# Perspectives Series: Cell Adhesion in Vascular Biology

## Genetic Manipulation of Vascular Adhesion Molecules in Mice

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Human genetic diseases have provided valuable information about vascular physiology and pathology. Well known examples in the specific area of cell adhesion include the insights into platelet function arising from analyses of Glanzmann's thrombasthenia and von Willebrand's disease and into leukocyte function from studies of the leukocyte adhesion deficiency (LAD) syndromes. Invaluable further information could be gleaned from additional mutations and from combinations of mutations. With the advent of genomic analysis, we can anticipate the discovery and isolation of many other such "disease genes," including those contributing to multigenic traits. However, human mutations obviously cannot be obtained or recombined at will and only those compatible with viability can be at all readily studied. What is needed is a system for exploiting both the wealth of new genetic information and our rapidly deepening understanding of the molecular and cellular bases for cell adhesion, allowing ready generation and manipulation of mutations in the genes for adhesion proteins. Such genetic engineering would allow detailed analyses of the roles of adhesion proteins in normal vascular processes and their involvement in various diseases. Fortunately, such a system now exists and the mouse is it.

Recent developments in molecular genetic analyses of mice make it possible to generate null mutations in any gene of interest; that is now routine and much useful information has already been obtained from such "knockout" mice. Transgenic mice, to which genes have been added, have been available for longer and provide another way of manipulating the genome of mice. Furthermore, it is now becoming possible to generate subtle mutations and tissue-specific or regulatable expression or ablation of specific genes (1, 2). Mice can be readily interbred to combine mutations in multiple genes, providing animal models for multigenic defects. In this brief article, we will consider some of the insights already obtained from studies of mice with mutations in genes for vascular adhesion molecules and, more importantly, will consider the rich possibilities they offer for future understanding of human physiology and disease.

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One area, in which "knockout" mutations in genes for adhesion molecules have already provided useful information, concerns embryonic development, including development of the heart and vasculature and of blood cells (3). Many null mutations in "adhesion genes" are embryonic lethals, frequently because of vascular defects. Some of these defects conform with expectations derived from other forms of analysis but others do not. Several useful general lessons can be drawn from these mutations. First, mutations in adhesion genes have revealed unexpected roles for adhesion molecules in vascular development. Many such embryonic lethal mutations would go unrecognized in humans but, now that increasingly detailed genetic maps of mice and men are available, there is a clear route to identifying genes contributing to birth defects such as heart malformations. Null mutations that cause lethality in mice can point the way to more subtle mutations in the same genes that cause birth defects in people. Mutations modeled on those seen in humans can then be generated in mice to produce a closer match to the human disease. Second, it is sometimes the case that the severity of murine mutations varies significantly depending on the genetic background (strain of mice). This presumably reflects the interaction of the engineered mutation with other genes, which vary between strains. Variations in the severity of disease caused by human mutations can result from similar genetic interactions. Again, advances in genetic mapping should allow identification of the interacting genes, revealing yet other genes involved in vascular development and possibly in genetically based birth defects.

Studies of developmental defects in knockout mice highlight several issues which have caused some confusion and which need to be considered in any genetic analysis. First is the issue of overlapping functions of, or compensation between, genes. If two gene products overlap in function, for example, if both contribute to angiogenesis or leukocyte recruitment, elimination of one of the genes may fail to block the process in question. That does not mean that the ablated gene is not involved in the function, only that it is not essential for that function. A distinct possibility is that elimination of one gene causes upregulation in expression or function of another gene product which compensates for the lost gene product. Some have suggested that these two possible consequences of gene ablation mean that the results are misleading. On the contrary, such results serve to reveal the complexity of the processes under consideration and, when appropriately interpreted and followed by further analyses, can uncover unexpected roles and interactions of genes. Overlapping functions and compensation undoubtedly play roles in the presentation of human diseases and we need to learn about them. Combination of mutations in the genes in question by interbreeding allows one to

probe these issues in detail and represents one of the strengths of molecular genetic analyses in mice. It is not that the approach is misleading. Rather, it is that the systems are complex and what is misleading are simplistic interpretations of the results of genetic manipulations, antibody blocking experiments or any other approach to complex systems. Recent advances in technology offer ways to refine the analyses further, by ablating genes only in specific tissues or cell types or in response to some inducer under experimental control (1, 2, 3). While not yet as routine as straightforward "knockouts," these methods are advancing rapidly and will make it possible to circumvent embryonic lethality (with potential compensatory upregulation of other genes) and will allow considerable refinement of the specificity of gene ablation approaches.

