells / cross section.

5. *In situ* hybridisation

A 480 bp fragment of rat PDGF-A cDNA, a 380 bp fragment of rat PDGF-B cDNA, a 564 bp fragment of rat PDGF-Rα cDNA, and a 411 bp fragment of rat PDGF- Rβ cDNA were each cloned into pBluescript II KS (Stratagene, La Jolla, CA) and used to generate corresponding antisense and sense cRNA probes. Radiolabelled RNA was synthesized using T3 and T7 RNA polymerase (Promega, Madison WI) and [^{35}S]UTP (Amersham Pharmacia Biotech, Piscataway, NJ). Briefly, cryostat rat tracheal sections (4-6 µm) were fixed with 4% PFA, dehydrated and stored at –70ºC until needed. Upon use, the sections were proteinase K treated, re-fixed in 4% PFA, treated with 50% formamide in 2x SSC, followed by acetylation with acetic anhydride in 0.1 M TEA buffer. Hybridization was performed o/n at +52ºC. After RNAse treatment and high stringency washes to remove unspecifically bound probe, the sections were dehydrated, air-dried, dipped into NTB-2 emulsion (Eastman Kodak, Rochester, NY), and exposed at +4ºC in the dark for 4 to 6 weeks. The sections were developed in D19 developer (Eastman Kodak), fixed in sodium fixative (Eastman Kodak), and counterstained in hematoxylin (Shandon, Pittsburgh, PA).

6. Statistical methods

All data are expressed as mean ± SEM and analysed using the Statview 512+ software (Brain Power Inc., Calabasas, CA). The non-parametric Mann-Whitney test was used for two group comparisons and Kruskal-Wallis test with Dunn correction was used for multiple group comparisons. For parametric comparisons, ANOVA-test was used. $P<0.05$ was considered statistically significant.

RESULTS

1. The rat tracheal allograft model (I-IV)

Altogether 384 tracheal transplantations were performed in this study. The histology of normal trachea, and syngeneic and allogeneic grafts is summarized in Figure 5.

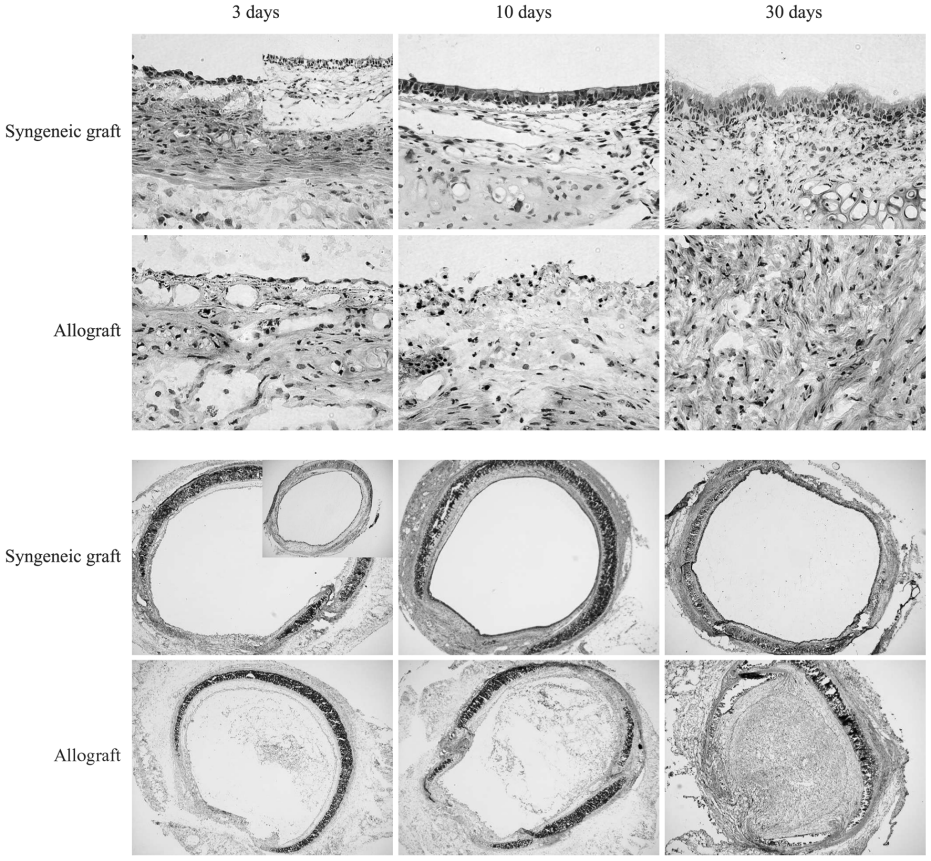

Figure 5. Photomicrographs of normal trachea (small photo in the upper right hand corner), and syngeneic, and allogeneic grafts 3, 10, 30 days after transplantation. In syngeneic grafts, the epithelium undergoes minor damage but recovers thereafter. The tracheal lumen remains completely open and the trachea is lined with normal, mucus-secreting epithelium 30 days after transplantation. On the other hand, in untreated allografts, the epithelium sustains progressive damage leading to nearly total epithelial necrosis 10 days after transplantation. Allografts develop a strong alloimmune response, which is associated with increased expression of cytokines, chemokines, and growth factors, culminating in the development of a fibroproliferative lesion obliterating the tracheal lumen. Magnification x80 in upper panel, x8 in lower panel.

The progressing epithelial destruction observed in allografts is associated with an intense infiltration of the allograft by ED1, CD4, and CD8 positive cells that is not seen in syngeneic grafts. After the epithelium has undergone total necrosis, allograft inflammation subsides but

luminal occlusion progresses and leads to total occlusion of the tracheal airway at one month (I-IV). The temporal kinetics of allograft inflammation, epithelial necrosis, and luminal occlusion are presented below in Figure 6.

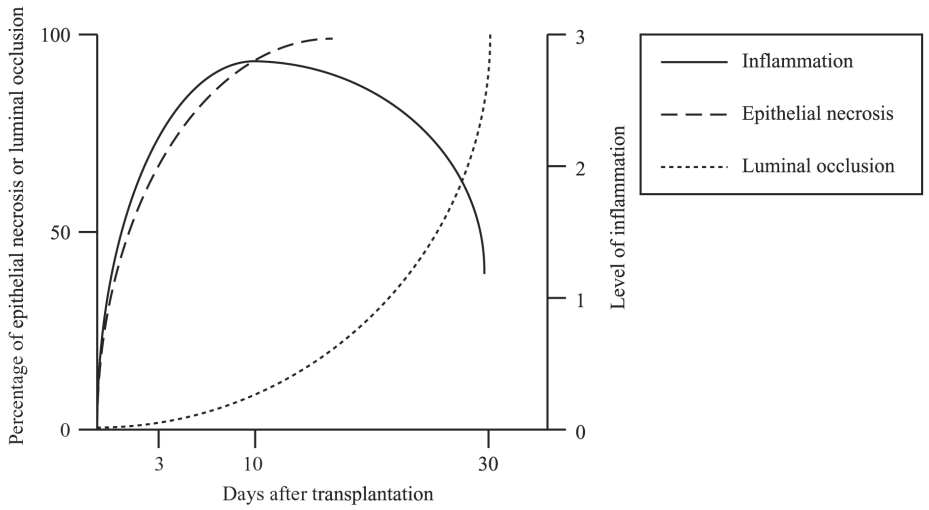

Figure 6. Kinetics of intragraft inflammation, epithelial necrosis, and luminal occlusion in untreated tracheal allografts

2. Role of the CD28/B7 costimulatory pathway in the development of experimental OB (I)

The expression of costimulatory molecules B7-1 (CD80) and B7-2 (CD86) were investigated in normal trachea, syngeneic grafts, and allografts after transplantation. B7-1 expression was very low at all time points and was detected from few scattered graft-infiltrating mononuclear cells. B7-2 expression, on the other hand, was constitutive and localized to mononuclear cells underlying the epithelium. Syngeneic transplantation had no influence on B7-2 expression. In allografts, B7-2 expression peaked at 10 days and was observed mainly in mononuclear cells of the allograft airway wall.

Murine and human CTLA4Ig (mCTLA4Ig, hCTLA4Ig) that block both CD28/B7-1 and B7-2 interaction and CTLA4IgY100F (mCTLA4IgY100F, hCTLA4IgY100F) that block only CD28/B7-1, but not CD28/B7-2, interaction were used to investigate the role of CD28/B7 costimulation in the development of OB. The murine forms failed to influence the

development of tracheal occlusion and they were left out of further analyses. Treatment by hCTLA4Ig significantly reduced epithelial necrosis at 10 days and tracheal occlusion 30 days after transplantation and induced a shift from the Th1- to Th2-like immune response as it reduced intragraft expression of IL-2 and IFN-γ compared to controls. The mutant form hCTLA4IgY100f had no significant effect on the cytokine levels or histological profile.

3. Role of endothelin-1 in the development of experimental OB (II)

The airway wall expression of ET-1 and its receptors was investigated at the time of peak inflammatory response 10 days after transplantation. In normal DA trachea and syngeneic grafts, ET-1 expression was observed only in ciliated epithelial cells. In allografts, the epithelium had undergone nearly total necrosis but widespread airway wall cell expression of ET-1 concentrating into the proliferative lesion was detected. No ET-RA expression was observed in normal DA trachea, while in syngeneic grafts, few ET-RA positive epithelial and airway wall cells were recorded. Allografts showed induced ET-RA expression in the proliferative lesion mononuclear and SMC-like cells. In normal DA trachea and syngeneic grafts, ET-RB expression was recorded only from few epithelial cells. In allografts, there was moderately elevated expression of ET-RB observed in the airway wall with strongest expression localizing to the proliferating lesion.

Inhibition of ET-1 activity by bosentan lead to reduced tracheal allograft epithelial necrosis at 10 days and attenuated tracheal occlusion at 30 days after transplantation compared to vehicle-treated controls. Bosentan treatment also decreased BrdU incorporation in both inflammatory cells of the airway wall and myofibroblasts of the myofibroproliferative lesion and downregulated tracheal allograft expression of IL-1β and IL-2 by 75% in comparison to vehicle-treated controls.

4. Effect of CMV infection on experimental OB (III,IV)

The effect of RCMV infection on the development of experimental OB was investigated in tracheal allograft recipients receiving base immunosuppression of CsA 1.5 mg/kg/d. Both

acute and chronic RCMV infection significantly enhanced epithelial necrosis and tracheal airway wall cell proliferation at 10 days and tracheal occlusion at 30 days after transplantation compared to non-infected controls. RCMV infection was associated with increased expression of proinflammatory cytokine TNF-α, IL-2 and decreased expression of IL-10 at 10 days compared to non-infected controls, suggesting a shift towards Th1-type immune responses. Furthermore, increased expression of PDGF-A and –B as well as PDGF-Rα and –Rβ was noted in allografts of RCMV-infected recipients.

5. Inhibition of RCMV infection-enhanced experimental OB (I, III)

The different treatment regimens and their efficacy on the development of RCMV infection-enhanced OB are shown in Figure 7.

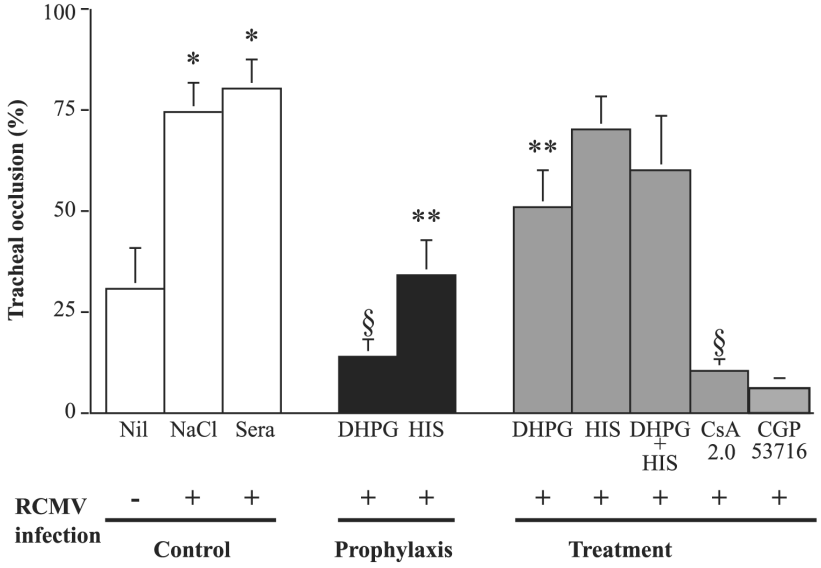

Figure 7. Effect of different treatment regimens on the degree of luminal occlusion in rat tracheal allograft recipients with acute RCMV infection. Acute RCMV infection was initiated 3 hours after transplantation with 5 x 10^5 PFU of Maastricht strain RCMV. All allograft recipients received cyclosporine 1.5 mg/kg/d s.c., except for one group which was given CsA 2 mg/kg/d. The CGP53716 group received additionally CGP 53716 50 mg/kg daily. Data are given as mean +/- SEM. *P<0.01 compared to non-infected controls, **P<0.05 and §P<0.01 compared to respective saline- or normal rat sera-treated controls by Mann-Whitney for two group comparison and Kruskal-Wallis and Dunn tests for multiple comparison. *n* = 10/group, CsA 2 mg/kg/d *n* = 5. Nil indicates non-infected allograft recipients (III,IV).

