The Immunolymphatic Theory of Chronic Rejection

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CHRONIC rejection (CR) primarily manifests itself as a progressive obliterative arteriopathy (OA) that most frequently affects medium-sized muscular arteries of vascularized organ allografts. The lack of similar changes in isografts argues strongly for an immunologic basis. The most widely accepted hypothesis proposed to explain CR suggests that direct immunologic injury to the graft endothelium mediated by alloreactive cells and/or antibodies triggers a vascular wall repair response driven by cytokines and growth factors. It will be referred to here as the "direct injury" model. This response can be made worse by hyperlipidemia or ameliorated by drugs that block the immunologic insult or the vessel wall response.

Despite successful application of this line of reasoning, several morphologic observations on failed allografts with CR are not readily explained by the direct injury model, as illustrated by the following examples: (1) OA preferentially involves medium-sized muscular arteries with accentuation of the changes near first and second order branch points; (2) OA changes are less severe in smaller arteries and veins, despite an antigenically similar endothelium; (3) OA is not a complication of graft-versus-host disease, despite being an immunologic mirror image of rejection; (4) OA is not always preceded by intimal inflammation or endothelial disruption detectable by light microscopy; (5) mechanical factors in medium-sized arteries are not conducive to lymphocyte attachment and exocytosis, except during severe immunologic reactions; (6) the reasons for an antigen-dependent and antigen-independent phases of OA are not readily apparent. Many of these peculiarities suggest that mechanical factors also contribute to the development of OA. Moreover, the direct injury model does not address the immunopathogenic mechanisms responsible for the persistent immunologic injury.

An effort has been made in recent years by our group to reconcile these inconsistencies in clinical tissue samples and a small animal model of CR, which was inadvertently uncovered while conducting experiments on the significance of microchimerism.[1,2] In that model, Brown-Norway (BN) rats are pretreated with a Lewis (LEW) bone marrow (BM) infusion or an orthotopic liver allograft (OLT) and a short, 2-week course of immunosuppression with Tacrolimus.[3] These pretreated recipients are then challenged 100 days later with a heterotopic LEW heart allograft without immunosuppression.

The heart grafts in both groups undergo a transient acute rejection between 5 and 15 days, marked by an influx of inflammation and an upregulation of both Th1- and Th2-type cytokine mRNA as detected by RT-PCR. Although all of the rats are operationally tolerant, in that the heart allografts are accepted and are beating for 100 days,[3] progressive CR and OA develop by 60 to 100 days in the challenge heart grafts of BM-pretreated recipients but not in those conditioned with OLT.

The development of CR and OA in the BM-pretreated group is associated with persistent low-grade inflammation and altered leukocyte trafficking through the heart allograft, loss of hematolymphoid chimerism, and focal disruption of microvascular and larger caliber lymphatic channels.[3] Freedom from CR in the OLT-pretreated group is associated with the persistence of donor MHC class II+ hematolymphoid cells (microchimerism), including OX62+ donor dendritic cells, which are thought to be deleterious to graft survival.

Histopathologic examination of the cardiac allografts in both groups showed successive stages in the development of OA. The early phase was seen in both groups and characterized by arterial adventitial and interstitial mononuclear inflammation, edema, focal disruption of lymphatic channels, segmental medial degeneration of arteries, and the onset of medial myocyte proliferation.[3] A slight increase in medial myocyte proliferation was also seen in isograft controls during the same time, but the magnitude was significantly less than in the allografts.[3] All of these changes occurred in the absence of intimal inflammation and an intact, but hypertrophied endothelium and resulted in mild intimal thickening in the OLT group by 60 days, which was not progressive at 100 days. These observations suggest that factor(s) associated with acute rejection, other than direct damage to the endothelium can contribute to the development of OA.

In contrast, the later phase of OA developed only in the BM-pretreated group. It was characterized by moderate to severe OA by 60 days that progressed by 100 days.[3] The

progressive changes were characterized by a marked increase in myointimal proliferation, and adventitial, medial, and intimal inflammation—some of which appeared to invade in an "outside-in" fashion from the adventitia. There were also nodular aggregates of endocardial and adventitial inflammation that localized near draining lymphatics.

Examination of a series of human liver allografts that failed at progressively longer periods of time after transplantation revealed that arteriopathic changes were most common in medium-sized muscular arteries contained within the liver hilum. During acute rejection, some of the earliest arterial changes were similar to those seen in the above animal model, which include focal medial myocyte degeneration, medial edema, and endothelial cell hypertrophy. These changes appear to be followed by infiltration of macrophages into the media, apparently from the adventitia, and then accumulation of foam cells in the subendothelial space. Eventually, the foam cells are replaced by a subendothelial band of donor myofibroblasts, which is largely devoid of inflammation. However, the foam cells often persist in the deep intima, focally disrupt the media, and cuff the adventitia.

Taken together, these observations suggest that a major driving force for arterial wall remodeling and the development of OA appears early after transplantation and affects the artery in a segmental distribution. We propose that focal disruption of lymphatic drainage is a key pathogenic factor and results in abnormal intraorgan fluid fluxes, as shown before.[4] In our experience, early changes are seen in the media and adventitia of both isografts and allografts, are present in all layers, and are more pronounced in allografts. This driving force can be ameliorated in allografts by the disappearance of inflammation and regrowth of lymphatics. However, it indolently progresses when chronic inflammation and fibrosis permanently alter the lymphatic drainage bed, which in turn would alter leukocyte trafficking through the organ, regardless of the stimulus for inflammation. In such an environment, arterial pulsation alone can likely drive the remodeling, which is made worse if the interstitial fluid contains cytokines.

Lymphatic obstruction alone without organ transplantation can result in arteriopathic changes including edema, medial degeneration, and adventitial fibrosis. This is followed by a shift to anaerobic catabolism of carbohydrate and increased lactate and glycosamine content in the vessel wall, eventually producing decreased distensibility and greater elastic stiffness.[5] Studies done long ago have shown that lymphatics from a renal allograft eventually reconnect to recipient lymph vessels within 10 to 14 days,[6] unless retarded by corticosteroid therapy or disrupted by rejection.[3,6-8] During acute rejection, there is an increased production of lymph fluid and disruption of the lymphatic microvascular endothelial junctions, which retards lymphatic flow.[6-8] The same is true of recurrent[6] or late rejection episodes: Ruggiero et al[9] showed total cessation of lymph flow from late-rejecting lung allografts.

It is not our intention to discount the notion that direct intimal arterial injury can lead to OA: this clearly occurs with intense immunologic injury. Instead, complementary pathways are suggested that may be more common than previously realized. Moreover, this hypothesis incorporates both immunologic and mechanical factors into the pathogenetic scheme.

Last, the persistence of donor hematolymphoid cells in long-term recipients is associated with freedom from progressive CR. Our conclusion at this time is that the robust "tolerogenic" state induced by OLT is a result of the hematolymphoid chimerism. However, the liver could also apply other selection pressures to the recipient T-cell repertoire that could ameliorate the graft inflammation. Thus, our studies suggest a possible mechanism underlying the persistent immunologic injury and a reason for the association between immunologic injury and the development of OA, other than direct endothelial injury.

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