Another issue arising from the results of some gene knockout experiments occurs when the resulting mice show no immediately obvious defects. Does this mean that the gene has no function, that it is "redundant?" Of course not. Failure to observe a phenotype does not mean that one is not present; it may simply mean that the physiological analyses are not yet sufficiently refined to reveal it. Mice, unlike people, do not present themselves at the clinic complaining of pains in their chest or joints or shortness of breath. Subtle defects likely to be representative of many human clinical problems, such as circulatory diseases and inflammation or defects in wound healing or defense against pathogens, will only reveal themselves after experimental challenges. Understanding of the involvement of adhesion molecules in the outcomes of important clinical interventions such as organ transplants or angioplasty also requires further experimentation. There is a significant need for physiological analyses of mutant mice and one can anticipate many new findings forthcoming from such studies.

With these issues in mind, let us consider as examples two areas of vascular biology, in which adhesion plays important roles, and ask what we have already learned from murine mutations and what we can hope to learn in the future. Among the best understood examples of cell adhesion are the interactions of lymphoid and myeloid cells and their precursors with endothelial cells during their normal development and traffic or at sites of infection or inflammation (4, 5). These interactions clearly involve multiple classes of adhesion molecules; selectins and integrins and their respective counterreceptors, carbohydrate-bearing mucins and immunoglobulin superfamily proteins, as well as extracellular matrix molecules such as fibronectin. Each of these families of adhesion receptors has multiple members. Ablations of β2 integrins or of selectin ligands in the human genetic diseases, LADI and LADII, respectively, clearly implicate these two families of adhesion molecules in leukocyte recruitment and function. However, these diseases are rare and difficult to study extensively in people and, furthermore, do not allow a decision as to which specific integrins, selectins or selectin ligands function in individual cases of leukocyte recruitment. This is where the murine systems come into play. In the past four years, mice lacking each of the selectins have been generated. The phenotypes proved that each does, indeed, play a different role in leukocyte traffic but also revealed likely overlaps in functions and these were confirmed by the double knockout of both endothelial selectins (P- and E-; 6, 7). The phenotypes of the double knockout mice are similar to, but somewhat more severe than, those of LADII patients, thought to lack all ligands for these selectins, which may mean that selectins play additional roles or that other unsuspected selectin ligands exist. Mice deficient in all possible combinations of the three selectin genes should be available before too long. Similarly, some selectin ligands and glycosylation enzymes involved in their functions have been knocked out and we can anticipate that the others will eventually be completed along with knockouts of each of the several genes encoding subunits of β2 integrins. This set of mice already is providing material for analyses of the involvement of these different adhesion receptors in various models of inflammation and transplantation and, undoubtedly, they will be subjected to many other models of human clinical conditions in the coming years. Since leukocyte recruitment plays a role not only in inflammation but also in atherosclerosis and in ischemia/reperfusion injuries in myocardial infarction, stroke and organ transplantation, it is easy to imagine considerable medical impact from studies of these mice.