Antiviral therapy. Antiviral prophylaxis was initiated 12 h before transplantation while antiviral treatment was commenced 5 days after transplantation. Both ganciclovir and HIS prophylaxis prevented RCMV-induced tracheal occlusion whereas treatment with the same agents did not significantly reduce obliterative changes. Ganciclovir prophylaxis was equally beneficial during both acute and chronic RCMV infection in preventing epithelial necrosis and airway obliteration. Antiviral prophylaxis by ganciclovir was also associated with reduced BrdU incorporation both in cells of the myofibroproliferative lesion and the airway wall, and decreased airway wall expression of IFN-γ, while HIS prophylaxis lead to a reduction in graft-infiltrating ED1-positive cells and decreased airway wall expression of IFN-γ. Regardless of the antiviral treatment regimen, viable RCMV could be recovered from the salivary glands of nearly all tracheal allograft recipients, indicating that antiviral treatment did not prevent RCMV infection.

Immunosuppression. Augmented CsA immunosuppression at the dose of 2.0 mg/kg/d was used to evaluate the effect of immunosuppression on RCMV infection-enhanced OB. This dose results in 24-h whole blood trough levels of 450-550 μg/L compared to 250-350 μg/L in the 1.5 mg/kg/d group. Augmented CsA immunosuppression lead to a clear reduction in epithelial necrosis and tracheal obliteration compared to base immunosuppressed tracheal allograft recipients and was associated with decreased expression of IL-2 and TNF-α in tracheal allografts.

PDGF pathway. CGP53716 was used to inhibit PDGF receptor protein tyrosine kinase activity. CGP53716 does not affect PDGF ligand or receptor expression (data not shown) nor does it affect epithelial necrosis at 10 days. However, CGP53716 treatment totally abolished the deleterious effect of RCMV infection on the development of experimental OB.

DISCUSSION

1. Early alloimmune activation is central for the development of OB

Except for the transient ischemic damage, the histological picture of untreated tracheal syngeneic grafts is similar to that of normal trachea. No fibroproliferation is seen and infiltration of the syngeneic graft by inflammatory cells is modest at most. Therefore, it is apparent that the changes seen in this model are alloimmune-dependent and it seems that the epithelium has a central role in the process. First of all, epithelial cells are an important source of antigen for host immune cells and may express both MHC class I and II molecules in an alloimmune setting (Ibrahim et al. 1993, Koskinen et al. 1997). Secondly, loss of epithelium in most cases precedes and is required for the development of the fibroproliferative lesion, suggesting that intact epithelium inhibits obliteration of the tracheal lumen (Ikonen et al. 2000). In one study, complete destruction of the epithelium by protease digestion induced a fibroproliferative lesion in syngeneic grafts (Adams et al. 2000b).

The major factor leading to epithelial loss is the alloimmune-driven inflammatory response characterized by intense migration of recipient inflammatory cells and the following production of a myriad of different proinflammatory cytokines. T cell activation and proliferation are central for the development of the alloimmune response leading to OB. The activation of T cells requires a secondary signal in addition to the signal mediated by binding of MHC molecule/antigen complex on the APC to the TCR. The most important costimulatory pathway is the CD28/B7 pathway. The CD28/B7 costimulatory pathway can be blocked using CTLAIg, and several studies have shown CTLA4Ig treatment to effectively inhibit the development of allograft rejection and have raised hopes of ultimately achieving true tolerance towards the transplant (Lin et al. 1993, Azuma et al. 1996, Larsen et al. 1996, Russell et al. 1996, Yamada et al. 2000). However, the efficacy of costimulatory blockade seems to be greatest when initiated right after transplantation at the time of peak alloimmune activation together with donor-specific transfusion and myelosuppressive recipient conditioning (Wekerle et al. 1998, Shirasugi et al. 2002).

In our study, B7-2 but not B7-1 expression was upregulated in allografts at 10 days (I). A similar pattern of expression was reported by Israel-Assayag and coworkers in mice with hypersensitivity pneumonitis, suggesting a specific role for B7-2 in the initiation and sustenance of the alloimmune response (Israel-Assayag et al. 1999). The central role of B7-2 is further supported by the notion that selective inhibition of CD28/B7-2 but not of CD28/B7-1 costimulation by monoclonal antibodies resulted in equal attenuation of ovalbumin-induced airway hyperresponsiveness as did CTLA4Ig treatment (Tsuyuki et al. 1997, Larche et al. 1998). However, the results do not exclude a complementary role for CD28/B7-1 costimulation as in a few studies CD28/B7-2 inhibition alone failed to increase allogeneic pancreatic islet survival (Lenschow et al. 1995, Zheng et al. 1997).

Thus, if early T cell activation is prevented by costimulatory blockade (I), (Shiraishi et al. 2002) or immunosuppressive drugs aimed at the IL-2 pathway (i.e. CsA, tacrolimus, sirolimus), the alloimmune response remains weak and the epithelial structures intact resulting in the prevention of OB (Fahrni et al. 1997, Koskinen et al. 1997, Adams et al. 2000, Yamada et al. 2000). However, immunosuppression with MMF or 15-deoxyspergualin (DSG) did not affect OB development, suggesting that inhibition of distal steps of lymphocyte activation in the absence of IL-2 inhibition is not sufficient to inhibit the alloimmune injury leading to growth factor production and fibroproliferation (Koskinen et al. 1997, Adams et al. 2000). The effects of different immunosuppressive drug regimens on tracheal allograft OB development are summarized in Figure 8.

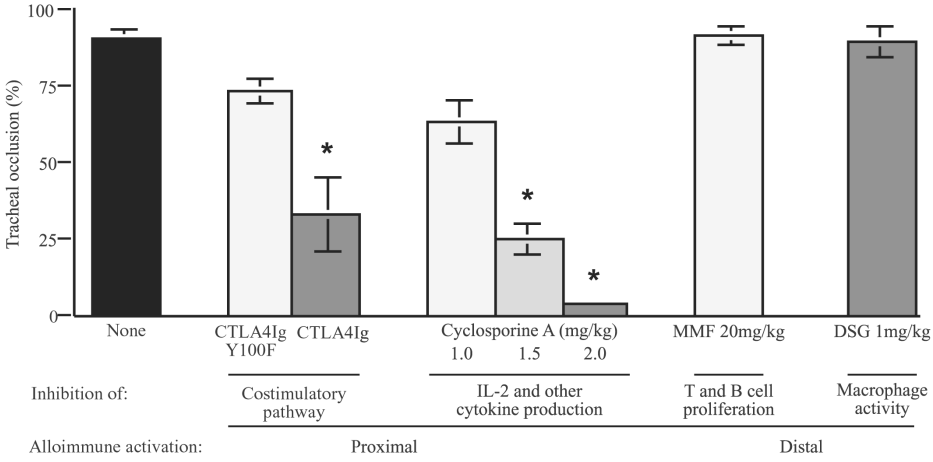

Figure 8. The effect of different immunosuppressive regimens on development of OB. While inhibition of proximal steps of T cell activation with either costimulatory blockade (CTLA4Ig) or cyclosporine A treatment resulted in significant inhibition of OB, interruption of later steps of the lymphocyte activation with mycophenolate mofetil (MMF) and deoxyspergualin (DSG) failed to have impact on OB development. Data from II, Koskinen et al, 1997. *P<0.05 compared to untreated control (None).

2. The dualistic role of the epithelium

When untreated, the alloimmune response leads to total epithelial necrosis 10-14 days after transplantation in allografts (Davreux et al. 1993, Kallio et al. 1997, Kallio et al. 2000). Coinciding with the strong Th1-dominated alloimmune response and increasing epithelial loss, prominent expression of SMC growth factors, such as PDGF, ET-1, EGF, and FGF is observed together with intense SMC proliferation (Koskinen et al. 1997, Kallio et al. 1999, Aris et al. 2002). At this stage, initiation of conventional immunosuppressive therapy does not influence the development of OB (Adams et al. 2000). Furthermore, if untreated allografts are removed at 14 days and retransplanted into syngeneic recipients, OB develops in the absence of alloimmune responses. However, if the same procedure is performed on day 7 before the loss of epithelium, OB does not develop (King et al. 2002).

As stated, epithelial cells are a major source of antigen and may present both MHC class I and II molecules to recipient lymphocytes. In addition, unlike donor-derived leukocytes, epithelial cells have the ability to regenerate and form a replenishing source of antigen that continuously fuels the alloimmune response. Therefore, in the presence of a strong alloimmune response, such as in our model, donor-derived epithelium is an important factor driving the alloimmune response and rejection.

The mechanism by which epithelium may confer protection against airway obliteration is unclear. It is possible that the injured epithelium itself produces and secretes cytokines and growth factors that then induce SMC migration and proliferation. However, it is also possible that the intact epithelium actively produces a negative inhibitory signal and, upon epithelial injury, this negative feedback loop is interrupted. In an innovative study, Tsurumi and coworkers describe a reciprocal relationship between tissue injury and endothelial cells (Tsurumi et al. 1997). When the arterial endothelium is damaged by balloon injury, there is a notable increase in vascular endothelial growth factor (VEGF) production. VEGF is known to actively promote re-endothelialization and to induce the production of NO (Asahara et al. 1995, van der Zee et al. 1997). They also showed that intact endothelium produces NO that in turn negatively regulates VEGF production via the protein kinase C pathway thereby providing a negative feedback signal and controlling the response to injury (Tsurumi et al. 1997). Should the damage to the endothelium be continuous and excessive, the amount of NO production would not be increased to the level necessary to inhibit VEGF and other growth

factor production as in an alloimmune setting. Such a reciprocal pathway has not been reported in the lung, but the lung epithelium is able to produce NO and supplementation of L-arginine, a precursor of NO, reduced SMC proliferation and OB in tracheal allografts, suggesting that NO may have a similar regulatory role in tracheal allografts (Kallio et al. 1997).

3. ET-1 has both proinflammatory and proproliferative properties in the pathogenesis of OB

After lung transplantation, there is a surge in ET-1 expression due to alloimmune activation and ischemic injury (Schersten et al. 1994, Aarnio et al. 1996, Jeppsson et al. 1998). ET-1 protein and mRNA expression are also upregulated during OB development (IV) (Aris et al. 2002). The overexpression of ET-1 after transplantation is probably induced by cytokines produced by activated macrophages such as IL-1β and TNF-α which are known to upregulate ET-1 production (Barnes 1994). Also, PDGF and TGF-β are capable of stimulating vascular SMC to produce ET-1, and the expression of ET-RA and –RB on vascular SMC is upregulated by PDGF (Hahn et al. 1990). In addition, activated macrophages themselves are capable of producing ET-1 (Ehrenreich et al. 1990), and ET-1 alone has the ability to stimulate ET-1 production by vascular SMC (Hahn et al. 1990).

There are several mechanisms by which ET-1 may promote SMC proliferation and thereby the development of OB. ET-1 has direct proproliferative effects *in vitro* both on vascular (Hirata et al. 1989) and airway SMC (Glassberg et al. 1994). However, the majority of studies support an indirect proproliferative effect through synergistic action with other growth factors, namely PDGF-BB (Weissberg et al. 1990) and TGF-β (Yeh et al. 1991), suggesting significant cross-talk between PDGF and ET-1 in promoting the fibroproliferative response. Blockade of both pathways might have resulted in a more prominent effect.

Bosentan has been accepted for clinical use for the treatment of primary pulmonary hypertension where it improves exercise ability and slows the rate of clinical worsening (Channick et al. 2001, Rubin et al. 2002). In addition, bosentan has been shown to decrease inflammatory reactions, vascular permeability and remodeling, and to prevent development of fibrosis (Chen et al. 1995, Filep et al. 1995, Park et al. 1997). Therefore, the results of this

study suggest a novel therapeutic strategy for the prevention of BOS by a drug that has already been successfully introduced to clinical practice in patients with pulmonary disease.

4. RCMV infection enhances alloimmune activation and SMC growth factor production

We have previously shown that RCMV infection enhances the development of OB compared to non-infected allograft recipients. RCMV infection enhanced OB is alloimmune-related, as RCMV-infected recipients of tracheal syngeneic grafts had no evidence of obliterative changes. Furthermore, RCMV infection was associated with an increased number of allograft-infiltrating CD4+ T cells and macrophages and upregulation of MHC class II expression (Koskinen et al. 1997b).

The findings of this study underline the importance of efficient early inhibition of CMV infection as antiviral treatment initiated 5 days after infection did not prevent RCMV infection-enhanced OB but prophylaxis initiated before infection totally prevented RCMV-induced OB (I). Several clinical studies have implicated CMV infection as a risk factor for OB but, more often than not, CMV infection is a univariate but not multivariate risk factor (Keenan et al. 1991, Bando et al. 1995, Keller et al. 1995, Baudet et al. 1996, Reichenspurner et al. 1996, Soghikian et al. 1996, Kroshus et al. 1997). Our results suggest that early CMV infection promotes the acute alloimmune response and may mediate OB development via increased acute rejection. In support of this, Reichenspurner and his coworkers showed that CMV infection increased the number and severity of acute rejection episodes and that CMV infection and acute rejection together formed a marked risk factor for the development of OB (Reichenspurner et al. 1996). In a recent study, CMV DNAaemia during the first six months after transplantation correlated strongly with the development of BOS despite 2-3 month ganciclovir prophylaxis and none of the patients negative for CMV DNAaemia developed BOS during the study period (Westall et al. 2003).

