GENESIS AND DIAGNOSIS OF BRONCHIOLITIS OBLITERANS POST-LUNG TRANSPLANTATION: LESSONS LEARNED FROM BRONCHOALVEOLAR LAVAGE

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INTRODUCTION

Over the last decade, lung transplantation has emerged as a therapeutic alternative for selected patients with end stage pulmonary parenchymal and/or vascular discase[1-3]. Technical and carly success has been tempered by the longterm development of obliterative bronchiolitis (OB)[4-5]. OB is defined histologically by the presence of dense subrnucosal eosinophilic collagen in the walls and/or lumens of the airways[6] or clinically as a progressive obstructive and restrictive pulmonary function impairment in the absence of infection, acute rejection or any other abnormality that could impair pulmonary function[7]. A drop in the FEV_1 of greater than 20% from the previous best baseline value is regarded as diagnostic of the bronchiolitis obliterans syndrome. The development of OB is the most significant complication that affects long-term morbidity and mortality because the cumulative probability of developing this complication at 5 years post-transplant has been 50% in the Pittsburgh and 60% in the Stanford series and because recipients who develop this complication experience lower long-term survival compared to recipients without OB[5,8].

THE IMMUNE RESPONSE

Although the cause(s) of post-transplant OB is (are) not fully understood, the bulk of the scientific evidence suggests that this occurs as a result of chronic allograft rejection. The generation of an allogeneic or immune response is the central event involved in the rejection of an allograft by the recipient[9-13]. Donor HLA antigens are presented by donor or recipient antigen presenting cells (APC) macrophages or dendritic cells (DC) to the T lymphocytes of the recipients. Donor derived APCs present donor HLA antigens directly to recipient T lymphocytes while recipient derived APCs present donor HLA antigens to recipient T lymphocytes after they have been processed by the recipient's APC. The direct pathway of antigen presentation stimulates the immune response involved in acute allograft rejection while indirect antigen presentation plays a more important role in chronic rejection.

In the indirect pathway, processed donor HLA antigen is presented by the APC to a T lymphocyte by the binding of specific receptors or ligands on both cells. The interaction between T-cell receptor and APC is facilitated by CD3 peptides and either CD4 receptors which preferentially bind to HLA class II molecules on the APC or CD8 receptors which preferentially bind to HLA class I molecules on the APC. Activation of the T-cell receptor leads to activation of a series of protein tyrosine kinases. These protein tyrosine kinase activate two key early signal transduction pathways; the calcineurin and ras-MAP kinase pathways. In the calcincurin pathway, protein tyrosine kinases activate the inositol phospholipid pathway. This mobilizes calcium from sequestered stores in the endoplasmic reticulum which, in turn, causes a flux of calcium across the cell membrane into the cell. The high level of cytosolic calcium binds to such as calcineurin. The immunosuppressants, cyclosporine and tacrolimus, inhibit the activity of calcineurin.

Activated calcincurin dephosphorylates the nuclear factor of activated T-cells (NFATp) whichis a cytokine synthesis promotor. Dephosphorylated NFATp translocates to the nucleus and binds to consensus sites on several other cytokine promotors such as activated protin-1 (AP-1) and nuclear factor kappa-B (NF-Kb). Both the calcineurin and the ras-MAP kinase pathways activate AP-1. Corticosteroids bound to its receptor inhibit the activation of AP-1 and NF-kB which prevents the transcription of most pro-inflamatory cytokines by lymphocytes and APCs such as IL-1, 2, 3, 4, 5, 6, 8, 11, 12, 13 as well as tumor necrosis alpha (TNF) and δ interferon (IFN) [14,15]. The immunosuppressant, 15-deoxysperqualine, inhibits heat shock or stress proteins which promote the function of NFkB by translocating NF-kB from the cytoplasm to the nucleus.

The production of cytokines indicates that a T-lymphocyte has progressed from a resting (G0) to the activated state (G1). These cytokines engage their receptors on the cell surface which activates other protein tyrosine kinases and sigmal transduction pathways. Stimulation of these pathways causes the T-lymphocyte, to progress from the activated (G1) to the growth phase (S) where DNA synthesis occurs which leads to mitosis and elonal expansion. One of these signal transduction pathways is the "rapamycin sensitive pathway". The steps in this pathway remain poorly understood but it is inhibited by rapamycin which prevents the cell from progressing from the G1 to the S phase.

