What's Potentially More Effective than a Roto-Rooter and Less Toxic than Drano? Anti-Adhesion Mimetics!

Editorial

It was with a great deal of trepidation that I agreed to comment on the report by Molossi et al. (1) in this issue of *The Journal.* Not being trained as a physician or a cardiologist and never having seen a person with a transplanted heart, I was hesitant (even though the Editors told me that this was my chance "to be famous"). However, once I saw the manuscript, I was more than pleased to make a few remarks.

Despite technical advances, we still find, in the case of cardiac transplants, that within five years, 91% of recipients will suffer severe heart failure (2). This occurs in the absence of marked signs of rejection, and is the result of arterial occlusion due to concentric intimal thickening. As transplantation associated intimal thickening involves many coronary vessels at once, therapies requiring catheterization and mechanical expansion of the vascular lumen are impractical. It is therefore essential to devise nontoxic approaches that can effectively access the entire coronary "plumbing."

In this issue of *The Journal,* Molossi et al. (1) describe a series of experiments in which transplantation arteriopathy is markedly reduced after the application of a synthetic peptide mimetic of the CS1 (connecting segment) of fibronectin. Initially, they hypothesized that "cellular fibronectin plays a pivotal role in the progression of allograft arteriopathy by directing the transendothelial trafficking of inflammatory cells through interactions of the CS1 motif with the VLA-4 integrin." This hypothesis is based upon the rapid advances in our understanding of the relationship between the extracellular matrix and the cell. We now know that extracellular matrices serve as more than a scaffolding upon which cells can stick and/or move. There is considerable information stored within the matrix that determines cell shape, movement, physiology, and differentiation (3). This information is transduced into the cell via specific adhesion receptors, in this case, members of the integrin family. Integrins are heterodimeric transmembrane molecules consisting of an α and a β subunit. There are over two dozen different integrins currently described. The particular integrin of interest here is $\alpha 4\beta 1$ known also as VLA-4 (very late antigen). The molecular and biological properties of VLA-4 were the subject of an excellent recent review in *The Journal* (4).

VLA-4 was originally thought to be expressed by white cells and to facilitate diapedesis. It has recently been shown to be expressed on other cell types particularly in the developing embryo (5). It serves as a receptor for two different ligands, vascular cell adhesion molecule (VCAM-1) and fibronectin. VCAM-1 is expressed on activated endothelial cells and facilitates diapedesis through binding to VLA-4 on lymphocytes, eosinophils, and basophils. Indeed, the administration of monoclonal antibodies specific for VCAM-1 or VLA-4 has been shown to reduce tissue injury following ischemia and reperfusion (3). Fibronectin is a major component of the extracellular matrix with several different binding motifs, the best known includes the RGD peptide (6) recognized by numerous different integrins and of importance in wound healing and thrombogenesis (7). VLA-4, however, interacts with a different motif located in the CS1 alternatively spliced domain of fibronectin (8). The VLA-4 recognition motif includes the amino acid sequence EILDVPST. Soluble peptides mimicking this motif will block VLA-4 mediated lymphocyte binding to fibronectin (8).

The expression of VLA-4 and its ligands is regulated, and occurs most commonly in response to inflammatory cytokines. Stimulated lymphocytes express VLA-4; activated endothelial cells express VCAM-1 and stimulated vascular smooth muscle cells express VLA-4, VCAM-1, and fibronectin. The interaction of VLA-4 on lymphocytes with VCAM-1 on endothelial cells not only results in heterotypic adhesion, but also triggers the release of inflammatory cytokines, and initiates cell division or cell death, depending upon the molecular environment (4). Thus, the hypothesis put forward by Molossi et al. (1) is based upon the concept of a cascade of self-perpetuating events in which VLA-4–VCAM binding facilitates lymphocyte-endothelial cell interactions and movement of white cells across the endothelium into a fibronectin-rich environment. Once there, the lymphocytes adhere to the surrounding fibronectin and release growth factors that subsequently stimulate intimal smooth muscle cell division and migration. This is, at least in part, the result of CS1 fibronectin peptide interacting with up-regulated VLA-4 on the activated intimal smooth muscle cells. To interupt this cascade of events, Molossi et al. synthesized a tetrapeptide (Phenylacetic acid-Leu-Asp-Phe-d-Pro-amide) capable of inhibiting VLA-4–fibronectin binding at less than micromolar concentrations. This peptide, or a scrambled control peptide, was administered daily for one week to rabbits maintained on an elevated cholesterol diet after heterotopic cardiac transplantation. The coronary vessels of both host and donor hearts were examined for evidence of arteriopathy. The results were striking. Coronary vessels of donor hearts grafted into animals receiving the active CS1 peptide exhibited little initial thickening beyond that seen in host coronary arteries. The coronary arteries of donor hearts from control animals receiving the scrambled peptide showed a marked increase in intimal formation and vascular occlusion. Not only was intimal thickening reduced in the treated animals, but there were fewer T cells within the subintimal space, almost undetectable levels of fibronectin was synthesized and little evidence of marked VCAM-1 up regulation was noted when compared to controls.