Absence of ganciclovir prophylaxis has been implicated as a risk factor for OB (Bando et al. 1995, Speich et al. 1999). Ganciclovir prophylaxis delays the onset of CMV infection but does not prevent it completely (Duncan et al. 1994). However, as our results underline the importance of early CMV infection, it is possible that delaying CMV infection episodes is enough to reduce the risk of later development of OB. Lately, the ISHLT Registry reported a

decrease in OB prevalence (Hertz et al. 2002) which could, in part, result from decreased early episodes of CMV infection due the widespread use of ganciclovir prophylaxis in lung transplant recipients. However, although the evidence supporting the need for adequate anti-CMV prophylaxis after solid organ transplantation to prevent chronic allograft rejection is mounting (Valantine et al. 1999, Valantine et al. 2001), the field of lung transplantation still lacks a randomised prospective study addressing the need for and duration of antiviral prophylaxis in the prevention of CMV infection-enhanced BOS.

Finally, this study suggests that RCMV infection-enhanced alloimmune activation culminates in increased PDGF ligand and receptor expression in the fibroproliferative lesion and that selective inhibition of PDGF receptor tyrosine kinase activity negates tracheal allograft occlusion without having any effect on alloimmune activation or epithelial injury (III). CGP53716, the drug used to block PDGF receptor activity, is a predecessor of imatinib (CGP57148B), that has been introduced to the clinic in the treatment of Philadelphia chromosome negative CML and gastrointestinal tumours (Druker et al. 2001, Cohen et al. 2002). The results suggest that blockade of PDGF activity by imatinib may be of therapeutic value in the prevention of OB in the clinic.

On the basis of the findings of this and previous studies, it is likely that there is a bi-directional relationship between CMV and the alloimmune response. Acute rejection activates latent or chronic CMV infection and early RCMV infection promotes acute rejection leading to increased PDGF expression and the development of OB (Figure 9).

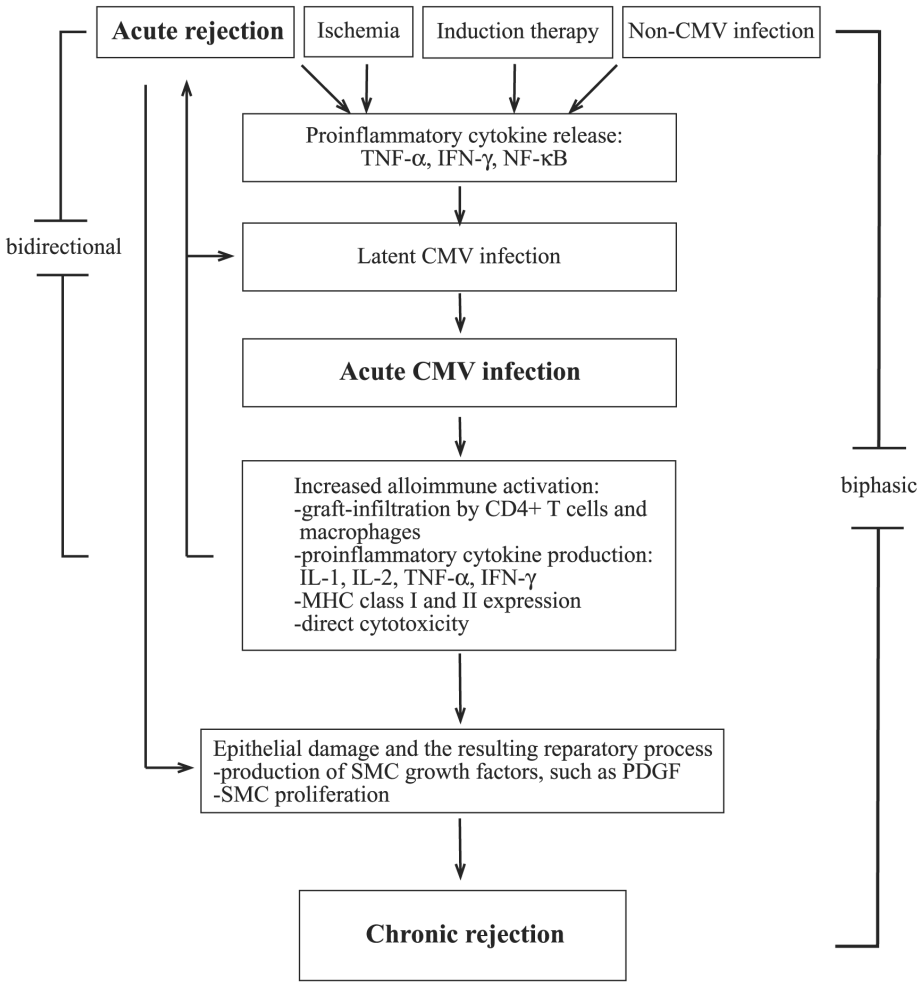

Figure 9. The effect of CMV infection on tracheal allograft rejection is bi-directional and biphasic. The proinflammatory cytokine production associated with the acute alloimmune response, lytic induction therapy, ischemia, and infections other than CMV can activate latent CMV infection. On the other hand, CMV infection is associated with increased immune activation leading to epithelial injury and growth factor production culminating in increased OB formation. The biphasic role of CMV on OB development emphasizes the need for antiviral prophylaxis during the early alloimmune response to prevent the enhancing impact of CMV infection on the later development of obliterative lesions.

5. Limitations of the tracheal allograft model

In the attempt to extrapolate the findings of this study to the clinical situation, one has to take into account the limitations of the tracheal allograft model. First of all, the anatomy of the trachea is considerably different from that of bronchioli. Trachea is surrounded by cartilage not seen in bronchioli. Furthermore, the obliterative changes affecting bronchioles do not

extend to large airways in man. Additionally, the tracheal allograft is not vascularized which makes the interpretation of findings related to ischemia difficult. The tracheal allograft has no airflow and is not in contact with foreign pathogens. The trachea contains less lymphoid tissue than lung allografts and may reduce the impact of direct allorecognition in this model. Finally, in our model, the obliterative lesion develops in one month compared to years in lung transplant patients. However, the obliterative lesion seen in tracheal allografts is similar to that seen in bronchioles in man and forms a reproducible and simple model for investigation of the pathogenesis of OB.

6. Conclusions of the study

The findings of this study show that B7-2, but not B7-1, is upregulated during the development of experimental OB in the rat. Selective blockade of the CD28/B7 costimulatory pathway leads to a shift from the Th1- to Th2-dominated immune response and reduces tracheal allograft epithelial necrosis and markedly inhibits tracheal luminal occlusion. Selective blockade of CD28/B7-1 costimulation failed to affect OB development, suggesting an important role for CD28/B7-2-mediated T cell costimulation in the development of OB. (I)

ET-1 ligand and receptor expression are upregulated during OB development. ET-1 expression is associated with increased alloimmune activation and SMC proliferation, leading to enhanced epithelial injury and obliteration of tracheal allograft lumen. Selective ET-receptor antagonism with bosentan resulted in reversal of the deleterious effects of ET-1 and the results imply that blockade of ET-1 action with bosentan may be useful for the prevention of clinical OB (II).

We modified the base immunosuppression of tracheal allograft recipients infected with RCMV resulting in an improved and more sensitive model for the investigation of RCMV infection-enhanced OB. This study confirms the role of RCMV infection as a significant risk factor for experimental OB. Although RCMV was detected from only a few inflammatory cells in tracheal allografts, RCMV infection enhanced the Th1-like alloimmune response and led to increased expression of PDGF ligands and receptors. Allografts of RCMV-infected recipients showed markedly increased epithelial necrosis and a three-fold increase in tracheal allograft occlusion compared to non-infected controls. Both acute and chronic RCMV

infection were equally detrimental. Antiviral prophylaxis with ganciclovir or RCMV hyperimmune serum negated the deleterious effect of RCMV infection but treatment initiated five days after transplantation failed to do so. The findings underline the importance of early and prophylactic treatment of RCMV infection in the prevention of OB. Additionally, our results support the indirect role of RCMV infection as a promoter of alloimmune activation as increased immunosuppression with CsA inhibited the development of RCMV infection-enhanced OB.

Finally, CGP53716-mediated PDGF-receptor blockade inhibited OB development in RCMV-infected recipients, suggesting that the final pathway in the development of the fibroproliferative lesion is mediated through PDGF receptor activation and that specific inhibition of this pathway almost totally abolishes OB development.

7. Summary

In the tracheal allograft model, the early alloimmune response causes allograft injury that is mediated by graft-infiltrating T cells and macrophages. The following epithelial damage leads to a reparative process that is characterized by SMC growth factor production. If the early alloimmune response is attenuated by immunosuppressive treatment aimed at T cell activation and proliferation, such as calcineurin inhibition or costimulatory blockade, the development of OB can be prevented. However, after sufficient alloimmune injury, immunosuppressive treatment does not suffice any more but instead, the obliterative process is alloimmune-independent and may only be affected by specific anti-SMC proliferative mechanisms. RCMV infection may be activated by the alloimmune response and, in turn, early RCMV infection promotes the alloimmune response causing increased allograft injury and production of SMC growth factors, such as PDGF. Effective antiviral prophylaxis, high dose immunosuppression, and inhibition of PDGF receptor activity result in attenuation of obliterative changes associated with RCMV infection. The results of this study suggest that rigorous immunosuppression and anti-CMV prophylaxis are called for in the treatment of lung transplant recipients but if BOS develops despite of these measures, antiproliferative agents such as bosentan and imatinib may result in a better outcome than augmentation of immunosuppression alone (Figure 10).

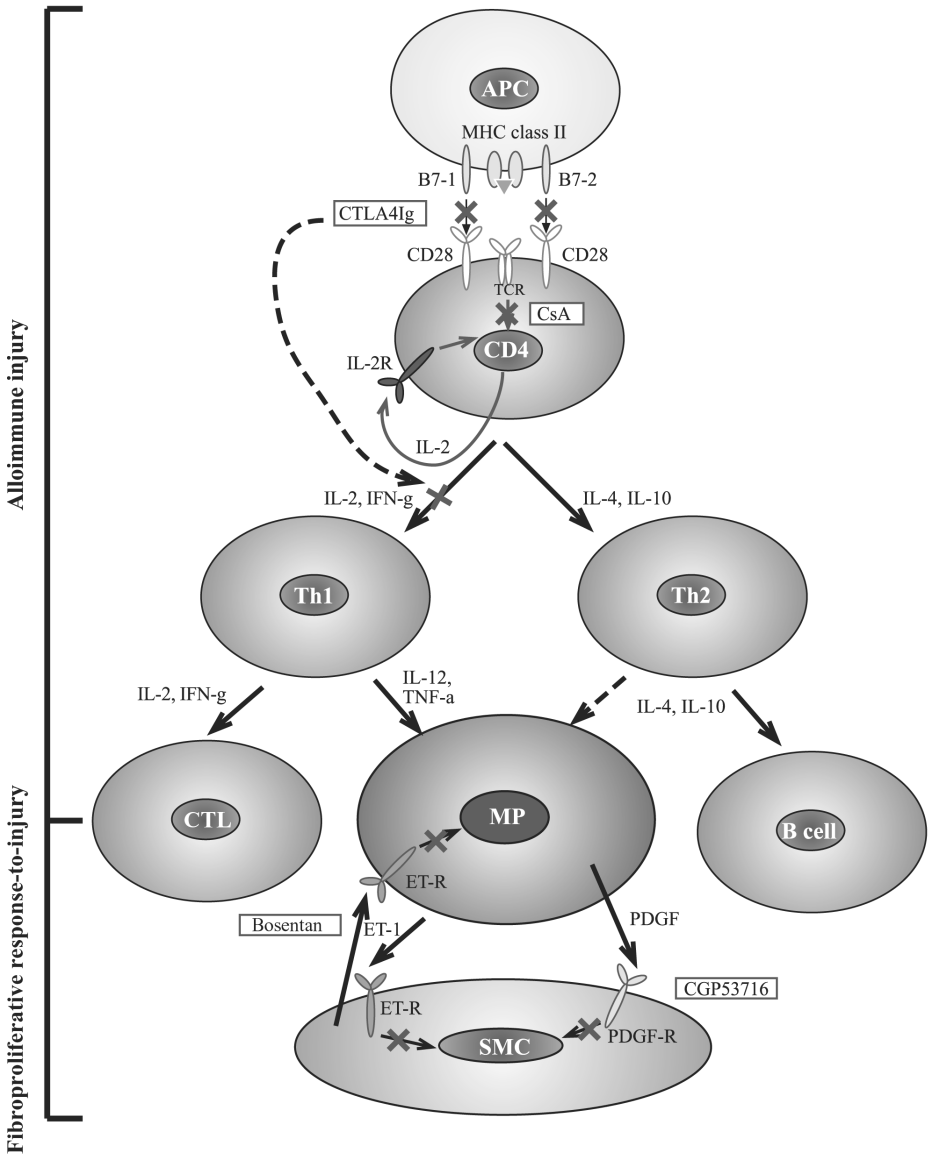

Figure 10. Potential targets of medical intervention in preventing OB. In addition to alloimmune-mediated graft injury, non-alloimmune factors such as ischemia and RCMV infection promote the alloimmune response and cause direct allograft injury. Epithelial cells serve as an important antigen-presenting cell type and fuel the alloimmune response. Prevention of the alloimmune response with immunosuppressive drugs attenuates epithelial cell injury and prevents the development of OB. After sufficient graft injury, the damaged epithelium either fails to inhibit or promotes growth factor production leading to SMC migration and proliferation into the airway lumen. At this time, the process is no longer sensitive to enhanced immunosuppression. Antiproliferative agents such as bosentan and imatinib may provide a novel approach to treatment and prevention of OB by targeting the "response-to-injury" phase. The dashed line depicts an inhibory effect. Abbreviations: APC, antigen-presenting cell; TCR, T cell receptor; CsA, cyclosporine A, CTLA4Ig, cytotoxic T lymphocyte-associated antigen immunoglobulin, Th, helper T cell; CTL, cytotoxic T lymphocyte; MP, macrophage; SMC, smooth muscle cell.

