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Targeted Mutations in Integrins and their Ligands: Their Implications for Vascular Biology

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Adhesion of cells to one another and to the underlying or surrounding extracellular matrix plays crucial roles in the development, physiology and pathology of all metazoans. This is as true in the vasculature as anywhere else. Indeed, cell adhesion has been most effectively studied in the context of vascular biology; we know more about cell adhesion of vascular cells than about any others and the concepts developed there serve as models for thinking about cell adhesion in other contexts (1).

During development of the vasculature, cell adhesion plays roles in the assembly of the heart and vessels; the cells need to migrate to the correct locations and assemble into tubes, which need to be arrayed correctly. These morphogenetic events rely on cell adhesion; both cell-cell and cell-matrix. If adhesion is improperly regulated during development, abnormalities in the heart or blood vessels can ensue.

During normal functioning of the vasculature, circulating cells such as lymphocytes need to adhere in appropriate locations during their traffic around the body. In response to infection or injury, leukocytes need to adhere and extravasate at sites of infection and platelets need to adhere to damaged vessel walls and to each other to prevent bleeding. When these adhesion processes are defective, disease results; susceptibility to infections or bleeding, respectively. While defective adhesion of blood cells is deleterious, so is excessive adhesion. Inappropriate adhesion of leukocytes or platelets can lead to inflammation or thrombosis, respectively. Therefore, adhesion must be tightly regulated to give sufficient adhesion when needed and to prevent inappropriate or excessive adhesion.

We now understand quite a bit about the adhesion receptors mediating these events and some inferences can be made about how adhesion is regulated (1,2). There are dozens of cell surface receptors contributing to cell adhesion in one system or other. Fortunately many of them fall into a few families. The major families of receptors involved in cell-cell adhesion are members of the *immunoglobulin superfamily* (IgSF) and *cadherins*, both of which can mediate homophilic (like with like) adhesion between cells. IgSF members can also function in heterophilic interactions (between unlike molecules) with members of another family of adhesion receptors, the *integrins* (1–3). IgSF/integrin interactions are important in many instances of blood cell-vessel wall adhesion (see below). Another important set of heterophilic interactions is that between *selectins*, carbohydrate-binding lectins, and their counterreceptors, glycoproteins presenting the relevant carbohydrate recognition motifs (4,5). The main family of cell-matrix adhesion receptors are the *integrins*, and, in this review, we will concentrate on them and, in particular, on what has been learned from studies of mice with mutations in the genes encoding integrins and their ligands (2,6,7).

Integrins in vascular biology

There are around twenty known integrins, each of which is a heterodimer of α and β subunits. There are currently 16 α subunits and 8 β subunits known, but not all combinations occur and not all the 20 which do are relevant for vascular biology (Fig. 1). There is degeneracy in the interactions of

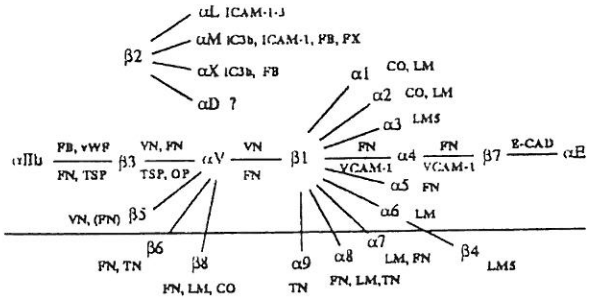

Fig. 1. Integrins of potential relevance in vascular biology. The figure depicts the αβ pairings and major ligand specificities of integrins (2). Those integrins below the line are not known to play any part in the vascular system but are included here for completeness.
Abbreviations: CO, collagen(s); E-cad, E-cadherin; FB, fibrinogen; FN, fibronectin; LM, laminin; OP, osteopontin; TN, tenascin-C; TSP, thrombospondin-1; VN, vitronectin; vWF, von Willebrand factor; iC3b, inactivated C3 component of complement; FX, factor X.