The point at which the CS1 peptide interrupts intimal thickening has yet to be determined. Perhaps T cells responding to inflammatory stimuli were unable to adhere. Alternatively, in the presence of the CS1 peptide, other integrins could become dominant resulting in rapid T cell migration through the intima without sufficient time for them to release stimulatory cytokines. The CS1 peptide could block smooth muscle cell fibronectin binding and prevent the secondary events required to establish an autocrine pathway for the maintenance of cell replication, migration, and continued biosynthetic and secretory activity. Refinement of this approach will require careful pharmacokinetic analysis of the peptide as well as further in vivo evaluation of CS1 depressed neointimalization. This is especially important in light of conflicting reports of the ability of CS1 peptides

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to block VLA-4-mediated adhesion ot VCAM-1 as well as to fibronectin in vitro (4).

In spite of the fact that intimal thickening was blocked, massive tissue rejection was taking place in the surrounding myocardium. As noted by Molossi et al. (1) peptide therapy was not sufficient to suppress tissue rejection, and therefore, would have to be used in conjunction with immunosuppressants if it were to effectively extend the functional life of the transplanted heart. This raises other questions concerning the half life of the tetrapeptide in vivo. For routine use, it will be necessary to develop second generation nonimmunogenic mimetics able to survive longer in the host, and preferably with increased binding affinities. Given our advances in synthetic chemistry and our increased knowledge of the structure of complex biological molecules, it is likely that the ultimate mimetic will be constructed to structurally mimic the active peptide configuration in solution, but may not, itself, be a polypeptide.

Developmental biologists are discovering new and unanticipated isoforms and functions for adhesion receptors (9), it is likely therefore, that long term exposure to blocking agents may have unexpected side effects. Since ligand mimetics are of relatively low molecular weight and can cross tissue barriers, there are considerations of the effects of such therapies on fertility and embryonic development, particularly at the very early stages of pregnancy when the cardiovascular and nervous system are being assembled. For example, VLA-4 and VCAM-1 were found to be important in placentation and heart development (10–12). Further, successful fertilization likely involves sperm–egg interactions mediated by "disintegrins," molecules with high affinity binding constants that involve ligand–receptor binding mechanisms similar to those employed by the integrins (13). The CS1 peptide might also enhance allograft rejection while maintaining fully patent coronary vessels. It is known that levels of peripheral blood lymphocyte are elevated upon prolonged exposure to monoclonal antibodies against VLA-4 or VCAM-1 (4). This is thought to be due to interference with lymphocyte trafficking through the lymphatic system. Since diapedesis and tissue rejection can evidently continue in the absence of VLA-4 or VCAM-1 function, it could be that levels of immune cell circulation through grafted tissue could also be increased necessitating the use of more aggressive methods to control tissue rejection while maintaining organ function. Further, integrins and their ligands have proven to be involved in numerous other biological processes. There are obvious questions concerning the side effects of extended exposure to bioactive peptides on hematopoiesis, inflammatory responses, and clearing of infectious agents.

In conclusion, the study reported by Molossi et al. (1) in this issue of *The Journal* is highly significant and represents a careful and well-planned first step towards preventing atherogenic changes in grafted organs. Like any good piece of research, however, their studies raise fundamental biological questions to which we have, as yet, no answers. They suggest that there are several different levels at which inflammatory stimuli may act to maintain homeostasis. They also suggest possible approaches to controlling dietary atherosclerosis. They raise new questions with respect to possible alternative pathways of bioregulation via adhesive interactions and emphasize the importance of understanding the signal transduction pathways activated by such adhesive events. Thus, while we look forward to future investigations into the efficacy of peptide mimetic and immunosuppressive therapy to prolong the functional life of grafted tissue, we look forward with greater expectation to insights into new biological interactions or molecular mechanisms which these kinds of experiments reveal.

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