YHTEENVETO (FINNISH SUMMARY)

Keuhkonsiirto on ainoa parantava hoitomuoto loppuvaiheen keuhkotautia sairastavalle potilaalle. Vaikka lyhytaikaisennuste on kohentunut huomattavasti ja 70% keuhkonsiirtopotilaista on elossa vuoden kuluttua siirrosta, keuhkosiirrännäisen kroonisen hyljinnän eli bronkiolitis obliterans-syndrooman (BOS) esiintyvyys ei ole laskenut. BOS on keuhkonsiirtopotilaiden johtava kuolinsyy ensimmäisen vuoden jälkeen, ja valtaosa potilaista sairastuu siihen. BOS ilmenee keuhkoputkien etenevänä ahtautumisena, eikä siihen ole toimivaa hoitoa. BOS:n kaksi tärkeintä riskitekijää ovat akuutti hyljintäreaktio sekä sytomegalovirus (CMV)-infektio. Tässä tutkimuksessa pyrittiin rotan trakeansiirtomallin avulla tunnistamaan BOS:n kehittymiselle tärkeitä molekyyliteitä ja katkaisemaan niitä kokeellisilla täsmälääkkeillä.

Trakeansiirron koe-eläinmallissa luovuttajarotan henkitorvi poistetaan ja siirretään vastaanottajarotan vatsaonteloon. Syngeenisissä (samaperimäinen) siirrännäisissä henkitorven epiteeli vaurioituu ensin lievästi hapenpuutteen vuoksi, mutta toipuu siitä pian. Kuukauden kuluttua siirrosta henkitorvi on täysin avoin, ja sitä verhoaa normaali limaa tuottava hengitystie-epiteeli. Sen sijaan lääkitsemättömissä allogeenisissä (eriperimäinen) siirrännäisissä epiteelin tuhoutuminen jatkuu ja kymmenen päivää siirrosta lähes koko epiteeli on tuhoutunut. Samanaikaisesti siirrännäiseen kehittyy voimakas alloimmuunivaste, johon liittyy tulehdusvälittäjäaineiden, kemokiinien ja kasvutekijöiden tuotanto, mikä puolestaan johtaa sileälihassolujen liikkumiseen henkitorven sisään, niiden jakautumiseen siellä ja lopulta henkitorven tukkeutumiseen. Histologisesti tämä fibroproliferatiivinen leesio muistuttaa ihmisellä BOS:ssa nähtävää löydöstä.

Tämä tutkimus painottaa varhaisen alloimmuunivasteen merkitystä BOS:n kehittymisessä. T-soluaktivaation esto hCTLA4Ig:lla, joka katkaisee T-solujen kostimulaation, johti tulehdusreaktion lievittymiseen ja siirtymään Th1-painotteisesta Th2-painotteiseen immuunivasteeseen. hCTLA4Ig-hoito vähensi myös epiteelivauriota sekä lopulta henkitorven tukkeutumista. Tulokset antavat viitettä siitä, että varhaisen alloimmuunivasteen heikentäminen hCTLA4Ig-hoidolla voisi olla tehokas myös kliinisessä käytössä BOS:n ehkäisyssä (I).

Endoteliini-1 ja sen reseptorien ilmentyminen oli neljä kertaa voimakkaampaa allogeenisissä henkitorvisiirteissä kuin syngeenisissä kontrolleissa. Endoteliini-1 reseptorien salpaus bosentaanilla heikensi alloimmuunivastetta, lievitti epiteelivauriota sekä vähensi sileälihassolukasvua ja johti näin hidastuneeseen BOS:n kehittymiseen henkitorvisiirteissä. Tulokset osoittavat, että endoteliinillä on biologisesti merkittävä rooli BOS:n kehittymisessä ja että bosentaani, joka on jo kliinisessä käytössä, voisi olla hyödyksi myös keuhkonsiirtopotilailla (II).

Kehitimme trakeansiirtomallin, jonka avulla tutkimme rotan sytomegalovirus (RCMV) – infektion ja BOS:n välistä yhteyttä. Tutkimus osoittaa, että RCMV-infektio voimistaa varhaista Th1-painotteista alloimmuunivastetta ja kiihdyttää BOS:n kehittymistä. RCMV-infektio pahensi epiteelivauriota, nopeutti sileälihassolujen jakautumista ja lisäsi PDGF-kasvutekijän tuotantoa. Nämä vaikutukset eivät olleet riippuvaisia virusmääristä, sillä siirrännäisistä pystyttiin tunnistamaan vain muutamia RCMV-infektoituneita soluja. Profylaktinen viruslääkitys joko gansikloviirilla tai RCMV hyperimmuuniseerumilla ehkäisi RCMV-infektion haitalliset vaikutukset, mutta viisi päivää siirron jälkeen näillä lääkkeillä aloitettu hoito ei vaikuttanut BOS:n kehittymiseen. Myös korkea-annoksinen siklosporiinihoito sekä PDGF-reseptorityrosiinikinaasin salpaus CGP53716:lla ehkäisivät RCMV-infektion BOS:n kehittymistä lisäävän vaikutuksen. Tulokset näyttävät, että RCMV-infektio lisää BOS:n kehittymistä epäsuorasti voimistamalla varhaista alloimmuunivastetta ja että vain ennaltaehkäisevä RCMV-infektion hoito estää sen haitalliset vaikutukset (III, IV).

Tutkimuksen tulokset tukevat voimakkaan immunosuppression ja antiviraalisen lääkityksen merkitystä keuhkosiirtopotilaiden hoidossa. Mikäli BOS kehittyy parhaasta mahdollisesta lääkehoidosta huolimatta, sileälihassolujen kasvutekijäreseptorien täsmäsalpaus bosentaanilla ja imatinibilla, CGP53716 uudemmalla johdannaisella, voi parantaa keuhkonsiirtopotilaiden ennustetta.

SAMMANFATTNING (SWEDISH SUMMARY)

Den viktigaste faktorn som försämrar lungtransplantationpatientens långtidsprognos är bronchiolitis obliterans-syndrom (BOS) eller kronisk rejektion. BOS leder till förträngning av luftrör. Det finns ingen effektiv terapi för prevention av BOS. Akuta rejektionsepisoder och cytomegalovirus (CMV) infektion är viktiga riskfaktorer för BOS, för både akut rejektion och CMV infektion förstärker Th1-dominerad immunrespons, som har anknytits till BOS. Avsikten med denna doktorsavhandling var klarläggning av vad som sker på cellulär och molekylär nivå efter trakeatransplantation hos råttor, så väl som nedbrytning av cytokin-växtfaktornätverket med hjälp av specifika och selektiva läkemedel.

I trakeatransplantationsmodellen transplanteras en trakea av DA-råttan till DA- (syngen transplantation) eller till WF-råttan (allogen transplantation). Trakean placeras in i omentum majus av mottagaren. Syrebristen efter syngen transplantation skadar epitelcellerna i trakean till en mild grad. En månad efter transplantationen liknar den histologiska bilden en normal trakea. Epitelcellerna i en allogen trakeatransplant blir totalt nekrotiserade under de första tio dagarna efter transplantationen. Samtidigt ser man en kraftig immunrespons i transplanten, som karakteriseras av produktion av cytokiner, kemokiner och växtfaktorer. Överekspression av sytokiner och växtfaktorer leder till migration av glattmuskelceller från luftrörsväggen till trakeans lumen. Förträngning av luftröret i vår modell liknar den histologiska bilden som observeras vid BOS hos människan.

Vi undersökte rollen av T-cell kostimulering vid utvecklingen av BOS. Behandlingen av råttor efter allogen trakeatransplantation med hCTLA4Ig, som inhiberar CD28/B7 kostimulering, försvagade inflammationen i trakean och förändrade Th1/Th2 kvoten i rikting av Th2-accentuerad immunrespons. Behandling med hCTLA4Ig minskade också epitelcellnekros och utveckling av BOS. Våra resultat poängterar den avgörande vikten av den tidiga alloimmunaktivationen efter lungtransplantation (I).

Vi påvisar, att ekspression av endotelin-1 (ET-1) och dess receptorer var fyra gånger högre i allogena jämfört med syngena trakeatransplanter. Selektiv ET-1 inhibition med bosentan dämpade alloimmunresponsen, epitelnekrosen och proliferationen av glattmuskelceller, vilket ledde till saktad utveckling av BOS. De här resultaten påvisar, att ET-1 spelar en betydlig

biologisk roll vid utvecklingen av BOS. Detta betyder att bosentan, som redan är i klinisk användning, kunde vara av nytta till lungtransplantationspatienter (II).

Vi undersökte betydelsen av råttans cytomegalovirus (RCMV) infektion vid utvecklingen av eksperimentell BOS. Vi påvisade, att RCMV infektion förstärkte Th1-dominerad immunrespons och accelererade BOS. RCMV-infektionen förvärrade epitelskadan, accelererade glattmuskelcellernas proliferation och ökade PDGF-faktorns ekspression. De här effekterna var oberoende av virusmängderna därför att man kunde identifiera bara några få RCMV-infekterade celler i transplanterna. Profylax med antingen gansiklovir eller RCMV-hyperimmunserum inhiberade RCMV-infektionen skadliga konsekvenser, men om behandlingen med dessa läkemedel startade fem dagar efter transplantationen hade den ingen effekt på BOS. Högdoserad siklosporinbehandling och hämning av PDGF-reseptorer med CGP53716 inhiberade utvecklingen av BOS. Våra resultat påvisar, att RCMV-infektionen accelererar utvecklingen av BOS indirekt genom att förstärka den tidiga alloimmunresponsen och att bara RCMV-profylax hindrar dess skadliga påföljder (III,IV).

Resultaten stöder vikten av effektiv immunosuppresiv och antiviral behandling efter lungtransplantation. Om BOS ändå utveklas, kan nedbrytning av växfaktornätverket med specifika läkemedel som bosentan och imatinib förbättra patienternas långtidsprognos.

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REFERENCES

Aarnio P, Ikonen T, Fyhrquist F, Taskinen E and Harjula A. 1998. Raised endothelin content in bronchoalveolar lavage fluid during lung allograft rejection in pigs. Scandinavian Cardiovascular Journal. 32:301-304.

Aarnio P, Tukiainen P, Taskinen E, Harjula A and Fyhrquist F. 1996. Endothelin in bronchoalveolar lavage fluid is increased in lung-transplanted patients. Scandinavian Journal of Thoracic & Cardiovascular Surgery. 30:113-116.

Adams BF, Berry GJ, Huang X, Shorthouse R, Brazelton T and Morris RE. 2000. Immunosuppressive therapies for the prevention and treatment of obliterative airway disease in heterotopic rat trachea allografts. Transplantation. 69:2260-2266.

Adams BF, Brazelton T, Berry GJ and Morris RE. 2000b. The role of respiratory epithelium in a rat model of obliterative airway disease. Transplantation. 69:661-664.

Akalin E, Sehgal V, Ames S, Hossain S, Daly L, Barbara M and Bromberg JS. 2003. Cytomegalovirus disease in high-risk transplant recipients despite ganciclovir or valganciclovir prophylaxis. Am J Transplant 3:731-735.

al-Dossari GA, Jessurun J, Bolman RM, 3rd, Kshettry VR, King MB, Murray JJ and Hertz MI. 1995. Pathogenesis of obliterative bronchiolitis. Possible roles of platelet-derived growth factor and basic fibroblast growth factor. Transplantation. 59:143-145.

Antoniades HN, Bravo MA, Avila RE, Galanopoulos T, Neville-Golden J, Maxwell M and Selman M. 1990. Platelet-derived growth factor in idiopathic pulmonary fibrosis. Journal of Clinical Investigation 86:1055-1064.

Antoniades HN, Scher CD and Stiles CD. 1979. Purification of human platelet-derived growth factor. Proceedings of the National Academy of Sciences of the United States of America. 76:1809-1813.