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integrins with their ligands: multiple integrins can recognize a given ligand and many integrins can recognize multiple ligands.

Among the integrins, a subset participates in heterotypic cell-cell adhesion and all these play important roles in vascular biology (2,3). This subset includes the β2 integrins, which bind IgSF molecules such as ICAMs 1-3, the two α4 integrins (α4β1 and α4β7) which recognize the IgSF counterreceptor VCAM-1 (α4β7 also recognizes another cell surface receptor, MAdCAM-1), αvβ3 which is reported to bind to CD31/PECAM-1 (8), and αEβ7, which is only expressed by intraepithelial lymphocytes and binds E-cadherin on epithelial cells (9,10). These cell-cell adhesion-mediating integrins are widely expressed on circulating blood cells and their counterreceptors are expressed on many cell types, including other leukocytes and endothelial cells. The platelet-specific integrin αIIbβ3 also mediates cell-cell adhesion of platelets, by binding the dimeric molecule fibrinogen which can bridge between adjacent platelets (11). The other integrins are concerned with cell-matrix adhesion and many of them , especially β1 and β3 integrins, are expressed on platelets, leukocytes and endothelial cells.

It is clearly a challenge to assign functions to individual integrins in a given vascular adhesion process; cells frequently express multiple integrins with partially overlapping ligand specificities. Various approaches have been taken to address such questions. Much useful information has been gained using antibodies specific for given integrins, their counterreceptors or ligands to block specific adhesion events. Indeed this is how many integrins were first identified. Similarly, those integrins which recognize the sequence RGD in their ligands (the αv and β3 integrins and α5β1) can all be blocked by peptidomimetics based on this sequence. Furthermore, peptidomimetics selective for given integrins in this group have been developed and have been useful in probing their involvement in specific processes (12). Similar approaches have been used to a lesser extent for α4 integrins but have not yet been particularly useful for other integrins, whose binding sites have not been so precisely defined.

Another obvious approach would be to use genetic approaches to ablate specific integrin subunits. In fact, two human genetic diseases have integrin genes as their targets and provided crucial early evidence for the importance of integrins in vascular biology. The bleeding disorder, Glanzmann's thrombasthenia (GT) arises from genetic defects in the genes for the integrin, αIIbβ3, the major fibrinogen receptor on platelets, which plays a central role in hemostasis (11). Studies of αIIbβ3 integrin and of the mutations leading to GT have played an important role in our understanding of integrin function. Similarly, the rare genetic disease, leukocyte adhesion deficiency I, affects the gene for the β2 integrin subunit. LAD I patients lack β2 and, therefore, all β2 integrins and their leukocytes are severely compromised in adhesion and extravasation at sites of infection (13). So far, there are few reports of human mutations affecting other integrin subunits, perhaps because they result in embryonic lethality (see below). An exception is the report of a defect in α2β1 integrin affecting platelet function and leading to a bleeding disorder (14) but the actual genetic defect has not been defined. Well known examples of genetic diseases affecting integrin ligands of importance to vascular function are afibrinogenemia and von Willebrand's disease (15).

Useful as these human mutations have been, they have significant limitations. They are rare diseases and there are obvious limits on the experimental analyses that can be performed on the affected patients. Many integrins lack known human genetic defects and it is not possible to combine even those that are known with other mutations. It would be invaluable to our understanding of integrin functions to have an experimental system allowing genetic analyses of any integrin or combination of integrins. Fortunately, technical advances in the past decade have made possible such genetic manipulations in mice and these have provided much valuable information about the functions of integrins and their ligands in the development and functioning of the vasculature.