Lymphocytes that enter the S phase need an adequate supply of purine and pyrimidine nucleotides in order to synthesize DNA. Nucleotides come from recycling via the salvage pathway or from de novo synthesis. Mycophenolic acid inhibits the enzyme, inosine monophosphate dehydrogenase (IMPDH), which is the rate limiting step in de novo purine synthesis. Azathioprine, a purine analogue, inhibits production of nucleotides via the salvage pathway by serving as a substrate for enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGPRT), insted of guanine. This leads to depletion of guanine containing nucleotides and inhibition of DNA synthesis. Mizoribine, brequinar and leflunomide are also competitive inhibitors of enzymes involved in de novo nucleotide synthesis. These drugs arrest cell growth at the end of G1 before DNA synthesis or the S phase of cell proliferation can occur.

After this complex series of biochemical changes, these is an ordered sequence of genetically regulated events that guide the process of T-lymphocyte differentiation. Cytokines produced by T lymphocytes lead to the activation and differentiation of other cell types. For instance, interlerikin 3 stimulates the proliferation of stem cells which, in turn, differentiate into granulocytes and macrophages. INF induces the expression of MHC Class II antigens on inducible cells and triggers macrophage function. IL-4, IL-5 and IL-6 induce the expansion of Blymphocytes which leads to the production of specific antibodies. IL-2 via its receptor stimulates the proliferation of the T lymphocyte that produced it as well as that of nearby cells. CD4 helper inducer lymphocytes are produced if the original binding between T lymphocyte and APC was with FILA Class II molecules and CD8 cytotoxic lymphocytes are produced of the original binding was with an HLA Class I molecule.

Functionally, there are two subsets of CD4 helper lymphocytes, TH1 and TH2, which are characterized by unique patterns of lymphokine secretion that simulate reciprocal immune functions. TH1 lymphocytes elaborate IL2, IFN and TNF and TNFB which promote delayed type hypersensitivity reactions involved in autoimmunity, allograft rejection and cellular responses to infectious pathogens. TH2 lymphocytes elaborate IL4, 5, 6, 10 and 13 which support antibody production involved in allergy and antibody responses to infectious pathogens. The net consequence of citokine production and the acquisition of activation induced cell surface receptors is the emergence of antigen specific graft infiltrating destructive T-cells.

Cytokines produced by these cells facilitate the activation of macrophages and other inflammatory cells and the production of anti-donor antibodies by stimulated B-cells. The allograft infiltrated by these immune and inflammatory cells is eventually destroyed by mechanisms that remain poorly understood.

PATHOGENESIS: ANIMAL MODELS

A lesion histologically similar to that in humans can be produced in long-term rat and swine allograft recipients who receive only minimal perioperative immunosuppression[16-19]. A lesion histologically similar to that in humans can also be produced in mice and rats when tracheal explants from one animal are placed subcutaneously or into the omentum of a different strain of animal from the same species[20-21].

PATHOGENESIS: CLINICAL CORRELATIONS

The primary target of the allogenic response in lung allografts appears to be cells that are epithelial in origin. This also appears to be the primary target of the process of chronic allograft rejection in bone marrow[22-23], liver[24-25] and kidney allografts[26-27]. The prevalence lo this disorder in kidney recipients can be decreased when there are no HLA mismatches between the donor and the recipient[28] and by pre-transplant blood transfusions which appear to induce some degree of tolerance in the recipient to the HLA antigens of the donor[29-30]. This suggets an allogeneic etiology as efforts to increase tolerance or decrease allogeneic influences appear to decrease the prevalence of this disorder. The strongest evidence that post-transplant OB is the consequence of an allogeneic event comes from the observation that the strongest risk factor the development of OB is frequency and severity of previous episodes of acute rejection (AR)[31-32].

Prior CMV disease or severe postoperative airway ischemia have also been associated with subsequent OB but the association is weaker than for acute rejectionll. Gene products of CMV appear to block the ability of cyclosporine to inhibit IL-2 transcription[33-34] . Restoration of IL-2 production despite the presence of eyelosporine or tacrolimus can result in normal T-lymphocyte function with resultant allograft rejection. A relationship between CMV disease and chronic rejection has also been described in heart[35-36] and liver[37] recipients.