Appelbaum FR. 2003. The current status of hematopoietic cell transplantation. Annual Review of Medicine 54:491-512.

Aris RM, Walsh S, Chalermskulrat W, Hathwar V and Neuringer IP. 2002. Growth factor upregulation during obliterative bronchiolitis in the mouse model. American Journal of Respiratory & Critical Care Medicine. 166:417-422.

Asahara T, Bauters C, Pastore C, Kearney M, Rossow S, Bunting S, Ferrara N, Symes JF and Isner JM. 1995. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in balloon-injured rat carotid artery. Circulation. 91:2793-2801.

Aubert JD, Pare PD, Hogg JC and Hayashi S. 1997. Platelet-derived growth factor in bronchiolitis obliterans-organizing pneumonia. American Journal of Respiratory & Critical Care Medicine. 155:676-681.

Azuma H, Chandraker A, Nadeau K, Hancock WW, Carpenter CB, Tilney NL and Sayegh MH. 1996. Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection. Proceedings of the National Academy of Sciences of the United States of America 93:12439-12444.

Bailey TC, Trulock EP, Ettinger NA, Storch GA, Cooper JD and Powderly WG. 1992. Failure of prophylactic ganciclovir to prevent cytomegalovirus disease in recipients of lung transplants. J Infect Dis 165:548-552.

Bando K, Paradis IL, Komatsu K, Konishi H, Matsushima M, Keena RJ, Hardesty RL, Armitage JM and Griffith BP. 1995. Analysis of time-dependent risks for infection, rejection, and death after pulmonary transplantation. Journal of Thoracic & Cardiovascular Surgery 109:49-57; discussion 57-49.

Bando K, Paradis IL, Similo S, Konishi H, Komatsu K, Zullo TG, Yousem SA, Close JM, Zeevi A and Duquesnoy RJ. 1995b. Obliterative bronchiolitis after lung and heart-lung transplantation. An analysis of risk factors and management. Journal of Thoracic & Cardiovascular Surgery 110:4-13; discussion 13-14.

Bankier AA, Van Muylem A, Knoop C, Estenne M and Gevenois PA. 2001. Bronchiolitis obliterans syndrome in heart-lung transplant recipients: diagnosis with expiratory CT. Radiology 218:533-539.

Barnes PJ. 1994. Endothelins and pulmonary diseases. Journal of Applied Physiology 77:1051-1059.

Barrett TB, Shu G and Clark EA. 1991. CD40 signaling activates CD11a/CD18 (LFA-1)-mediated adhesion in B cells. J Immunol 146:1722-1729.

Baudet EM, Dromer C, Dubrez J, Jougon JB, Roques X, Velly JF, Deville C and Couraud L. 1996. Intermediate-term results after en bloc double-lung transplantation with bronchial arterial revascularization. Bordeaux Lung and Heart-Lung Transplant Group. Journal of Thoracic & Cardiovascular Surgery 112:1292-1299; discussion 1299-1300.

Beisser PS, Kaptein SJ, Beuken E, Bruggeman CA and Vink C. 1998. The Maastricht strain and England strain of rat cytomegalovirus represent different betaherpesvirus species rather than strains. Virology 246:341-351.

Belperio JA, DiGiovine B, Keane MP, Burdick MD, Ying Xue Y, Ross DJ, Lynch JP, 3rd, Kunkel SL and Strieter RM. 2002. Interleukin-1 receptor antagonist as a biomarker for bronchiolitis obliterans syndrome in lung transplant recipients. Transplantation. 73:591-599.

Belperio JA, Keane MP, Burdick MD, Lynch JP, 3rd, Xue YY, Berlin A, Ross DJ, Kunkel SL, Charo IF and Strieter RM. 2001. Critical role for the chemokine MCP-1/CCR2 in the pathogenesis of bronchiolitis obliterans syndrome. Journal of Clinical Investigation. 108:547-556.

Belperio JA, Keane MP, Burdick MD, Lynch JP, 3rd, Xue YY, Li K, Ross DJ and Strieter RM. 2002b. Critical role for CXCR3 chemokine biology in the pathogenesis of bronchiolitis obliterans syndrome. Journal of Immunology. 169:1037-1049.

Bittner HB, Kendall SW, Chen EP, Craig D and Van Trigt P. 1995. The effects of brain death on cardiopulmonary hemodynamics and pulmonary blood flow characteristics. Chest. 108:1358-1363.

Boehler A, Bai XH, Liu M, Cassivi S, Chamberlain D, Slutsky AS and Keshavjee S. 1999. Upregulation of T-helper 1 cytokines and chemokine expression in post-transplant airway obliteration. American Journal of Respiratory & Critical Care Medicine. 159:1910-1917.

Boehler A, Chamberlain D, Xing Z, Slutsky AS, Jordana M, Gauldie J, Liu M and Keshavjee S. 1998. Adenovirus-mediated interleukin-10 gene transfer inhibits post-transplant fibrous airway obliteration in an animal model of bronchiolitis obliterans. Human Gene Therapy. 9:541-551.

Boehler A and Estenne M. 2003. Post-transplant bronchiolitis obliterans. European Respiratory Monograph 26:158-178.

Boscoe MJ, Goodwin AT, Amrani M and Yacoub MH. 2000. Endothelins and the lung. Int J Biochem Cell Biol 32:41-62.

Bowden RA, Ljungman P and Paya CV. 1998. Transplant Infections. Lippincott-Raven Publishers.

Bruggeman CA, Meijer H, Bosman F and van Boven CP. 1985. Biology of rat cytomegalovirus infection. Intervirology 24:1-9.

Buchdunger E, Zimmermann J, Mett H, Meyer T, Muller M, Regenass U and Lydon NB. 1995. Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. Proceedings of the National Academy of Sciences of the United States of America 92:2558-2562.

Campos L, Naji A, Deli BC, Kern JH, Kim JI, Barker CF and Markmann JF. 1995. Survival of MHC-deficient mouse heterotopic cardiac allografts. Transplantation. 59:187-191.

Carlquist JF, Edelman L, Bennion DW and Anderson JL. 1999. Cytomegalovirus induction of interleukin-6 in lung fibroblasts occurs independently of active infection and involves a G protein and the transcription factor, NF-kappaB. J Infect Dis 179:1094-1100.

Chalermskulrat W, Neuringer IP, Schmitz JL, Catellier DJ, Gurka MJ, Randell SH and Aris RM. 2003. Human leukocyte antigen mismatches predispose to the severity of bronchiolitis obliterans syndrome after lung transplantation. Chest 123:1825-1831.

Channick RN, Simonneau G, Sitbon O, Robbins IM, Frost A, Tapson VF, Badesch DB, Roux S, Rainisio M, Bodin F and Rubin LJ. 2001. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. Lancet 358:1119-1123.

Charpin JM, Stern M, Grenet D and Israel-Biet D. 2000. Insulinlike growth factor-1 in lung transplants with obliterative bronchiolitis. Am J Respir Crit Care Med 161:1991-1998.

Chen SJ, Chen YF, Meng QC, Durand J, Dicarlo VS and Oparil S. 1995. Endothelin-receptor antagonist bosentan prevents and reverses hypoxic pulmonary hypertension in rats. Journal of Applied Physiology 79:2122-2131.

Chevalier MS and Johnson DC. 2003. Human cytomegalovirus US3 chimeras containing US2 cytosolic residues acquire major histocompatibility class I and II protein degradation properties. J Virol 77:4731-4738.

Cohen MH, Dagher R, Griebel DJ, Ibrahim A, Martin A, Scher NS, Sokol GH, Williams GA and Pazdur R. 2002. U.S. Food and Drug Administration drug approval summaries: imatinib mesylate, mesna tablets, and zoledronic acid. Oncologist 7:393-400.

Cooper JD, Billingham M, Egan T, Hertz MI, Higenbottam T, Lynch J, Mauer J, Paradis I, Patterson GA and Smith C. 1993. A working formulation for the standardization of nomenclature and for clinical staging of chronic dysfunction in lung allografts. International Society for Heart and Lung Transplantation. Journal of Heart & Lung Transplantation 12:713-716.

Corry DB, Reiner SL, Linsley PS and Locksley RM. 1994. Differential effects of blockade of CD28-B7 on the development of Th1 or Th2 effector cells in experimental leishmaniasis. Journal of Immunology. 153:4142-4148.

Daar AS, Fuggle SV, Fabre JW, Ting A and Morris PJ. 1984. The detailed distribution of MHC Class II antigens in normal human organs. Transplantation. 38:293-298.

Daar AS, Fuggle SV, Fabre JW, Ting A and Morris PJ. 1984b. The detailed distribution of HLA-A, B, C antigens in normal human organs. Transplantation. 38:287-292.

Date H, Lynch JP, Sundaresan S, Patterson GA and Trulock EP. 1998. The impact of cytolytic therapy on bronchiolitis obliterans syndrome. Journal of Heart & Lung Transplantation. 17:869-875.

Davreux CJ, Chu NH, Waddell TK, Mayer E and Patterson GA. 1993. Improved tracheal allograft viability in immunosuppressed rats. Annals of Thoracic Surgery. 55:131-134.

de Perrot M, Liu M, Waddell TK and Keshavjee S. 2003. Ischemia-Reperfusion-induced Lung Injury. Am. J. Respir. Crit. Care Med. 167:490-511.

de Perrot M, Sekine Y, Fischer S, Waddell TK, McRae K, Liu M, Wigle DA and Keshavjee S. 2002. Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation. American Journal of Respiratory & Critical Care Medicine. 165:211-215.

Diamantstein T and Osawa H. 1986. The interleukin-2 receptor, its physiology and a new approach to a selective immunosuppressive therapy by anti-interleukin-2 receptor monoclonal antibodies. Immunological Reviews. 92:5-27.

Diamond DA, Michalski JM, Lynch JP and Trulock EP, 3rd. 1998. Efficacy of total lymphoid irradiation for chronic allograft rejection following bilateral lung transplantation. International Journal of Radiation Oncology, Biology, Physics. 41:795-800.

DiGiovine B, Lynch JP, 3rd, Martinez FJ, Flint A, Whyte RI, Iannettoni MD, Arenberg DA, Burdick MD, Glass MC, Wilke CA, Morris SB, Kunkel SL and Strieter RM. 1996. Bronchoalveolar lavage neutrophilia is associated with obliterative bronchiolitis after lung transplantation: role of IL-8. Journal of Immunology 157:4194-4202.

Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R and Talpaz M. 2001. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. New England Journal of Medicine. 344:1038-1042.

Duncan AJ, Dummer JS, Paradis IL, Dauber JH, Yousem SA, Zenati MA, Kormos RL and Griffith BP. 1991. Cytomegalovirus infection and survival in lung transplant recipients. Journal of Heart & Lung Transplantation 10:638-644; discussion 645-636.

Duncan SR, Grgurich WF, Iacono AT, Burckart GJ, Yousem SA, Paradis IL, Williams PA, Johnson BA and Griffith BP. 1994. A comparison of ganciclovir and acyclovir to prevent cytomegalovirus after lung transplantation. American Journal of Respiratory & Critical Care Medicine 150:146-152.

Dusmet M, Maurer J, Winton T and Kesten S. 1996. Methotrexate can halt the progression of bronchiolitis obliterans syndrome in lung transplant recipients. Journal of Heart & Lung Transplantation. 15:948-954.

Egan JJ, Lomax J, Barber L, Lok SS, Martyszczuk R, Yonan N, Fox A, Deiraniya AK, Turner AJ and Woodcock AA. 1998. Preemptive treatment for the prevention of cytomegalovirus disease: in lung and heart transplant recipients. Transplantation 65:747-752.

Ehrenreich H, Anderson RW, Fox CH, Rieckmann P, Hoffman GS, Travis WD, Coligan JE, Kehrl JH and Fauci AS. 1990. Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. Journal of Experimental Medicine 172:1741-1748.

Eisen HJ, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, Starling RC, Sorensen K, Hummel M, Lind JM, Abeywickrama KH, Bernhardt P and Group RBS. 2003. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. New England Journal of Medicine. 349:847-858.

El-Gamel A, Sim E, Hasleton P, Hutchinson J, Yonan N, Egan J, Campbell C, Rahman A, Sheldon S, Deiraniya A and Hutchinson IV. 1999. Transforming growth factor beta (TGF-beta) and obliterative bronchiolitis following pulmonary transplantation. Journal of Heart & Lung Transplantation. 18:828-837.

Elssner A, Jaumann F, Dobmann S, Behr J, Schwaiblmair M, Reichenspurner H, Furst H, Briegel J and Vogelmeier C. 2000. Elevated levels of interleukin-8 and transforming growth factor-beta in bronchoalveolar lavage fluid from patients with bronchiolitis obliterans syndrome: proinflammatory role of bronchial epithelial cells. Munich Lung Transplant Group. Transplantation. 70:362-367.

Elssner A and Vogelmeier C. 2001. The role of neutrophils in the pathogenesis of obliterative bronchiolitis after lung transplantation. Transplant Infectious Disease. 3:168-176.

Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI and Yousem S. 2002. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. Journal of Heart & Lung Transplantation 21:297-310.

