BRONCHIOLITIS OBLITERANS

Chronic rejection of the lung allograft is defined as a fibrosing process affecting the lung, which primarily affects the conducting airways and the pulmonary vasculature. The process affecting the conducting airways has been labeled bronchiolitis obliterans, while that affecting the pulmonary arteries and veins has been named graft atherosclerosis/graft phlebosclerosis.

Bronchiolitis Obliterans:

The concept of bronchiolitis obliterans is a difficult one because the term has been utilized as both a morphologic descriptor and clinicopathologic syndrome. Using the lexicon of pathologists, bronchiolitis obliterans(OB) describes an intraluminal polypoid plug of granulation tissue found within the terminal and respiratory bronchioles. This granulation tissue polyp is a very non-specific histologic finding as it is seen in most infectious pneumonias, diffuse alveolar damage, aspiration, usual interstitial pneumonia, cryptogenic organizing pneumonia, among other conditions. To the pulmonologist however bronchiolitis obliterans implies a chronic scarring process affecting the small airways of the lung which results in progressive obliteration of the small airways with resultant obstructive lung disease. In the setting of lung transplantation, we utilize the term bronchiolitis obliterans in a hybrid fashion - in the lung allograft, bronchiolitis obliterans represents dense irreversible eosinophilic scarring of the terminal and respiratory bronchioles which may either partially or totally obliterate the lumen of the airway. By utilizing this definition bronchiolitis obliterans is usually an irreversible process (in contrast to the reversibility of granulation tissue) and therefore has a strong correlation with diminished pulmonary function test scores, primarily forced expiratory volume at one second. It is strongly correlated with the recently proposed clinical grade for the bronchiolitis obliterans syndrome (BOS), which is based on obstructive functional alterations.

Bronchiolitis obliterans may develop in the allograft lung through several means. In the usual proposed sequence (which is assumed but not necessarily mandatory) the bronchioles of the lung have their submucosa infiltrated by a mononuclear cell population, primarily small round, small cleaved, and occasional large lymphoid cells. These lymphocytes home to the basement membrane of the airways and migrate through the basement membrane into the overlying respiratory mucosa. This infiltration may occur as part of acute rejection reactions, lymphocytic bronchitis/ bronchiolitis, or through other pathways. Because of cytotoxic alloreactive injury to the epithelium, individual cell necrosis occurs. When confluent areas of necrosis develop, a fibropurulent exudate forms within the lumen of the airway. This may either be focal and segmental or diffuse throughout the length of the airway, and frequently is associated with skip regions of preserved epithelium. Regions of ulceration are accompanied by the proliferation of fibroblasts, myofibroblasts, and endothelial cells which migrate from the denuded submucosa into the fibropurulent exudate forming intraluminal polypoid masses of loose fibromyxoid tissue. This tissue frequently contains lymphocytes, occasional neutrophils, macrophages,

hemosiderin, and foamy histiocytes. Over time this myxoid tissue is re-epithelialized through the growth of the adjacent metaplastic epithelium which incorporates the loose granulation tissue into the wall of the airway.

Several consequences of this inflammatory reaction are possible. If there is severe epithelial damage and the granulation tissue tethers opposite sides of the airway, the bronchiole may be completely occluded by connective tissue. Over time this granulation tissue may convert into dense eosinophilic scar tissue with total obliteration of the airway lumen. With partial injury to the airway an eccentric or concentric plaque of dense eosinophilic connective tissue may be interposed between the submucosa and the regenerating epithelium. This may require trichrome or elastic tissue stains to highlight these submucosal plaques, whose compromise of the airway diameter increases airway resistance and induces obstructive airway disease. In other instances the granulation tissue of the airway my completely resorb and the airway may return to normal with little evidence of residual scarring. In any scenario, the diagnosis of bronchiolitis obliterans by a pathologist, should be restricted to those cases containing dense eosinophilic scar tissue, not loose myxoid granulation tissue. What determines whether granulation tissue converts into dense scar tissue is unclear. We believe it is in part related to the severity of injury to the airway, to the persistence of the injury, and to the presence of severe basement membrane damage to the respiratory mucosa. Healing may also be affected by the absence of a bronchial circulation.