Murine mutations in integrins and their ligands

In recent years, "knockout" or null mutations have been made in the genes for most integrins (see Table 1; refs 6,7). Among

Table 1. Integrin Knockout Mutations

β1	Early embryonic lethality. No vasculature
α1	Viable. No obvious vascular defects
*α2	ND
α3	Perinatal lethality. Defects in kidneys, lung and skin. No obvious defects
*α4	Defects in placentation, heart development and lymphocyte and homong
*α5	Embryonic lethality. Defects in embryonic and extraembryonic vasculature
α6	Perinatal lethality. Major skin defects. No obvious vascular defects
α7	Viable. Muscular dystrophy. No reported vascular defects
α8	Perinatal lethality. Defects in kidney development. No reported vascular defects
α9	Early postnatal death; chylothorax
*αv	Embryonic and perinatal lethality. Hemorrhage
*αIIb	ND
*β3	Viable
β4	Perinatal lethality. Major skin defects. No obvious vascular defects
*β5	Viable. No obvious defects
β6	Viable. Inflammation. No obvious vascular defects
β8	ND
*β7	Viable. Defects in gut-associated lymphoid tissue
*αE	Viable. Some defectsin gut-associated lymphoid tissue
*β2	Viable. Defects in inflammatory responses and trans plant rejection
*αL	Viable. Defects in mixed lymphocyte response and allograft rejection
*αM	Viable. Defects in neutrophil apoptosis
*αX	ND
*αD	ND

*marks integrins of relevance to vascular biology.
ND = not done
References are given in the text for those integrins of known relevance to vascular biology. For other references see 6,7.

integrins of potential relevance for vascular biology (Fig. 1) only α2, αIIβ, αX and αD remain unscathed. Equally, many of the relevant extracellular matrix ligands and counterreceptors have been "knocked out" (6).

What have we learned from these mutations? One set of mutations has major effects on vascular development and are lethal mutations. These include mutations in fibronectin and two of its receptor integrins, α5β1 and αv. Fibronectin-null embryos die 2-3 days after implantation with heart defects and defective vasculature, both within the embryo itself and in the yolk sac (16,17). The severity of the defects depends on the genetic background and lies in the execution of cardiogenesis and vasculogenesis (18). The progenitors of cardiac myocytes and endothelial cells are induced at the correct times and places but, to one degree or another, fail to form heart and vessels. The α5-null mutation produces similar but milder defects (19), clearly indicating that another fibronectin receptor must be operating in addition to α5β1. Analyses of α5-null cells suggested that αvβ1 could substitute for α5β1 (20). Furthermore, αv integrins (αvβ3 and αvβ5) have been implicated in vasculogenesis and angiogenesis (21). For these reasons, we ablated the αv gene (Bader and Hynes, unpublished data). Surprisingly, there was extensive development of the heart and vasculature. Around 20% of αv-null progeny develop to term, are born alive but die soon afterward. These pups show hemorrhage in the brain and intestine and further analyses of the αv-null embryos and pups should provide valuable insights into the roles of αv integrins in vascular development. One interpretation would be that much of cardiogenesis and vasculogenesis is αv-independent but that development and/or maintenance of the brain vasculature does depend on αv integrins. It is possible that different forms of angiogenesis exist, which differ in their requirements for av integrins. It is interesting to note that knockouts of β3, β5 and β6 are all viable with no obvious defects in vascular development (Table 1), although more analyses are needed to investigate these issues further and double mutants will need to be generated. It is, furthermore, of some interest that knockouts of most ligands for αv integrins yield viable mice (6); something of a surprise. Overall, the effects of mutations in av integrins or their ligands are all milder than might have been anticipated. This shows that many of these proteins are not *essential* for development, although overlapping or subtle functions are not yet ruled out. It seems plausible to suggest that this set of receptors and ligands may have evolved to play roles in repair processes.

As discussed, αv integrins may be angiogenic in some situations. In contrast, it appears that thrombospondin-1 (TSP) can act as a suppressor of angiogenesis (22,23). TSP can be a ligand for αvβ3 and αIIbβ3 integrins, although it also has other receptors (23). Cells from TSP-null mice show a reduction in their ability to suppress angiogenesis (24). Thus, further analyses of αv and TSP mutant mice should provide insights into the regulation of angiogenesis.