Further refinement of these mouse models is also easy to envisage. Given the development of promoter elements which drive gene expression in specific leukocyte subtypes or selectively in particular vascular beds, it should be possible to express or ablate adhesion receptor genes selectively. Thus, one can anticipate that mice will be developed with defects in targeting of specific subsets of white blood cells and/or to specific sites. These mice (existing and prospective) will serve as versatile models for human genetic diseases and autoimmune or inflammatory conditions, both for understanding their molecular basis and for testing means for controlling them. For example, mutant mice can be used to ask which selectin(s) or integrin(s) play the dominant role(s) in a given inflammatory event and mice showing an inflammatory response (for example because of overexpression of some adhesion molecule or chemoattractant) could be used to test drugs designed to block specific adhesion events. Information about overlapping functions could be used to design and test strategies for deliberate compensatory upregulation and mutant mice could be used as models for testing the efficacy of gene therapy. Another potential application of these mice is in understanding the development and dissemination of lymphoid and myeloid precursors, a key issue in stem cell and bone marrow transplantation. Studies on mice defective in certain integrins have already revealed functions for specific integrins during lymphoid development and homing (3). Further analyses along these lines using mice lacking other integrins, selectins or their counterreceptors will provide more detailed understanding of the key adhesive interactions involved in seeding and retention of blood cell precursors at different sites, allowing manipulation of transfusion and transplantation.

Another area in which cell adhesion receptors clearly play an important role is in hemostasis and thrombosis. We mentioned at the outset the human genetic diseases, Glanzmann's thrombasthenia (GT) and von Willebrand's disease, due to mutations in, respectively, a platelet integrin and an extracellular adhesive ligand of platelets. Although murine models for these two diseases are not yet available, they should be forthcoming soon and other adhesion receptors on platelets should also be ablated in mice, especially given the possibility of megakaryocyte-specific knockouts. Two different integrin subunits can be targets of mutations in GT and one of these (β3) is widely expressed, raising questions about other defects in those patients, since β3 integrins are also thought to be involved in bone remodelling and angiogenesis, key events in osteoporosis, wound healing and cancer. All of these clinically

important processes should be open to experimental studies obviously not possible with GT patients. As in the case of leukocyte adhesion-deficient mice discussed above, mice engineered to lack or misexpress adhesion genes involved in hemostasis and thrombosis should provide valuable experimental material for analysis of the relative importance of different adhesion receptors, for testing drugs targeted against platelet adhesion and as substrates for testing the efficacy of gene therapy. Similarly, comparison of fibrinogen-deficient mice with von Willebrand factor-deficient mice should be informative as to the relative importance of these two platelet adhesion molecules in hemostatic and thrombotic events.

We mentioned earlier the fact that mice can be intercrossed to combine various mutations. This facility is clearly of value in analyzing multigenic disorders. Atherosclerosis and cancer are two important human diseases, both of which are multigenic in origin and both of which involve cell adhesion. Although it is obvious that other genes, having nothing directly to do with cell adhesion, play central roles in these two diseases, mutations in these genes can be combined in mice with genes affecting cell adhesion to test for enhancement or suppression of the disease process. For example, mutations affecting cholesterol clearance can be combined with mutations affecting monocyte adhesion to investigate the role of monocyte recruitment in progression of atherosclerotic lesions and mutations in tumor suppressor genes, predisposing mice to cancer, can be combined with mutations in integrins or selectins or their ligands to investigate the possible roles of these adhesion molecules in metastasis.

While some of the experiments discussed above are intentionally futuristic and speculative, they seem to us realistic. It is clear that, in the 3–4 years since the first reports of mice engineered to lack specific cell adhesion molecules, we have already learned a significant amount from their study. The technology is advancing rapidly; knockouts and transgenics are now routine, subtle mutations are straightforward and cell-type–specific and regulatable induction of mutations and control of gene expression are becoming more common. So, the availability of variously engineered mouse strains will be increasing ever more rapidly. While each of these mouse strains takes a year or more to make, many years of experimentation are required to yield all that can be learned from them. Unlike humans with genetic defects or diseases affecting or involving cell adhesion, mutant mice can be bred in large numbers and can be intercrossed with other mutant strains. They can serve as models for human diseases, as sources of other interacting genes and as substrates for the testing of drugs, transplants and gene therapy prior to their testing in humans. Mice are not people and there will undoubtedly be differences in detail, but their genomes and those of humans are closely related and the genome programs are increasingly revealing genetic parallels. Those same programs will also be yielding a rich harvest of new genes and natural mutations and mice will provide the mammalian test vehicle for their detailed investigation. It seems clear that many insights into human biology and disease will flow from assiduous study of these genetically manipulated mice.

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