Although the mechanism(s) whereby airway ischemia might mediate the development of OB is unknown, an ischemic airway injury is associated with increased expression of Class II HLA antigens on epithelial cells[38] which may[39] or may not[40] increase the risk of acute rejection and subsequent OB. The ischemic injury to epithelial cells may occur more indirectly via an immunologic injury to endothelial cells. An occlusive arterial lesion is frequently associated with the loss of bile ducts in liver[24-25] and of renal tubules in kidney allografts[26-27]. A histologically similar lesion to thar which occurs in cardiac allografts[35-36] also occurs in the coronary arteries and pulmonary arteries and veins of heart-lung recipients with OB[41-42]. Enhanced expression of Class II HLA antigens on endothelial cells has also been observed in lung allografts with OB[43]. Thus, an immune response may be directed against endothelial antigens while results in an occlusive arteriopathy with secondary airway mucosal ischemia. This ischemic injury may up regulate Class II antigens on epithelial cells which leads to a second immune mediated injury directed against epithelial cells.

PATHOGENESIS: CELLULAR EVENTS

Histologic active OB is associated with an infiltration of lymphocytes and neutrophils in the walls and lumen of the airways. Hence, OB has been associated with a significant increase in the proportion and number of neutrophils recovered by bronchoalveolar lavage (BAL)[44]. Although the proportion and the ratio of CD4 to CD8 lymphocytes was increased when OB was present, the differences were not significant[45]. There has been a significant relationship between donor specific alloreactivity of BAL lymphocytes as assessed in the primed lymphocyte test (PLT) and the presence of chronic and especially acute rejection[46-47] and this assay has been significantly predictive of the subsequent involvement of OB[48]. The citotoxic activity of BAL lymphocytes against donor antigen has also been

increased when chronic and aspecially acute rejection has been present[46-47] . The PLT and cytotoxicity of these lymphocytes has correlated well with the same activity of lymphocytes propagated from transbronchial lung biopsies[46-47] . The clinical reliability, however, of the PLT assay has not been sufficiently accurate to allow it to be a reliable indicator of the presence of acute or chronic rejection.

The allograft comes with a full complement of lymphocytes and alveolar macrophages (AM) from the donor. Although most of these cells are replaced by those of the recipient by 12 weeks post-transplant, some donor derived AM and lymphocytes persist in the air spaces of the transplanted lung for many weeks after transplantation[49]. The presence of acute or chronic rejection has been associated with a more rapid transition of AM from donor to recipient phenotype and the degree of PLT activity of BAL lymphocytes has been inversely related to the proportion of donor derived AM in the BAL[50]. Thus, the persistence of donor derived AM in the lung allograft has been associated with less severe acute and absent chronic rejection and diminished alloreactivity of BAL lymphocytes in vitro. The persistence of donor AM in the allograft may be a manifestation of micro chimerism.

The level of IL-6, a pro-inflammatory cytokine produced by macrophages, TH2 lymphocytes, endothelial cells and fibroblasts, was significantly elevated in BAL specimens from 13 episodes of rejection (type unspecified) and especially 24 episodes of cytornegalovirus pneumonia (CMVp) in 27 lung recipients[51]. A significant number of IL-6 gene expressing BAL cells were also recovered from these allografts. There was no correlation between serum and BAL IL-6 levels[51].

The level of soluble receptors for tumor necrosis factor alpha (TNF-SR) but not TNF, another proinflammatory cytokine producted mononuclear phagocytes and THI lymphocytes, was also significantly increased BAL specimens from 5 episodes of unspecified rejection and 4 episodes of CMVp in 29 lung recipients[52] . The serum levels of both TNF and its receptor were both significantly elevated during 12 episodes of CMVp but not during 12 episodes of unspecified rejection. The hypothesis is that soluble receptor probably functions as an anticytokine by forming a complex with the cytokine[52]. The amount of IL-6 and TNF produced by AM recovered by BAL was significantly increased during 8 episodes of bacterial pneumonia, 12 episodes of CMVp, 21 episodes of AR but not in 15 specimens obtained when OB was present[53].

The amount of IL-6 produced by AM recovered by BAL was significantly elevated duling 12 episodes of AR and 9 episodes of CMVp but not after these events or in 19 specimens when OB was present[54]. The amount of transforming growth factor-beta (TGF-B), an anti-inflammatory monokine produced by AM which organizes tissue repair by stimulating synthesis of extracellular matrix proteins and angiogenesis and by inhibiting lymphocyte, fibroblast, epithelial and endothelial replication and proliferation, was significantly increased after 12 episodes of AR, 9 episodes of CMVp and in 19 specimens when OB was present but not during these episodes of AR or CMVp. The amount of TGF-B in lung tissue as assessed by immunocytochemistry was also significantly increased during and after AR, CMVp and especially during OB[54].