Ettinger NA, Bailey TC, Trulock EP, Storch GA, Anderson D, Raab S, Spitznagel EL, Dresler C and Cooper JD. 1993. Cytomegalovirus infection and pneumonitis. Impact after isolated lung transplantation. Washington University Lung Transplant Group. American Review of Respiratory Disease 147:1017-1023.

Fahrni JA, Berry GJ, Morris RE and Rosen GD. 1997. Rapamycin inhibits development of obliterative airway disease in a murine heterotopic airway transplant model. Transplantation. 63:533-537.

Farver CF, Raychaudhuri B, Malur A, Drazba J, Maurer J, Tubbs R, Mehta AC, Schilz R and Thomassen MJ. 2000. Increased alveolar macrophage nuclear factor-kappa B activation and macrophage inhibitory protein-1alpha levels in lung transplant patients. Transplantation. 70:1599-1603.

Faulds D and Heel RC. 1990. Ganciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. Drugs 39:597-638.

Filep JG, Fournier A and Foldes-Filep E. 1995. Acute pro-inflammatory actions of endothelin-1 in the guinea-pig lung: involvement of ETA and ETB receptors. British Journal of Pharmacology 115:227-236.

Fiser SM, Tribble CG, Long SM, Kaza AK, Kern JA, Jones DR, Robbins MK and Kron IL. 2002. Ischemia-reperfusion injury after lung transplantation increases risk of late bronchiolitis obliterans syndrome. Annals of Thoracic Surgery 73:1041-1047.

Fisher AJ, Gabbay E, Small T, Doig S, Dark JH and Corris PA. 1998. Cross sectional study of exhaled nitric oxide levels following lung transplantation. Thorax. 53:454-458.
Fishman JA and Rubin RH. 1998. Infection in organ-transplant recipients. N Engl J Med 338:1741-1751.

Furukawa Y, Becker G, Stinn JL, Shimizu K, Libby P and Mitchell RN. 1999. Interleukin-10 (IL-10) augments allograft arterial disease: paradoxical effects of IL-10 in vivo. American Journal of Pathology. 155:1929-1939.

Geist LJ, Monick MM, Stinski MF and Hunninghake GW. 1991. The immediate early genes of human cytomegalovirus upregulate expression of the interleukin-2 and interleukin-2 receptor genes. American Journal of Respiratory Cell & Molecular Biology 5:292-296.

Glassberg MK, Ergul A, Wanner A and Puett D. 1994. Endothelin-1 promotes mitogenesis in airway smooth muscle cells. American Journal of Respiratory Cell & Molecular Biology 10:316-321.

Gould DS and Auchincloss H, Jr. 1999. Direct and indirect recognition: the role of MHC antigens in graft rejection. Immunology Today. 20:77-82.

Griffiths PD. 2002. The 2001 Garrod lecture. The treatment of cytomegalovirus infection. J Antimicrob Chemother 49:243-253.

Gu Y, Takao M, Kai M, Lu L, Shimamoto A, Onoda K, Shimono T, Tanaka K, Shimpo H, Shiraishi T and Yada I. 2000. The role of cyclosporine A and interleukin-2 in obliterative airway disease in a rat tracheal transplant model. Annals of Thoracic & Cardiovascular Surgery. 6:224-231.

Haagmans BL, Teerds KJ, van den Eijnden-van Raaij AJ, Horzinek MC and Schijns VE. 1997. Transforming growth factor beta production during rat cytomegalovirus infection. J Gen Virol 78:205-213.

Hahn AW, Resink TJ, Scott-Burden T, Powell J, Dohi Y and Buhler FR. 1990. Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: a novel autocrine function. Cell Regulation 1:649-659.

Hardy JD, Webb WR, Dalton Jr. ML and Walker Jr. GR. 1963. Lung homotransplantation in man. JAMA 186:1065-1074.

Harringer W and Haverich A. 2002. Heart and heart-lung transplantation: standards and improvements. World Journal of Surgery 26:218-225.

Hashimoto M, Nakanishi R, Muranaka H, Umesue M, Eifuku R and Yasumoto K. 2000. Short-course immunosuppression using FK506 for rat tracheal allografts. Journal of Cardiovascular Surgery. 41:487-492.

Heldin CH, Eriksson U and Ostman A. 2002. New members of the platelet-derived growth factor family of mitogens. Archives of Biochemistry & Biophysics 398:284-290.

Heldin CH and Westermark B. 1999. Mechanism of action and in vivo role of platelet-derived growth factor. Physiological Reviews 79:1283-1316.

Hele DJ, Yacoub MH and Belvisi MG. 2001. The heterotopic tracheal allograft as an animal model of obliterative bronchiolitis. Respiratory Research. 2:169-183.

Heng D, Sharples LD, McNeil K, Stewart S, Wreghitt T and Wallwork J. 1998. Bronchiolitis obliterans syndrome: incidence, natural history, prognosis, and risk factors. Journal of Heart & Lung Transplantation. 17:1255-1263.

Hertz MI, Henke CA, Nakhleh RE, Harmon KR, Marinelli WA, Fox JM, Kubo SH, Shumway SJ, Bolman RM, 3rd and Bitterman PB. 1992. Obliterative bronchiolitis after lung transplantation: a fibroproliferative disorder associated with platelet-derived growth factor. Proceedings of the National Academy of Sciences of the United States of America. 89:10385-10389.

Hertz MI, Jessurun J, King MB, Savik SK and Murray JJ. 1993. Reproduction of the obliterative bronchiolitis lesion after heterotopic transplantation of mouse airways. American Journal of Pathology. 142:1945-1951.

Hertz MI, Jordan C, Savik SK, Fox JM, Park S, Bolman RM, 3rd and Dosland-Mullan BM. 1998. Randomized trial of daily versus three-times-weekly prophylactic ganciclovir after lung and heart-lung transplantation. J Heart Lung Transplant 17:913-920.

Hertz MI, Taylor DO, Trulock EP, Boucek MM, Mohacsi PJ, Edwards LB and Keck BM. 2002. The Registry Of The International Society For Heart And Lung Transplantation: Nineteenth Official Report--2002. The Journal of Heart and Lung Transplantation 21:950-970.

Higenbottam T, Otulana BA and Wallwork J. 1990. Transplantation of the lung. Eur Respir J 3:594-605.

Hirata Y, Takagi Y, Fukuda Y and Marumo F. 1989. Endothelin is a potent mitogen for rat vascular smooth muscle cells. Atherosclerosis 78:225-228.

Hirt SW, You XM, Moller F, Boeke K, Kretschmar J, Starke M, Steinmann J and Wottge HU. 1999. The influence of ischemia on the severity of chronic rejection in a rat lung transplant model. Transplantation Proceedings. 31:189-190.

Hirt SW, You XM, Moller F, Boeke K, Starke M, Spranger U and Wottge HU. 1999b. Development of obliterative bronchiolitis after allogeneic rat lung transplantation: implication of acute rejection and the time point of treatment. Journal of Heart & Lung Transplantation. 18:542-548.

Hosenpud JD, Bennett LE, Keck BM, Boucek MM and Novick RJ. 2000. The Registry of the International Society for Heart and Lung Transplantation: seventeenth official report-2000. Journal of Heart & Lung Transplantation. 19:909-931.

Hosenpud JD, Bennett LE, Keck BM, Boucek MM and Novick RJ. 2001. The Registry of the International Society for Heart and Lung Transplantation: eighteenth Official Report-2001. Journal of Heart & Lung Transplantation. 20:805-815.

Iacono AT, Keenan RJ, Duncan SR, Smaldone GC, Dauber JH, Paradis IL, Ohori NP, Grgurich WF, Burckart GJ, Zeevi A, Delgado E, O'Riordan TG, Zendarsky MM, Yousem SA and Griffith BP. 1996. Aerosolized cyclosporine in lung recipients with refractory chronic rejection. American Journal of Respiratory & Critical Care Medicine. 153:1451-1455.

Ibrahim L, Dominguez M and Yacoub M. 1993. Primary human adult lung epithelial cells in vitro: response to interferon-gamma and cytomegalovirus. Immunology. 79:119-124.

Ikonen TS, Brazelton TR, Berry GJ, Shorthouse RS and Morris RE. 2000. Epithelial re-growth is associated with inhibition of obliterative airway disease in orthotopic tracheal allografts in non-immunosuppressed rats. Transplantation. 70:857-863.

Inkinen K, Holma K, Soots A, Krogerus L, Loginov R, Bruggeman C, Ahonen J and Lautenschlager I. 2003. Expression of TGF-beta and PDGF-AA antigens and corresponding mRNAs in cytomegalovirus-infected rat kidney allografts. Transplant Proc 35:804-805.

Israel-Assayag E, Dakhama A, Lavigne S, Laviolette M and Cormier Y. 1999. Expression of costimulatory molecules on alveolar macrophages in hypersensitivity pneumonitis. American Journal of Respiratory & Critical Care Medicine 159:1830-1834.

Jaramillo A, Smith MA, Phelan D, Sundaresan S, Trulock EP, Lynch JP, Cooper JD, Patterson GA and Mohanakumar T. 1999. Development of ELISA-detected anti-HLA antibodies precedes the development of bronchiolitis obliterans syndrome and correlates with progressive decline in pulmonary function after lung transplantation. Transplantation. 67:1155-1161.

Jeppsson A, Tazelaar HD, Miller VM and McGregor CG. 1998. Distribution of endothelin-1 in transplanted human lungs. Transplantation. 66:806-809.

June CH, Ledbetter JA, Gillespie MM, Lindsten T and Thompson CB. 1987. T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. Mol Cell Biol 7:4472-4481.

Kallio EA, Koskinen PK, Aavik E, Buchdunger E and Lemstrom KB. 1999. Role of platelet-derived growth factor in obliterative bronchiolitis (chronic rejection) in the rat. American Journal of Respiratory & Critical Care Medicine 160:1324-1332.

Kallio EA, Koskinen PK, Aavik E, Vaali K and Lemstom KB. 1997. Role of nitric oxide in experimental obliterative bronchiolitis (chronic rejection) in the rat. Journal of Clinical Investigation 100:2984-2994.

Kallio EA, Lemstrom KB, Hayry PJ, Ryan US and Koskinen PK. 2000. Blockade of complement inhibits obliterative bronchiolitis in rat tracheal allografts. American Journal of Respiratory & Critical Care Medicine. 161:1332-1339.

Kanasky WF, Jr., Anton SD, Rodrigue JR, Perri MG, Szwed T and Baz MA. 2002. Impact of body weight on long-term survival after lung transplantation. Chest 121:401-406.

Keenan RJ, Lega ME, Dummer JS, Paradis IL, Dauber JH, Rabinowich H, Yousem SA, Hardesty RL, Griffith BP and Duquesnoy RJ. 1991. Cytomegalovirus serologic status and postoperative infection correlated with risk of developing chronic rejection after pulmonary transplantation. Transplantation 51:433-438.

Keller CA, Cagle PT, Brown RW, Noon G and Frost AE. 1995. Bronchiolitis obliterans in recipients of single, double, and heart-lung transplantation. Chest 107:973-980.

Kelly J, Hurley D and Raghu G. 2000. Comparison of the efficacy and cost effectiveness of pre-emptive therapy as directed by CMV antigenemia and prophylaxis with ganciclovir in lung transplant recipients. J Heart Lung Transplant 19:355-359.

Kelly JL, Albert RK, Wood DE and Raghu G. 1995. Efficacy of a 6-week prophylactic ganciclovir regimen and the role of serial cytomegalovirus antibody testing in lung transplant recipients. Transplantation 59:1144-1147.

Kesten S, Chaparro C, Scavuzzo M and Gutierrez C. 1997. Tacrolimus as rescue therapy for bronchiolitis obliterans syndrome. Journal of Heart & Lung Transplantation. 16:905-912.

King MB, Pedtke AC, Levrey-Hadden HL and Hertz MI. 2002. Obliterative airway disease progresses in heterotopic airway allografts without persistent alloimmune stimulus. Transplantation. 74:557-562.

Kirkman RL, Barrett LV, Gaulton GN, Kelley VE, Koltun WA, Schoen FJ, Ythier A and Strom TB. 1985. The effect of anti-interleukin-2 receptor monoclonal antibody on allograft rejection. Transplantation. 40:719-722.

Koskinen P, Lemstrom K, Kallio E and Hayry P: The inhibitory effect of cyclosporine, 15-deoxyspergualin and mycophenolate mofetil (RS 61443) on the development of bronchiolitis obliterans in rat tracheal allografts. The first international symposium on the etiology and pathobiology of transplant vascular sclerosis. Southampton, Bermuda, 1995

Koskinen PK, Kallio EA, Bruggeman CA and Lemstrom KB. 1997b. Cytomegalovirus infection enhances experimental obliterative bronchiolitis in rat tracheal allografts. American Journal of Respiratory & Critical Care Medicine 155:2078-2088.

Koskinen PK, Kallio EA, Krebs R and Lemstrom KB. 1997. A dose-dependent inhibitory effect of cyclosporine A on obliterative bronchiolitis of rat tracheal allografts. American Journal of Respiratory & Critical Care Medicine 155:303-312.