The development of clinical bronchiolitis obliterans is related to the number of airways affected and the extent of airway injury. For example, if a biopsy demonstrates only one injured airway out of several thousand, one would not expect significant pulmonary function abnormalities. This may account for some instances of "bronchiolitis obliterans" developing in the setting of normal pulmonary function tests. Other times the observation of bronchiolitis obliterans represents a harbinger of a subsequent poor clinical response if for example 100 airways are affected and 120 affected airways are required for detection of pulmonary function abnormalities. Obviously the extent of injury to the airways would have some effect on pulmonary function alterations.

It should be noted that, while the small airways are destroyed, the bronchi (large airways) become dilated, chronically inflamed, and fibrotic. Why bronchi dilate and small airways scar down is unclear - it may simply be due to luminal diameter, but this paradox exists. Ectasia accounts for exuberant production of mucus and its potential fungal colonization. Bronchocentric granulomatosis like reactions to fungi may occur in these cylindrically dilated air passages.

The development of bronchiolitis obliterans is associated with several conditions. First it is related to the number, frequency, and intensity of acute rejection episodes. Second there is a suggestion that bronchiolitis obliterans may be related to previous pulmonary infection, particularly cytomegalovirus infection. Third, there is some evidence that early ischemic damage to the lung may be associated with the development of bronchiolitis obliterans. Milne et al and Yousem have also shown that organizing pneumonia like reactions in airways and airspaces may induce bronchiolitis obliterans.

The development of bronchiolitis obliterans in the allograft lung does not necessarily mean that it is due to immunologic activity. Numerous causes of small airway scarring have been recognized in lung transplant and non-transplant patients. Particularly relevant to the lung allograft recipient is the role of infection. Numerous infectious agents (to which the immunocompromised lung recipient is exposed) have been documented to cause bronchiolitis obliterans. These include many bacterial infections, viral infections, and some atypical organisms, including mycoplasma and chlamydia. In our practice, if we note histologic OB after a non-rejection related inflammatory process, we are certain to note this etiology in our pathology reports so that patients are not treated as for rejection induced OB. Second the development of acute harvest (ischemic) injury to the lung parenchyma has been associated with airway and interstitial scarring. This is particularly relevant in individuals who experience severe diffuse alveolar damage with secondary airway scarring similar to that in pediatric bronchopulmonary dysplasia. Third, because of the loss of their cough reflex lung transplant recipients are predisposed to aspiration. Aspiration has been recognized as one cause of bronchiolitis obliterans and obstructive lung disease, however it is extremely rare to identify aspirated food stuffs in the airways of lung recipients who are several months post-transplant or who have OB.

In the differential diagnosis of bronchiolitis obliterans one must recognize histologic mimics especially to the intra-airway granulation tissue phase of the OB reaction. First acute cellular rejection with small airway inflammation can have perivascular mononuclear infiltrates accompanied by intraairway granulation tissue polyps. It is important to recognize that this granulation tissue reaction is part of the acute lung injury associated with the acute rejection reaction, rather than bronchiolitis obliterans. Second, a few lung transplant recipients have developed bronchiolitis obliterans organizing pneumonia. Again these individuals have granulation tissue within their airways and airspaces and have a rather dramatic response to steroid therapy, much as one would anticipate in an acute rejection reaction. Finally in patients who experience diffuse alveolar damage, the proliferative phase of this reaction is frequently associated with intraluminal granulation tissue polyps. It is particularly important to highlight the need to distinguish loose granulation tissue reactions from dense eosinophilic scarring of the airways, an essential distinction in predicting prognosis.

Numerous immunohistochemical studies have been performed on patients with bronchiolitis obliterans. It has been noted these patients have increased expression of class II antigens by the epithelial cells of the bronchial mucosa. In addition, the cellular infiltrate appears rich in cytotoxic T cells rather than helper T cells, and is accompanied by a significant infiltrate of S100 positive Langerhans cells. Some individuals have also noted that Leu 7 positive natural killer cells are more prominent in bronchiolitis obliterans than other conditions. Recent studies have also indicated that granzyme and perforin producing T cells are more commonly noted in chronic airway rejection.

One of the most difficult problems in the diagnosis of OB is its means of histologic confirmation. The role of transbronchial biopsy(TBBx) has been controversial however at the University of Pittsburgh, TBBx is the method of choice for the diagnosis of OB, having at least a 60% sensitivity rate and 95% specificity. The utilization of TBBx requires several

components, the most important being a pulmonologist who is willing to obtain the necessary five pulmonary parenchymal fragments to achieve good results. Second, a pathologist committed to the interpretation of TBBx is essential. Third, adequate sectioning is necessary - at least three H&E slides at three levels is necessary, and should be accompanied by trichrome, elastic and Grocott stains.