The level of soluble receptors for interleukin-2 (IL-2R) in BAL was dignificantly elevated during 12 episodes of AR, but not during 3 episodes of bacterial pneumonia, 9 episodes of CMVp or in 5 specimens when lymphocytic bronchitis, the probable precursor lesion of OB, or in 10 specimens when OB was present[55]. Gene transcripts in BAL cells and/or lung tissue for TNF, IL-2, and IFN were present in 25%, 50% and 50% respectively of 4 specimens obtained when no infection or rejection was present; in 60%, 70% and 0% respectively of 10 specimens obtained when AR was present; in 50%, 83% and 33% respectively of 6 specimens obtained when infection was present; and in 0%, 100% and 100% respectively obtained from a single specimen when OB was presentIl. Thus,

gene expression for these proinflammatory cytokines, while usually present during episodes of infection and rejection, was also frequently present when no rejection or infection could be detected in the allograft. Significantly increased expression of the genes for IL-2, IL-5, TNF-B and especially IL-4 and IL-6 was observed in BAL cells obtained during 7 episodes of AR[57]. Significantly increased expression of the genes for IL-1 and IFN and decreased expression of the genes for IL-5, IL-7 and TNF-B were found in 4 specimens of BAL cells obtained when OB was present[57] . Gene expression for these cytokines in serum was not observed when AR or OB was present[57].

T lymphocyte receptors that binds to APCs via HLA restricted antigen recognition contain B chains (TCRB) while those receptors involved in non-HLA restricted antigen recognition contain chains (TCR). The usual ratio of TCRB to TCR lymphocytes in both blood and BAL in 12 :1-50 : 1. The proportion of TCR lymphocytes in 38 blood and especially in 43 BAL specimens obtained from 24 lung recipients was significantly increased and this was significantly decreased the ratio TCRB to TCR lymphocites in blood and especially in BAL[58]. This occurred in 14 specimens with no infection or rejection, 14 specimens with infection and 6 specimens with AR. While this ratio remained suppressed in 4 BAL specimens associated with OB, that in blood was significantly increased into the normal range due to an increased proportion of TCRB lymphocytes`. This suggests increased HLA restricted antigen recognition by lymphocytes in the blood but not in the allograft when OB was present. Treatment of OB (n=4) or refractory AR (n=5) with aerosol cyclosporine that resulted in histologic improvement correlated best with significant decreased in gene expression of 2-150 fold for IL-6 and 8-90 fold INF[59].

Endothelin- 1 (ET- 1) is a peptide produced by endothelial, epithelial and mononuclear cells such as macrophages and lymphocytes that causes vasoconstriction, bronchoconstriction, smooth muscle and fibroblast proliferation. In dogs, the level of ET-1 in BAL but not blood was significantly increased during 8 episodes of AR and these levels retumed to baseline with treatment of the AR[60]. In 64 BAL specimens obtained from 23 human lung recipients, the level of ET-1 in BAL was significantly increased at all time points up to 104 weeks post-transplant but it did not change when AR, CMVp or other infections were present in the allograft[61]. There is no information available regarding ET-1 levels in BAL or blood before or at the time of OB. Finally, the levels of and gene expression for platelet derived growth factor (P1)GF), a monokine secreted by macrophages which stimulates the migration and growth of fibroblasts, was significantly increased in the BAL of 5 lung recipients with OB[62].

These studies of the levels of cytokines and their gene products (mRNA) in BAL and lung tissue have yielded some conflicting results probably because of the absence of standardized assays, small numbers of observations, difficulties in establishing the diagnosis of OB and exclusion of co-existent infection and the complexities of the microenvironment in the allograft. For instance, the in vitro production of TNF and B by normal human AM and CD is increased in the presence of allogeneic T lymphocytes but this response is suppressed by of IL-10 and/or cyclosporine[63]. However, these studies generally do show that both gene and levels of the pro-inflammatory cytokines are elevated when infection or acute chronic rejection have been present. Also, these cytokines and their genes have generally not been present when no rejection or infection was present in the allograft. Additionally, certain growth factors such as TGF-B and PDGF that modulate the repair process that occurs after injury are increased in the presence of chronic rejection. The pattem of cytokine, production and secretion of growth factors found in BAL pretty much mirrors the expected findings where an immune response generates injury, inflammation and subsequent tissue repair.

SUMMARY

The success of lung transplantation has been tempered by the significant risk of post-transplant OB. The process is almost certainly the consequence of an immune response with subsequent scarring and fibrosis in the

airways as a result of the repir process. The technique of BAL has bcen a powerful tool to decipher immunologic events involved in the pathogenesis but not in the diagnosis of this process. It can also be useful to assess the effectiveness of new forms of immunosuppressant therapy that will become available in the future.

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