In contrast with the milder than expected phenotype of av integrin mutations, mutations in α4 integrin and VCAM-1 are more deleterious than anticipated (25–27). It had been thought that α4 was predominantly expressed on circulating blood cells and that VCAM-1 (vascular cell adhesion molecule 1) was solely expressed by endothelial cells. α4/VCAM-1-mediated adhesion was thought to be involved primarily in adhesion of lymphocytes, monocytes and eosinophils to inflamed endothelium. However, when α4 and VCAM-1 were knocked out, the phenotypes of the resulting mutants revealed unexpected roles for α4/VCAM-1 interactions in vascular development. Specifically this interaction plays a role in chorio-allantoic adhesion to form the placenta and in the development and maintenance of the epicardium and coronary vessels (25–27). Because of these defects, α4-null embryos die in utero (25) as do the vast majority of VCAM-1-null embryos (26,27).

Apart from FN, α4, α5, αv and VCAM-1, other integrins and their ligands do not appear to play major essential roles in vascular development (see Table 1 and refs 6 and 7). Mutations in several other integrins and ligands do have effects on vascular functions in adult animals. Those integrins which appear to be expressed only by white blood cells (α4β7, αEβ7, β2 integrins) might be expected to be dispensible for development and that appears to be the case (see lower part of Table 1). This set of mice does however shed light on the roles of integrins in leukocyte traffic. Absence of α4β7 and αEβ7 or of αEβ7 alone leads to deficits in recruitment of lymphocytes to the gut-associated lymphoid tissue (28,29), consistent with a role of these integrins in homing to those sites. Similarly, analyses of animals chimeric for the expression of α4 integrins reveals a role for these integrins in homing to Peyer's patches but not to the intestinal epithelium (30) and a similar analysis of β1 integrin chimeras reveals a role for some β1 integrins (but not α4β1, ref 30) in seeding the fetal liver with lymphoid precursors (31). The α4 chimeras also show that both B and T cell progenitors need α4 integrins for normal development in the bone marrow, though apparently not in the fetal liver or thymus (30). Further work along these lines should uncover other roles for specific integrins in the development and/or homing of distinct lymphoid and myeloid cells.

The mutations in β2 integrin genes and in ICAM-1 also provide information about myeloid cell traffic. As expected from the human LAD I patients, β2-deficient mice show reduced recruitment of neutrophils to sites of inflammation (32). The same is true of ICAM-1-deficient mice (33,34). However, since these mutations appear to be hypomorphic rather than null (32,35), the defects are not complete. It will be of interest to determine how the severity of the defects is altered as true null mutations are analyzed. Furthermore, the possibility of overlapping roles of the several ICAMs will need to be addressed by the ablation of the other genes and the generation of double mutations.

Ablation of the αL gene produced defects in mixed lymphocyte response (as did the ICAM-1 mutation) in conformity with expectation from in vitro analyses and also showed deficits in allograft rejection and in responses against tumors (36,37). In contrast, mice lacking αM showed a marked increase in neutrophils in a chemical peritonitis model and it was found that αMβ2 is important for phagocytosis-induced apoptosis; an unexpected result (38). Further analyses of these mice should be informative concerning the

roles of the different β2 integrins and will allow deeper analyses of the defects in LAD I patients.

Similarly, recently obtained strains with null mutations in β3 integrin (39) and von Willebrand factor (40) will serve as mouse models for the human bleeding disorders, Glanzmann's thrombasthenia and von Willebrand's disease. The availability of such mouse models will allow experimental analyses not feasible with human patients and should provide new insights into those diseases and, likely, new therapies.

Conclusions

The development of strains of mice defective in specific integrin subunits or in their ligands or counterreceptors has already provided new information about their roles in the development of the vasculature and in the behavior of blood cells. Further analyses of these mice and of cells there from, as well as the generation of mice with more subtle defects in the same genes, promise to provide further insights and valuable animal models of human disease states including inflammatory disorders and defects in thrombosis and hemostasis.

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