The pathologist must be aware that a slide displaying a well oriented cross section of a membranous or respiratory bronchiole is the exception rather than the rule in a TBBx. One needs to learn to recognize the interrupted fascicles of smooth muscle which define the respiratory bronchiole and to identify the amount of "normal" connective tissue surrounding the muscle and within the submucosa. This is essential because OB frequently is identified as bridging bands of scar tissue crisscrossing airway lumens and obliterating the conducting passage. Bands of blue collagen on trichrome connecting cords of red smooth muscle provide the key to early diagnosis. In our experience, the vast majority of histologic OB cases will have a clinical correlate in new onset pulmonary function abnormalities. In the absence of TBBx confirmation, a repeat TBBx will be performed which increases yield by 10 - 20%. If the diagnosis is still unconfirmed after two TBBx sessions, the patient is either given a clinical diagnosis of OB using the BOS criteria or taken to thoracoscopic wedge biopsy.

It should be emphasized that scarring of the respiratory bronchioles or even alveolar ducts requires that one assumption be made about the donor lung - that it is normal when harvested. In fact, many donor lungs are from cigarette smokers and show evidence of smokers (respiratory) bronchiolitis with fibrosis of the alveolar ducts and airways. This needs to be factored when a diagnosis of OB is considered. Be certain to compare the current biopsy to previous ones, especially the first after transplant where OB is rarely seen.

In the experience of the University of Pittsburgh approximately 30% of our patient population develops clinical and pathologic bronchiolitis obliterans usually late in the first year after transplant. The response of these patients to interventional therapy has been disheartening. Approximately two thirds of the patients experience a progressive unrelenting loss of pulmonary function which may continue for several years. The development of progressive obstruction is frequently accompanied by repetitive infectious episodes, primarily bacterial and fungal, which exacerbates this progressive decline. Ultimately patients expire of an infectious complication. Bronchiolitis obliterans develops on an average of 11 months post-transplant, however it has been noted to develop as early as two months after transplant and as late as several years after transplantation. New therapies are currently being developed to combat this chronic process, and at the University of Pittsburgh we have focused largely on the use of aerosolized cyclosporine.

Graft Atherosclerosis:

Developing hand-in-hand with bronchiolitis obliterans is a progressive myointimal thickening of the pulmonary arteries and veins which corresponds to the graft atherosclerosis seen in the coronary arteries of heart allografts. In fact the development of pulmonary atherosclerosis in heart/lung recipients correlates strongly with the development of coronary artery changes in the

cardiac allograft. The pulmonary vascular changes which develop seem to have little functional impact however and at this point in time are histologic curiosities. I have seen rare cases of marked vasculitis in acute rejection, which caused disproportionate vascular destruction and in these cases pulmonary hypertension was noted. We are unclear what isolated atherosclerosis in a TBBx means, in the absence of OB. It may occasionally be due to donor disease perhaps previous vascular damage from acute rejection, or a harbinger of OB. This finding needs to be further investigated.

Philosophical Aside:

Although we classify acute rejection by the degree of perivascular infiltrates, it is important to recognize that the long term survival of the graft is tied to a progressive airway obliterative process. To many of us, this paradox highlights the need to grade the degree of airway inflammation in acute rejection reactions (B1-B4) and to study airway damage over time in lung allografts. With mononuclear cell damage to epithelium and mesenchyme, there comes a point in time where scarring develops and bronchiolitis obliterans is diagnosed. How this fibrosis develops is a question. Is bronchiolitis obliterans simply an uncontrolled acute rejection? Does bronchiolitis obliterans develop some time after transplantation when specific lymphocytes and mononuclear cells are stimulated and become alloreactive? Is there a low level of airway rejection in all transplant patients that smolders along until enough airways are damaged to cause obstructive function tests? Probably each hypothetical scenario accounts for some cases of chronic rejection.

We also use fibrosis to differentiate bronchiolitis obliterans from the airway damage in acute rejection. In future grading schemes, it may be worthwhile to develop three scales of rejection grade: a perivascular inflammation grade, and airway inflammation grade, and a degree of fibrosis grade which, in the context of the number of vessels and bronchi/bronchioles affected, could generate a numerical score of rejection.