Koskinen PK, Kallio EA, Tikkanen JM, Sihvola RK, Hayry PJ and Lemstrom KB. 1999. Cytomegalovirus infection and cardiac allograft vasculopathy. Transplant Infectious Disease. 1:115-126.

Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV and Pestka S. 2000. Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci U S A 97:1695-1700.

Kramer MR, Stoehr C, Whang JL, Berry GJ, Sibley R, Marshall SE, Patterson GM, Starnes VA and Theodore J. 1993. The diagnosis of obliterative bronchiolitis after heart-lung and lung transplantation: low yield of transbronchial lung biopsy. Journal of Heart & Lung Transplantation 12:675-681.

Kroshus TJ, Kshettry VR, Savik K, John R, Hertz MI and Bolman RM, 3rd. 1997. Risk factors for the development of bronchiolitis obliterans syndrome after lung transplantation. Journal of Thoracic & Cardiovascular Surgery 114:195-202.

Kupiec-Weglinski JW, Padberg W, Uhteg LC, Towpik E, Lord RH, Ma L, Diamantstein T, Strom TB and Tilney NL. 1987. Anti-interleukin-2 receptor (IL-2R) antibody against rejection of organ grafts. Transplantation Proceedings. 19:591-593.

Kusaka M, Pratschke J, Wilhelm MJ, Ziai F, Zandi-Nejad K, Mackenzie HS, Hancock WW and Tilney NL. 2000. Activation of inflammatory mediators in rat renal isografts by donor brain death. Transplantation. 69:405-410.

Larche M, Till SJ, Haselden BM, North J, Barkans J, Corrigan CJ, Kay AB and Robinson DS. 1998. Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. Journal of Immunology 161:6375-6382.

Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C, Cho HR, Aruffo A, Hollenbaugh D, Linsley PS, Winn KJ and Pearson TC. 1996. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 381:434-438.

Lechler RI, Lombardi G, Batchelor JR, Reinsmoen N and Bach FH. 1990. The molecular basis of alloreactivity. Immunology Today. 11:83-88.

Lee ES, Gotway MB, Reddy GP, Golden JA, Keith FM and Webb WR. 2000. Early bronchiolitis obliterans following lung transplantation: accuracy of expiratory thin-section CT for diagnosis. Radiology 216:472-477.
Lemstrom KB, Aho PT, Bruggeman CA and Hayry PJ. 1994. Cytomegalovirus infection enhances mRNA expression of platelet-derived growth factor-BB and transforming growth factor-beta 1 in rat aortic allografts. Possible mechanism for cytomegalovirus-enhanced graft arteriosclerosis. Arterioscler Thromb 14:2043-2052.

Lemstrom KB, Bruning JH, Bruggeman CA, Koskinen PK, Aho PT, Yilmaz S, Lautenschlager IT and Hayry PJ. 1994. Cytomegalovirus infection-enhanced allograft arteriosclerosis is prevented by DHPG prophylaxis in the rat. Circulation 90:1969-1978.

Lemstrom KB, Bruning JH, Bruggeman CA, Lautenschlager IT and Hayry PJ. 1994. Triple drug immunosuppression significantly reduces immune activation and allograft arteriosclerosis in cytomegalovirus-infected rat aortic allografts and induces early latency of viral infection. Am J Pathol 144:1334-1347.

Lenschow DJ, Zeng Y, Hathcock KS, Zuckerman LA, Freeman G, Thistlethwaite JR, Gray GS, Hodes RJ and Bluestone JA. 1995. Inhibition of transplant rejection following treatment with anti-B7-2 and anti-B7-1 antibodies. Transplantation 60:1171-1178.

Lesslauer W, Koning F, Ottenhoff T, Giphart M, Goulmy E and van Rood JJ. 1986. T90/44 (9.3 antigen). A cell surface molecule with a function in human T cell activation. Eur J Immunol 16:1289-1296.

Leung AN, Fisher K, Valentine V, Girgis RE, Berry GJ, Robbins RC and Theodore J. 1998. Bronchiolitis obliterans after lung transplantation: detection using expiratory HRCT. Chest. 113:365-370.

Lin H, Bolling SF, Linsley PS, Wei RQ, Gordon D, Thompson CB and Turka LA. 1993. Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. Journal of Experimental Medicine 178:1801-1806.

Linsley PS and Ledbetter JA. 1993. The role of the CD28 receptor during T cell responses to antigen. Annual Review of Immunology. 11:191-212.

Ljungman P, Griffiths P and Paya C. 2002. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 34:1094-1097.

Lu KC, Jaramillo A, Lecha RL, Schuessler RB, Aloush A, Trulock EP, Mendeloff EN, Huddleston CB, Alexander Patterson G and Mohanakumar T. 2002. Interleukin-6 and interferon-gamma gene polymorphisms in the development of bronchiolitis obliterans syndrome after lung transplantation. Transplantation. 74:1297-1302.

Magnan A, Mege JL, Escallier JC, Brisse J, Capo C, Reynaud M, Thomas P, Meric B, Garbe L, Badier M, Viard L, Bongrand P, Giudicelli R, Metras D, Fuentes P, Vervloet D and Noirclerc M. 1996. Balance between alveolar macrophage IL-6 and TGF-beta in lung-transplant recipients. Marseille and Montreal Lung Transplantation Group. American Journal of Respiratory & Critical Care Medicine. 153:1431-1436.

Magro CM, Deng A, Pope-Harman A, James Waldman W, Bernard Collins A, Adams PW, Kelsey M and Ross P. 2002. Humorally mediated posttransplantation septal capillary injury syndrome as a common form of pulmonary allograft rejection: a hypothesis. Transplantation. 74:1273-1280.

Mal H, Dehoux M, Sleiman C, Boczkowski J, Leseche G, Pariente R and Fournier M. 1998. Early release of proinflammatory cytokines after lung transplantation. Chest. 113:645-651.

Markmann JF, Bassiri H, Desai NM, Odorico JS, Kim JI, Koller BH, Smithies O and Barker CF. 1992. Indefinite survival of MHC class I-deficient murine pancreatic islet allografts. Transplantation. 54:1085-1089.

Martinet Y, Rom WN, Grotendorst GR, Martin GR and Crystal RG. 1987. Exaggerated spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. New England Journal of Medicine. 317:202-209.

Mason D. 1988. The roles of T cell subpopulations in allograft rejection. Transplantation Proceedings 20:239-242.

Maurer JR, Snell G, deHoyos A, Kesten S and Winton T. 1993. Outcomes of lung transplantation using three different cytomegalovirus prophylactic regimens. Transplant Proc 25:1434-1435.

McArthur JG and Raulet DH. 1993. CD28-induced costimulation of T helper type 2 cells mediated by induction of responsiveness to interleukin 4. Journal of Experimental Medicine. 178:1645-1653.

McDyer JF, Li Z, John S, Yu X, Wu CY and Ragheb JA. 2002. IL-2 receptor blockade inhibits late, but not early, IFN-gamma and CD40 ligand expression in human T cells: disruption of both IL-12-dependent and -independent pathways of IFN-gamma production. Journal of Immunology. 169:2736-2746.

Michelson S, Dal Monte P, Zipeto D, Bodaghi B, Laurent L, Oberlin E, Arenzana-Seisdedos F, Virelizier JL and Landini MP. 1997. Modulation of RANTES production by human cytomegalovirus infection of fibroblasts. J Virol 71:6495-6500.

Minamoto K and Pinsky DJ. 2002. Recipient iNOS but not eNOS deficiency reduces luminal narrowing in tracheal allografts. Journal of Experimental Medicine. 196:1321-1333.

Moore TM, Khimenko P, Adkins WK, Miyasaka M and Taylor AE. 1995. Adhesion molecules contribute to ischemia and reperfusion-induced injury in the isolated rat lung. Journal of Applied Physiology. 78:2245-2252.

Mosmann TR, Cherwinski H, Bond MW, Giedlin MA and Coffman RL. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348-2357.

Myers JL and Katzenstein AL. 1988. Epithelial necrosis and alveolar collapse in the pathogenesis of usual interstitial pneumonia. Chest. 94:1309-1311.

Naidu B, Krishnadasan B, Whyte RI, Warner RL, Ward PA and Mulligan MS. 2002. Regulatory Role of IL-10 in Experimental Obliterative Bronchiolitis in Rats. Experimental and Molecular Pathology 73:164-170.

Neuringer IP, Aris RM, Burns KA, Bartolotta TL, Chalermskulrat W and Randell SH. 2002. Epithelial kinetics in mouse heterotopic tracheal allografts. American Journal of Transplantation. 2:410-419.

Neuringer IP, Walsh SP, Mannon RB, Gabriel S and Aris RM. 2000. Enhanced T cell cytokine gene expression in mouse airway obliterative bronchiolitis. Transplantation. 69:399-405.

Niimi M, Pearson TC, Larsen CP, Alexander DZ, Hollenbaugh D, Aruffo A, Linsley PS, Thomas E, Campbell K, Fanslow WC, Geha RS, Morris PJ and Wood KJ. 1998. The role of the CD40 pathway in alloantigen-induced hyporesponsiveness in vivo. Journal of Immunology. 161:5331-5337.

Okada Y, Zuo X-J, Marchevsky AM, Toyoda M, Pass JA, Matloff JM and Jordan SC. 1998. Pre-transplant donor-specific transfusions induce allograft rejection and IL-2 gene expression in the WKY -> F344 functional tolerance model of rat lung transplantation. Transplant Immunology 6:137-146.

Palmer SM, Davis RD, Hadjiliadis D, Hertz MI, Howell DN, Ward FE, Savik K and Reinsmoen NL. 2002. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. Transplantation 74:799-804.

Park SH, Saleh D, Giaid A and Michel RP. 1997. Increased endothelin-1 in bleomycin-induced pulmonary fibrosis and the effect of an endothelin receptor antagonist. American Journal of Respiratory & Critical Care Medicine 156:600-608.

Reichenspurner H, Girgis RE, Robbins RC, Yun KL, Nitschke M, Berry GJ, Morris RE, Theodore J and Reitz BA. 1996. Stanford experience with obliterative bronchiolitis after lung and heart-lung transplantation. Annals of Thoracic Surgery 62:1467-1472; discussion 1472-1463.

Riise GC, Andersson BA, Kjellstrom C, Martensson G, Nilsson FN, Ryd W and Schersten H. 1999. Persistent high BAL fluid granulocyte activation marker levels as early indicators of bronchiolitis obliterans after lung transplant. European Respiratory Journal. 14:1123-1130.

Roitt I, Brostoff J and Male D: Immunology, Churchill Livingstone, 1985

Rosen GM, Pou S, Ramos CL, Cohen MS and Britigan BE. 1995. Free radicals and phagocytic cells. FASEB Journal. 9:200-209.

Ross R, Raines EW and Bowen-Pope DF. 1986. The biology of platelet-derived growth factor. Cell 46:155-169.

Rozengurt N, Springall DR and Polak JM. 1990. Localization of endothelin-like immunoreactivity in airway epithelium of rats and mice. Journal of Pathology 160:5-8.

Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, Pulido T, Frost A, Roux S, Leconte I, Landzberg M and Simonneau G. 2002. Bosentan therapy for pulmonary arterial hypertension. New England Journal of Medicine 346:896-903.

Rubin RH. 2001. Cytomegalovirus in solid organ transplantation. Transplant Infectious Disease. 3:1-5.

Russell ME, Hancock WW, Akalin E, Wallace AF, Glysing-Jensen T, Willett TA and Sayegh MH. 1996. Chronic cardiac rejection in the LEW to F344 rat model. Blockade of CD28-B7 costimulation by CTLA4Ig modulates T cell and macrophage activation and attenuates arteriosclerosis. Journal of Clinical Investigation 97:833-838.

Sakagami K, Ohsaki T, Ohnishi T, Saito S, Matsuoka J and Orita K. 1989. The effect of anti-interleukin 2 monoclonal antibody treatment on the survival of rat cardiac allograft. Journal of Surgical Research. 46:262-266.

Sayegh MH, Akalin E, Hancock WW, Russell ME, Carpenter CB, Linsley PS and Turka LA. 1995. CD28-B7 blockade after alloantigenic challenge in vivo inhibits Th1 cytokines but spares Th2. Journal of Experimental Medicine. 181:1869-1874.

Sayegh MH and Turka LA. 1998. The role of T-cell costimulatory activation pathways in transplant rejection. New England Journal of Medicine. 338:1813-1821.

Schersten H, Aarnio P, Burnett JC, Jr., McGregor CG and Miller VM. 1994. Endothelin-1 in bronchoalveolar lavage during rejection of allotransplanted lungs. Transplantation. 57:159-161.

Scholma J, Slebos DJ, Boezen HM, van den Berg JW, van der Bij W, de Boer WJ, Koeter GH, Timens W, Kauffman HF and Postma DS. 2000. Eosinophilic granulocytes and interleukin-6 level in bronchoalveolar lavage fluid are associated with the development of obliterative bronchiolitis after lung transplantation. American Journal of Respiratory & Critical Care Medicine. 162:2221-2225.

Schwartz RH. 1992. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell. 71:1065-1068.

Schweizer RT, Rovelli M, Palmeri D, Vossler E, Hull D and Bartus S. 1990. Noncompliance in organ transplant recipients. Transplantation 49:374-377.

Scott JP, Higenbottam TW, Sharples L, Clelland CA, Smyth RL, Stewart S and Wallwork J. 1991. Risk factors for obliterative bronchiolitis in heart-lung transplant recipients. Transplantation 51:813-817.

Sedmak DD, Knight DA, Vook NC and Waldman JW. 1994. Divergent patterns of ELAM-1, ICAM-1, and VCAM-1 expression on cytomegalovirus-infected endothelial cells. Transplantation 58:1379-1385.

Serrick C, Adoumie R, Giaid A and Shennib H. 1994. The early release of interleukin-2, tumor necrosis factor-alpha and interferon-gamma after ischemia reperfusion injury in the lung allograft. Transplantation. 58:1158-1162.

Shiraishi T, Yasunami Y, Takehara M, Uede T, Kawahara K and Shirakusa T. 2002. Prevention of acute lung allograft rejection in rat by CTLA4Ig. American Journal of Transplantation. 2:223-228.

Shirasugi N, Adams AB, Durham MM, Lukacher AE, Xu H, Rees P, Cowan SR, Williams MA, Pearson TC and Larsen CP. 2002. Prevention of chronic rejection in murine cardiac allografts: a comparison of chimerism- and nonchimerism-inducing costimulation blockade-based tolerance induction regimens. Journal of Immunology. 169:2677-2684.

Sissons JG, Carmichael AJ, McKinney N, Sinclair JH and Wills MR. 2002. Human cytomegalovirus and immunopathology. Springer Semin Immunopathol 24:169-185.

Smith CR, Jaramillo A, Lu KC, Higuchi T, Kaleem Z and Mohanakumar T. 2001. Prevention of obliterative airway disease in HLA-A2-transgenic tracheal allografts by neutralization of tumor necrosis factor. Transplantation. 72:1512-1518.

Smith MA, Sundaresan S, Mohanakumar T, Trulock EP, Lynch JP, Phelan DL, Cooper JD and Patterson GA. 1998. Effect of development of antibodies to HLA and cytomegalovirus mismatch on lung transplantation survival and development of bronchiolitis obliterans syndrome. Journal of Thoracic & Cardiovascular Surgery. 116:812-820.

Smith PD, Saini SS, Raffeld M, Manischewitz JF and Wahl SM. 1992. Cytomegalovirus induction of tumor necrosis factor-alpha by human monocytes and mucosal macrophages. Journal of Clinical Investigation 90:1642-1648.

Snyder LS, Hertz MI, Peterson MS, Harmon KR, Marinelli WA, Henke CA, Greenheck JR, Chen B and Bitterman PB. 1991. Acute lung injury. Pathogenesis of intraalveolar fibrosis. Journal of Clinical Investigation. 88:663-673.

Soghikian MV, Valentine VG, Berry GJ, Patel HR, Robbins RC and Theodore J. 1996. Impact of ganciclovir prophylaxis on heart-lung and lung transplant recipients. Journal of Heart & Lung Transplantation 15:881-887.

Speich R, Boehler A, Thurnheer R and Weder W. 1997. Salvage therapy with mycophenolate mofetil for lung transplant bronchiolitis obliterans: importance of dosage. Transplantation. 64:533-535.

Speich R, Thurnheer R, Gaspert A, Weder W and Boehler A. 1999. Efficacy and cost effectiveness of oral ganciclovir in the prevention of cytomegalovirus disease after lung transplantation. Transplantation. 67:315-320.

Speir E, Modali R, Huang ES, Leon MB, Shawl F, Finkel T and Epstein SE. 1994. Potential role of human cytomegalovirus and p53 interaction in coronary restenosis [see comments]. Science 265:391-394.

Stanbrook MB and Kesten S. 1999. Bronchial hyperreactivity after lung transplantation predicts early bronchiolitis obliterans. American Journal of Respiratory & Critical Care Medicine. 160:2034-2039.

Steinhoff G, You XM, Steinmuller C, Boeke K, Stals FS, Bruggeman CA and Haverich A. 1995. Induction of endothelial adhesion molecules by rat cytomegalovirus in allogeneic lung transplantation in the rat. Scandinavian Journal of Infectious Diseases - Supplementum 99:58-60.

Streblow DN, Kreklywich C, Yin Q, De La Melena VT, Corless CL, Smith PA, Brakebill C, Cook JW, Vink C, Bruggeman CA, Nelson JA and Orloff SL. 2003. Cytomegalovirus-mediated upregulation of chemokine expression correlates with the acceleration of chronic rejection in rat heart transplants. J Virol 77:2182-2194.

Suga M, Maclean AA, Keshavjee S, Fischer S, Moreira JM and Liu M. 2000. RANTES plays an important role in the evolution of allograft transplant-induced fibrous airway obliteration. American Journal of Respiratory & Critical Care Medicine. 162:1940-1948.

Sundaresan S, Mohanakumar T, Smith MA, Trulock EP, Lynch J, Phelan D, Cooper JD and Patterson GA. 1998. HLA-A locus mismatches and development of antibodies to HLA after lung transplantation correlate with the development of bronchiolitis obliterans syndrome. Transplantation 65:648-653.

Suthanthiran M and Strom TB. 1994. Renal Transplantation. N Engl J Med 331:365-376.

Teder P and Noble PW. 2000. A cytokine reborn? Endothelin-1 in pulmonary inflammation and fibrosis. American Journal of Respiratory Cell & Molecular Biology. 23:7-10.

Tikkanen JM, Krebs R, Nykanen AI, Koskinen PK and Lemstrom KB: Overexpression of VEGF enhances experimental rat tracheal obliterative bronchiolitis. ESOT Congress. Venice, Italy, 2003

Trulock EP, Edwards LB, Taylor DO, Boucek MM, Mohacsi PJ, Keck BM and Hertz MI. 2003. The Registry of the International Society for Heart and Lung Transplantation: Twentieth Official adult lung and heart-lung transplant report--2003. Journal of Heart & Lung Transplantation 22:625-635.

Tsurumi Y, Murohara T, Krasinski K, Chen D, Witzenbichler B, Kearney M, Couffinhal T and Isner JM. 1997. Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. Nature Medicine. 3:879-886.

Tsuyuki S, Tsuyuki J, Einsle K, Kopf M and Coyle AJ. 1997. Costimulation through B7-2 (CD86) is required for the induction of a lung mucosal T helper cell 2 (TH2) immune response and altered airway responsiveness. Journal of Experimental Medicine 185:1671-1679.

Uyama T, Winter JB, Groen G, Wildevuur CR, Monden Y and Prop J. 1992. Late airway changes caused by chronic rejection in rat lung allografts. Transplantation. 54:809-812.

Valantine HA. 2003. Cardiac allograft vasculopathy: central role of endothelial injury leading to transplant "atheroma". Transplantation 76:891-899.

Valantine HA, Gao SZ, Menon SG, Renlund DG, Hunt SA, Oyer P, Stinson EB, Brown BW, Jr., Merigan TC and Schroeder JS. 1999. Impact of prophylactic immediate posttransplant ganciclovir on development of transplant atherosclerosis: a post hoc analysis of a randomized, placebo-controlled study. Circulation 100:61-66.

Valantine HA, Luikart H, Doyle R, Theodore J, Hunt S, Oyer P, Robbins R, Berry G and Reitz B. 2001. Impact of cytomegalovirus hyperimmune globulin on outcome after cardiothoracic transplantation: a comparative study of combined prophylaxis with CMV hyperimmune globulin plus ganciclovir versus ganciclovir alone. Transplantation 72:1647-1652.

van der Zee R, Murohara T, Luo Z, Zollmann F, Passeri J, Lekutat C and Isner JM. 1997. Vascular endothelial growth factor/vascular permeability factor augments nitric oxide release from quiescent rabbit and human vascular endothelium. Circulation. 95:1030-1037.

van Dorp WT, van Wieringen PA, Marselis-Jonges E, Bruggeman CA, Daha MR, van Es LA and van der Woude F. 1993. Cytomegalovirus directly enhances MHC class I and intercellular adhesion molecule-1 expression on cultured proximal tubular epithelial cells. Transplantation 55:1367-1371.

Vink C, Beuken E and Bruggeman CA. 2000. Complete DNA sequence of the rat cytomegalovirus genome. J Virol 74:7656-7665.

Waldman WJ, Knight DA, Adams PW, Orosz CG and Sedmak DD. 1993. In vitro induction of endothelial HLA class II antigen expression by cytomegalovirus-activated CD4+ T cells. Transplantation 56:1504-1512.

Wang X, Huong S-M, Chiu ML, Raab-Traub N and Huang E-S. 2003. Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. Nature 424:456-461.

Weissberg PL, Witchell C, Davenport AP, Hesketh TR and Metcalfe JC. 1990. The endothelin peptides ET-1, ET-2, ET-3 and sarafotoxin S6b are co-mitogenic with platelet-derived growth factor for vascular smooth muscle cells. Atherosclerosis 85:257-262.

Wekerle T, Sayegh MH, Hill J, Zhao Y, Chandraker A, Swenson KG, Zhao G and Sykes M. 1998. Extrathymic T cell deletion and allogeneic stem cell engraftment induced with costimulatory blockade is followed by central T cell tolerance. Journal of Experimental Medicine. 187:2037-2044.

Westall GP, Michaelides A, Williams TJ, Snell GI and Kotsimbos TC. 2003. Bronchiolitis obliterans syndrome and early human cytomegalovirus DNAaemia dynamics after lung transplantation. Transplantation 75:2064-2068.

Wilhelm MJ, Pratschke J, Beato F, Taal M, Kusaka M, Hancock WW and Tilney NL. 2000. Activation of the heart by donor brain death accelerates acute rejection after transplantation. Circulation. 102:2426-2433.

Worthy SA, Park CS, Kim JS and Muller NL. 1997. Bronchiolitis obliterans after lung transplantation: high-resolution CT findings in 15 patients. AJR. American Journal of Roentgenology 169:673-677.

Yamada A, Konishi K, Cruz GL, Takehara M, Morikawa M, Nakagawa I, Murakami M, Abe T, Todo S and Uede T. 2000. Blocking the CD28-B7 T-cell costimulatory pathway abrogates the development of obliterative bronchiolitis in a murine heterotopic airway model. Transplantation 69:743-749.

Yamaguchi T, Shinagawa Y and Pollard RB. 1988. Relationship between the production of murine cytomegalovirus and interferon in macrophages. Journal of General Virology. 69:2961-2971.

Yanagisawa M, Kurihara H, Kimura S, Goto K and Masaki T. 1988. A novel peptide vasoconstrictor, endothelin, is produced by vascular endothelium and modulates smooth muscle Ca2+ channels. Journal of Hypertension 6:S188-191.

Yeh YC, Burns ER, Yeh J and Yeh HW. 1991. Synergistic effects of endothelin-1 (ET-1) and transforming growth factor alpha (TGF-alpha) or epidermal growth factor (EGF) on DNA replication and G1 to S phase transition. Bioscience Reports 11:171-180.

Yoshida Y, Iwaki Y, Pham S, Dauber JH, Yousem SA, Zeevi A, Morita S and Griffith BP. 1993. Benefits of posttransplantation monitoring of interleukin 6 in lung transplantation. Annals of Thoracic Surgery. 55:89-93.

Yousem SA, Berry GJ, Cagle PT, Chamberlain D, Husain AN, Hruban RH, Marchevsky A, Ohori NP, Ritter J, Stewart S and Tazelaar HD. 1996. Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. Journal of Heart & Lung Transplantation 15:1-15.

Yousem SA, Burke CM and Billingham ME. 1985. Pathologic pulmonary alterations in long-term human heart-lung transplantation. Human Pathology. 16:911-923.

Yousem SA, Dauber JA, Keenan R, Paradis IL, Zeevi A and Griffith BP. 1991. Does histologic acute rejection in lung allografts predict the development of bronchiolitis obliterans? Transplantation 52:306-309.

Zamora MR. 2002. Controversies in lung transplantation: management of cytomegalovirus infections. Journal of Heart & Lung Transplantation 21:841-849.

Zheng L, Walters EH, Ward C, Wang N, Orsida B, Whitford H, Williams TJ, Kotsimbos T and Snell GI. 2000. Airway neutrophilia in stable and bronchiolitis obliterans syndrome patients following lung transplantation. Thorax 55:53-59.

Zheng XX, Sayegh MH, Zheng XG, Li Y, Linsley PS, Peach R, Borriello F, Strom TB, Sharpe AH and Turka LA. 1997. The role of donor and recipient B7-1 (CD80) in allograft rejection. Journal of Immunology 159:1169-1173.

Zhou YF, Shou M, Guetta E, Guzman R, Unger EF, Yu ZX, Zhang J, Finkel T and Epstein SE. 1999. Cytomegalovirus infection of rats increases the neointimal response to vascular injury without consistent evidence of direct infection of the vascular wall. Circulation 100:1569-1575.

Zhou YF, Yu ZX, Wanishsawad C, Shou M and Epstein SE. 1999b. The immediate early gene products of human cytomegalovirus increase vascular smooth muscle cell migration, proliferation, and expression of PDGF beta-receptor. Biochemical & Biophysical Research Communications 256:608-613.